

**International Conference on Emerging
Trends and Innovations in Life Sciences
(ETILS) - Emerging Trends in Life Sciences:
A Compendium of Innovative Research –
Volume I**

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Accredited with A⁺ by NAAC (4th Cycle)

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**International Conference on Emerging Trends and Innovations in Life Sciences (ETILS) -
Emerging Trends in Life Sciences: A Compendium of Innovative Research – Volume I**

© Dr. A. S. Maheswari, Dr. J. Viji, Dr. P. Anitha

First Edition: 2025

ISBN: 978-93-94638-69-3



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Publisher

Thanuj International Publishers,
Tamil Nadu,
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**The light that guides us all...
To them we owe all our achievements**



Our Revered Founder

**Padmabhushan Sri N. Ramaswami Ayyar
Srimathi Alamelu Seethalakshmi Ammal**

Titles Conferred on our Founder

**'Rao Sahib' by the British Government
'Rao Bahadur' by the British Government
'Diwan Bahadur' by the British Government
'Padma Sri' by the Government of India
'Padma Bhushan' by the Government of India**



Sri. R. Panchapakesan

Managing Trustee

Message

I am delighted to acknowledge the tremendous efforts of the Research Department of Zoology in organizing the *International Conference on Emerging Trends and Innovations in Life Sciences*. The department's commitment to advancing knowledge in the fields of Biology and interdisciplinary studies is truly commendable. The two-day conference, I believe has provided an invaluable platform for academicians, researchers, scholars and industry experts to come together, share their insights and explore emerging trends in Life Sciences.

The conference's objective of fostering collaboration, promoting innovation, and encouraging the exchange of research findings has undoubtedly made a significant impact in the academic community. The conference's focus on the latest advancements and innovations across diverse scientific fields is bound to inspire new ideas, enhance research initiatives and pave the way for future breakthroughs.

I extend my heartfelt appreciation to the Head, faculty members and students, whose unwavering dedication, has made this event a meaningful and impactful contribution to the academic and research community.

May the blessings of Goddess Akilandeswari, help the department attain greater achievements in its future academic endeavours.



Dr. M. V. Alli
Principal
Message

The field of life sciences is undergoing a rapid transformation, propelled by ground-breaking discoveries, technological advancements and an urgent global need to address complex challenges in health, agriculture and the environment. The **International Conference ETILS 2025** was conceptualized as a platform to bring together researchers, academicians, industry professionals and young scholars to share and discuss their innovative ideas, novel concepts and cutting edge research that are meaningful, impactful and socially relevant.

My appreciations to the Head and Faculty, Research Department of Zoology, Seethalakshmi Ramaswami College, Tiruchirappalli for taking this initiative of publishing a book on, **Emerging Trends and Innovations in Life Sciences**, the chapters of this book provides a comprehensive exploration on diverse topics including animal science, molecular biology, biotechnology, microbiology, environmental sustainability, bioinformatics and applied sciences.

It brings me great satisfaction to witness our vision of a multidisciplinary scientific discourse materialize successfully. The scholarly contributions compiled in this volume are the result of meticulous research, critical inquiry aiming to contribute positively to science and society. This book will not only serve as a record of the academic deliberations held during the conference but also as a valuable research repository for future researchers, I congratulate the authors of this book for presenting their expert insights.

I am confident that this initiative will grow in both stature and significance, inspiring many more such collaborative endeavours in the future.

Warm Regards



Dr. S. Santhi

Self-Financed Section In-charge

Message

It is with unbridled enthusiasm and unalloyed admiration that I extend my warmest felicitations to the visionary organizers and erudite contributors of the International Conference on Emerging Trends in Life Sciences. The release of this magisterial tome, a veritable treasure trove of cutting-edge research and innovative paradigms, marks a seminal moment in our collective pursuit of advancing knowledge and deciphering the complexities of life sciences.

As we convene to explore the latest trends and breakthroughs in this dynamic field, I am sanguine that this publication will serve as a crucible for the advancement of human understanding, inspiring us to push the boundaries of knowledge and harness the transformative potential of life sciences for the betterment of society.

I commend the authors, researchers, and editors for their indefatigable and unflinching efforts in bringing forth this invaluable resource, which promises to catalyze novel solutions, foster synergistic collaborations, and shape the trajectory of life sciences in profound and far-reaching ways.

Dr.S.Santhi

Self-Financed Section i/c



Dr. A. S. Maheswari

Convener, ETILS 2025

Preface

It is with great enthusiasm that we present the proceedings of the *International Conference on Emerging Trends and Innovations in Life Sciences (ETILS 2025)*, held on **February 5 & 6, 2025**, organized by the Research Department of Zoology, Seethalakshmi Ramaswami College (Autonomous), Tiruchirappalli, in collaboration with the Internal Quality Assurance Cell (IQAC) and the Institution's Innovation Council (IIC).

This international conference aimed to provide a dynamic platform for researchers, academicians, and industry professionals to exchange insights and recent advances in various branches of Life Sciences. The conference themes spanned a broad spectrum—including Applied Biology, Environmental Sciences, Biomedicine, Microbiology, Nanotechnology, and more—highlighting the relevance of scientific innovations in addressing real-world challenges and promoting sustainable development.

The research papers featured in this volume reflect the richness of academic inquiry and the depth of scientific exploration undertaken by contributors from diverse disciplines and institutions. We believe that these proceedings will serve as a valuable resource for future research, collaboration, and innovation in the life sciences community.

We extend our heartfelt thanks to all the authors, reviewers, and participants who contributed to the success of this conference and to the preparation of this publication.

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Rodent Pests of Agricultural Importance and their Eco-Friendly Management Methods

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Rodentia is one of the largest orders of mammals in the world and it comprises of 2277 species in 481 genera included in 33 families (Wilson and Reeder, 2005; Kumar *et al.*, 2013; Chakma *et al.*, 2018). In India, the present checklist reports valid rodent taxa up to subspecies level, includes 103 species and 89 subspecies under 46 genera which belong to 7 families (Pradhan and Talmale, 2011). Of them, the following 20 species have been reported to be of economic importance in India and the same is given in Table 1 (Sridhara and Tripathi, 2005).

Table 1: Major rodent pests of India

S. No	Common Name	Scientific Name	Major Groups
1	Indian crested Porcupine	<i>Hystrix indica</i> Kerr, 1792	Porcupines
2	Northern striped squirrel	<i>Funambulus pennanti</i> Wroughton, 1905	Squirrels
3	Southern palm squirrel	<i>Funambulus palmarum</i> Linnaeus, 1766	
4	Western Ghat squirrel	<i>Funambulus tristriatus</i> Waterhouse, 1837	
5	Indian gerbil	<i>Tatera indica</i> Hardwicke, 1807	Gerbils
6	Desert gerbil	<i>Meriones hurrianae</i> Jerdon, 1867	
7	Hairy-footed gerbil	<i>Gerbillus gleadowi</i> Murray, 1886	

8	Lesser bandicoot rat	<i>Bandicota bengalensis</i> Gray, 1835	Bandicoots
9	Larger bandicoot rat	<i>Bandicota indica</i> Bechstein, 1800	
10	Brown rat or Norway rat	<i>Rattus norvegicus</i> Berkenhout, 1769	Rats
11	The House rat	<i>Rattus rattus</i> Linnaeus, 1758	
12	Bruneusculus rat,	<i>Rattus brunneusculus</i> Hodgson, 1845	
13	Himalayan rat	<i>Rattus nitidus</i> Hodgson, 1845	
14	Wroughton's rat	<i>Rattus wroughtoni</i> Hinton, 1919	
15	Soft-furred field rat	<i>Millardiameltada</i> Gray, 1837	
16	Indian bush rat	<i>Gohundaelliotti</i> Gray, 1837	
17	Short-tailed mole rat	<i>Nesokia indica</i> Gray, 1830	
18	House mouse	<i>Mus musculus</i> Linnaeus, 1758	Mice
19	Field mouse	<i>Mus booduga</i> Gray, 1837	
20	Spiny field mouse	<i>Mus platythrix</i> Bennett, 1832	

The Cauvery delta, “the Granary of South India” is inhabited by four species of field rodents. Out of the four species, the following three rodents namely *Bandicota bengalensis*, *Millardiameltada* and *Mus booduga* is found in the bunds of crop fields (Sivaprakasam, 1988; Neelananarayanan *et al.*, 1996 and Neelananarayanan, 1997); and the fourth one *Tatera indica* is reported to occupy barren lands around the fields of crops (Sivaprakasam, 1988; Neelananarayanan *et al.*, 1996). All these four species are reported to inflict depredation to various stages of different crops of Cauvery delta (Neelananarayanan *et al.*, 1993a, b; 1995a, b; 1997; Neelananarayanan, 1997, 2008a, b). Besides, the human habitations are occupied by three species of rodents such as Brown rats (*Rattus norvegicus*) and house mice (*Mus musculus*) along with the house or black rats (*Rattus rattus*) (Sakthivel, 2012).

A large sized rat *i.e.*, the larger Bandicoot rat (*Bandicota indica*) is commonly found in rice fields of agriculture areas (San and Sein, 2019) and in villages as well as in urban habitats (Herbreteau *et al.*, 2005). This species is reported to cause significant damage to field crops

(grains and vegetables) in addition to stored foodstuffs (Marshall, 1988; Aplin *et al.*, 2003; Sagar and Bindra, 1976). Earlier studies from Thailand and India indicated that *B. indica* infested the following crops namely wheat, rice, groundnut, sugarcane and oil palm (Boonsong *et al.*, 1987; Parshad, 1999).

Rodents are regarded as small mammals and most of them weigh around 100g, squat, compact mammals with short limbs and a tail. The salient features of these animals are i) a pair of chisel-like front teeth, called incisors present in the upper and lower jaws, ii) absence of canine teeth iii) few molars on each of the jaw and iv) toothless gap between the incisors and cheek teeth called diastema. The incisors continue to grow during the life of rodents but are worn down by gnawing.

Rodents are versatile animals with acute sense of hearing, smell, taste and touch. They are highly adaptable and have a high rate of reproduction. Most rodent species will give birth 6-12 young ones in each litter and each of the female can give birth offspring every month with a gestation period of 21-23 days. The females can become pregnant again at post-partum oestrus within 24 hrs of giving birth (Greaves, 1982). In most species of the rodents, a female can reach sexual maturity at 2-5 months of age (about 3 months in rat).

The following reasons are attributed for rodents to attain pest status viz., i) they ingest and depredate food of humans both at the crop fields and at the storage places ii) their indiscriminate biting and burrowing behaviour which results in the destruction of numerous articles (packaging, clothes, furniture and structures like floors, buildings, bridges etc.,) iii) their gnawing habit on electrical cables cause fires and iv) contamination of food products through their urine and faecal droppings thus reducing the sales value and transmitting deadly diseases to man (Prakash, 1988).

Rodents are known to transmit various deadly diseases to human and domestic animals. They are Leptospirosis, Salmonellosis, Trichinosis, Hantavirus pulmonary syndrome, Hantavirus renal syndrome, Argentine hemorrhagic fever and lymphocytic choriomeningitis (Castillo *et al.*, 2003; Vanasco *et al.*, 2003). In developing countries, commensal rodents may attack crops shortly after they have been planted or sown, but they are usually most vulnerable close to harvest. Rodents can damage any crop, but the most important are rice, maize, millet, sorghum

and wheat (Meehan, 1984; Singleton, 2003). Oil seed crops such as groundnuts, sunflower, cotton, linseed and coconuts are also highly vulnerable to rodent attack. Rats may be very harmful to sweet potatoes, yams and other root crops (Lund, 1994).

Rice may be grown by direct seeding, or by transplanting from a nursery. As it grows, stem proliferates (tillering), the panicle formation (heading) and ripen, in a growing season of from three to five months. Two crops per year are often grown in the tropics. Planting is generally more or less synchronized in each locality, according to weather patterns and for flooded rice it is based on the availability of water. Rat damage can occur throughout the growing period. The ripe grain is a rich food source, but the cutting off growing plant is deemed to inflict a significant loss (Wood, 1994).

Several methods are being employed by farmers to control the rodent pests, such as cultural, mechanical (trapping and hunting techniques), biological (using biological agents such as predators/parasites), non-lethal agents (repellents) and chemicals (rodenticides). Trapping and hunting are very old practices to control rodents (Jain and Tripathi, 1987; Smith, 1994). Various types of traps have been developed and of these, live catch traps (Sherman trap and Wonder trap) are commonly used in human dwellings, shops, restaurants etc., whereas kill type trap (snap trap, arrow trap and *Thanjavur* bow trap) is often used for managing field rodents. Tribals in almost all parts of India, especially in the coastal zone, and in the northeastern region, have their designs of indigenous traps. Among these, the common ones are *Thanjavur* bow trap, arrow trap, Bamboo trap and stone trap. The *Thanjavur* bow trap is a very simple one but most effective in the rice fields (Neelamarayan *et al.*, 1995b; Sakthivel *et al.*, 2009). Biological method is an effective measure for controlling vertebrate pests and can help to bring down their population below economic injury level (Wood, 1985; Santhanakrishnan, 1987; Neelamarayanan, 1997; 2007b). Mechanical methods need more manpower involving more costs and are also difficult to operate on a large scale (Sridhara, 1985).

Rodents also serve as food for cats (Buccaman, 1907, 1908; Kirshnamurthy *et al.*, 1967), kites, owls, snakes (Deoras, 1964), hedgehogs, jungle cat, fox, mongoose and monitor lizards (Prakash and Mathur, 1987), though according to Minton (1966), rodents formed only a low percentage of food of monitor lizards and snakes. Whitaker and Dattatri (1986), on the other hand, found that 85 and 75 per cent scats of cobra (*Naja naja*) and russels viper (*Vipera russelli*)

contained remains of rodents, respectively. Further, Whitaker and Dattatri (1986) reported only 29 and 22 percent of the krait (*Bungarus caeruleus*) and saw scaled viper (*Echis carinatus*), respectively exhibited remnants of rodents in the scats. The feeding rates of captive snakes in the Madras snake park, India indicated that during a period of one-month, various species of snakes consumed 6 to 20 rats and mice; the average consumption per snake per month was one rodent in every three days (Whitaker and Dattatri, 1986). If the above consumption rate is considered towards predator - prey relationship between snakes and rodents, obviously the predatory pressure by snakes on rodents appears to be very low. With this low level of predation, rodent population with a high intensity of breeding cannot be controlled. Thus, it appears that snakes alone may not control rodent population in nature (Prakash, 1991). Prakash (1992) emphasized the need for undertaking biological control studies to estimate the role of barn owls in effective control of various field rodent pests.

Several natural predators such as falcons, eagles, kites, owls, jungle cats, domestic cats and snakes mainly feed on rodents. Owls are important biological control agents of agricultural pests as their diet is dominated by rodents (Neelananarayanan et al., 1999, 2007a; Pande et al., 2004, 2007). Even though the potential of owls in agricultural pest management was suggested earlier by Wodzicki (1973) and the possible utilization of natural enemies in Integrated Pest Management needs attention by researchers. Undoubtedly, owls play an important role in managing pest organisms in many terrestrial ecosystems.

Bearing the depredatory role of rodents in mind, our predecessors adopted simple, economic and effective control measures against them. However, the advent of rodenticides changed their attitudes and chemicals replaced the traditional methods which are simple, economic, effective and non-hazardous to non-targets. When man began to use novel methods of rodent control like, use of acute and chronic rodenticides and chemical fumigants, he was totally unaware of the dire consequences of these chemicals to his livestock and other non-target organisms.

In view of the side-effects of rodenticides on the non-targets and environment, the Scientists of today are searching for rodent control methods which will not create any havoc on the ecosystem. To accomplish this objective an attempt was made to review the results of some of the indigenous

rodent control methods of *Cauvery* Deltaic region, which are deemed to be eco-friendly and ecologically sustainable.

The following indigenous rodent control methods viz., *Thanjavur* bow-trap, Bio-control of rodent pests by Barn owl (perching poles for avian predators), Pit fall trap, Burrow fumigation, Burrow digging, etc., were reported to have been used by farmers who inhabited different parts of Nagapattinam district, Tamil Nadu.

1. *Thanjavur* bow-tap

Thanjavur bow-trap is an inexpensive and eco-friendly tool for rodent control but unfortunately many farmers of our country are unaware of it. In this method, no poison or chemical is used for killing the target animals.

Field trials in checking the rodent pests were conducted in a portion of *Cauvery* delta in three rice crop seasons viz., *Kuruvai*, *Samba* and *Thaladi*. An experienced traditional rodent trapper was hired for this purpose. During *Kuruvai* season 68.4 ha rice fields were subjected to the trials over a period of 53 days and during *Samba* and *Thaladi* seasons, simultaneously, 70 ha rice fields were covered over a period of 58 days.

Three hundred *Thanjavur* bow-traps were used every day by the trapper. He placed the traps in the evening hours in a grid pattern at 2-5 m interval and left them overnight. Parched paddy and raw rice mixed with 1% coconut oil was used as bait material to attract the rodents. The traps were planted over a tilted hill and on either side a little quantity of the bait material was placed. To enable the rodents to enter the trap and get entrapped, the hills around the trap were allowed to stand. This technique is said to improve trapping efficiency. The trapper took three hours to place all the 300 traps inside the rice fields.

During *Kuruvai* season 15,900 *Thanjavur* bow-traps were set while during *Samba* and *Thaladi* seasons there were 17,400 night-traps. Out of the traps, 4,029 rodent pests were trapped during *Kuruvai* season and 4,176 rodents, during *Samba* and *Thaladi* seasons. The mean number of rodents trapped per day with three hundred traps were 76.01 (25.33%) for *Kuruvai* season and 72 (24%) for *Samba* and *Thaladi* seasons. The computed number of rodents trapped/ha was 58.9 for *Kuruvai* and 59.65 for *Samba* and *Thaladi* seasons.

All the rodent species, that inhabit the rice fields except *T. indica* which inhabits the barren lands, were trapped and killed by this method. Of the three species viz., *B. bengalensis*, *M. meltda* and *M. booduga*, the *B. bengalensis* is the predominant rodent pest of rice crops of our area and its trapping accounted for 65.52% (2,640 nos.) during *Kuruvai* season and 74.06% (3,051 nos.) during *Samba* and *Thaladi* seasons, followed by *M. meltda* and *M. booduga*. Except, *M. booduga* all the other three species of rodents are eaten by the *Vedars* (a local tribal community) and some local men, and for them this is an important, high protein food supplement (Neelanarayanan *et al.*, 1995b; Sakthivel *et al.*, 2009).

Therefore, it is deemed that this tool is a simple, economical and effective one to combat rodents' menace in the crop fields. As there is no chance for rodents to attain endangered status, this method may very well be encouraged among peasant community. There is a large demand for dried rodent-meal for the livestock, poultry and fish for consumption. Besides, the rodents' skin has also proved to be good for making small leather products. Therefore, the trapped rodents may be used for the above purposes without wasting them.

Hence, the traditional *Thanjavur* bow-trappers are to be identified by the Central and State agricultural personnel and Non-Governmental Organizations and they must be encouraged to practice this ecofriendly method. I sincerely hope that this kind of action will certainly help the marginal and small-scale farmers mostly to keep the rodent pests under check in their rice fields. Further, studies on the effectiveness of this trap in other crop fields are also needed.

Advantages of this method

- requires less manpower
- simple, economical and effective method
- does not affect the non-targets
- an ecofriendly method
- offers good trappability
- all the ravaging rodent species are trapped and killed
- the trapped rodents form a good source of protein for human beings, livestock etc.

- the number of dead rodents is seen by the farmers,
- adds prosperity to the traditional trappers.

2. Biocontrol of Rodent Pests by Barn owl

The food habit of the barn owl has been studied in detail and more extensively than that of any other birds of prey, because of the wide distribution of this bird and the ease with which regurgitated pellets containing non-digestible remains of prey items can be found for analysis (Taylor, 1994). The literature reviews on the major diet of barn owls from six continents were reported to be rodents, the most important small mammalian group. Out of 52 key studies, analyzed on barn owl's diet by Taylor (1994), the rodents constituted more than 50% of all prey items in 47 studies and they formed more than 75% of all prey items by number in 33 studies, suggesting thereby that barn owls are excellent rodent hunters in nature.

Barn owl - Prey items - Frequency

Analysis of 4030 regurgitated pellets revealed that small mammals viz., *B. bengalensis*, *M. meltada*, *M. booduga*, *T. indica* and *Rattus rattus*; an insectivorous mammal, *Suncus murinus*; amphibians, birds and insects were the constituents of the diet of barn owls. Of the six species of small mammals, the first five species are of agricultural importance. Of these, *B. bengalensis* (1953 nos.) and *M. meltada* (1908 nos.) (the most destructive vertebrate pests of this study area) were found to be consumed by barn owls in great numbers during study period. *S. murinus* (567), unidentified rodent species (461), *R. rattus* (393), *M. booduga* (254), *T. indica* (129), insects (21) and birds (1) formed the diet (Neelanarayanan, 1997; 2007b).

Magnitude of Intake by Barn owls in Captivity

Barn owls normally regurgitate daily after every meal. In general, both adult and sub-adult barn owls' consumption of *B. bengalensis* was more (in terms of prey weight) than other species of rodents. The mean number of prey (all species of rodents) consumed by the adult and sub-adult barn owl/day was 1.58 and 1.56, respectively. The mean prey weight consumed/day by the adult and the sub-adult barn owls were 64.62 ± 17.14 g and 68.01 ± 15.49 g, respectively.

Studies on daily food requirements of adult barn owl when they are at reproductive stage and chicks are also required, to calculate and get more precise values on the predatory pressure of barn owls, over rodent pests.

The monthly prey requirement of one adult (non-breeding) and one sub-adult barn owl was worked out to be 47 prey, however in terms of biomass one sub-adult barn owl required more prey (2,040 g / month) than adults (1939 g / month). Obviously, the growing sub-adults require more food than the adults (Neelananarayanan and Kanakasabai, 2005).

In conclusion, the results of the present study vividly indicate that the barn owls are potential hunters of rodent pests and can therefore be used for controlling rodent pests in crop fields and fallow lands. Steps should be initiated to increase their population in the wild by way of installing nest boxes and to breed them in captivity. The barn owls propagated in captive conditions can be released near to crop fields thereby making way to exert greater predatory pressure on rodent pests in agricultural fields. The barn owls can be included as one of the components of Integrated Rodent Pest Management (IRPM) Programme for efficient and Eco-friendly control of rodent pests.

The Other Environment Friendly Methods

3. Pit fall trap

Pots of 20 *litre* capacity are used as traps, in this method. They are placed in the crop fields. Based on the size of the field, the number of pots to be implanted may be decided. Preferably, 4-6 pots may be implanted per (field of one) acre. Further, each pot must be implanted 5-10 m away from the bunds where the activity of rodents is high. About two-thirds of the pot is to be buried into the soil and the exposed one third portion of the pot is to be daubed with mud, to camouflage the presence of the pot. Semisolid cow dung should be prepared and poured into the implanted pot up to one third of the pot to make it to serve as an effective marshy trap. The prepared cow dung should neither be too solid nor too liquid. In order to maintain the semisolid condition of the cow dung ever in the implanted pot, it is to be stirred with the help of a stick in the evening hours of the day, and this will help the same to prevent from becoming solidified at the bottom. Every fortnight this medium needs to be changed.

In order to attract the rodents into the trap any one of the following bait materials *viz.*, parched paddy, dry fish, coconut etc., can be used. In *Cauvery* delta, the parched paddy mixed with coconut oil is the predominant bait. A little quantity of the prepared bait material is spilt around the implanted pot at a few places. The same bait must also be smeared over a layer of husk which is spread on the semisolid cow dung. After eating the bait spilt around the implanted pot, the rats are tempted to enter the pot to forage the bait inside, where they get entrapped. Next morning the pots are to be checked carefully (since at times snakes may also be inside the pot) for the trapped rodents. In an observation made in cotton fields near Andoor village, Nagapattinam district it was known that we recorded a trapping of 30 rodents in three days. Of them there were 16 *B. bengalensis*, 10 *M. melstada* and 4 *M. booduga*. The mean number of animals trapped per day was 10. Studies and documentation on the utilization of this trap in other crop fields are needed (Neelamarayanan and Kanakasabai, 2000).

4. Burrow fumigation

A mud pot of 2 litre capacity is used for this method, with a hole at the centre of the bottom to blow the air at the time of fumigation. A handful of straw is burnt, and smoke is generated from that. The burning straw with the smoke is then inserted into the pot carefully. The pot is now ready for fumigating the burrows. The active burrows of lesser bandicoot rat, *B. bengalensis* and Indian field mouse, *M. booduga* burrow entrance would have been plugged by the occupant rodent, and they are to be unplugged and then the pot is to be fixed at the burrow entrance. The air is blown for few minutes then the pot is detached. The smoke that enters the burrows makes the animals suffocate and they die inside the burrows. The fumigated burrows are opened, and the death of occupant rodents is confirmed. Small- and large-scale adoption of this method to contain rodents' menace is required in different crop fields and they would yield valuable information about whether this method is economical or not for farming community (Neelamarayanan and Kanakasabai, 2000).

5. Burrow digging

It is done by *Vedars* (a Tribal community of district) and local men. In Kancheepuram district of Tamil Nadu, it is done by *Irula* tribals (Whitaker and Murali, 1988). The active burrows of Lesser Bandicoot Rat *B. bengalensis* are identified and they are excavated for capturing the occupant rodent. This rat is chiefly hunted for its delicious flesh. The other burrows of field rodents

viz., Soft-furred Field Rat *M. melstada*, Indian Field Mouse *M. booduga* and Indian Gerbil *T. indica* are also excavated on request by the landowners and they are killed and either buried or eaten by them (Neelananarayanan and Kanakasabai, 2000).

Of all the indigenous methods of rodent control described above, only the following methods are currently being practiced by the farming community. They are *Thanjavur* bow-trap and burrow digging methods. The pit fall trap and burrow fumigation for rodent control are used here and there. The method of implanting perching poles for avian predators is also in use in few places. Very few people, who know this technique, unfortunately do not follow this technique and it may be because of the advent of rodenticides in the market. Therefore, it is hoped that this message is to be spread among the farmers about the significance of traditional rodent control techniques and this can certainly help them to some extent, to bring down the rodent population in the crop fields without affecting the environment.

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The evolving role of Engineered Microbial Consortia in Bioremediation: A Glance

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Abstract: Bioremediation has emerged as an environmentally sustainable approach in mitigating pollution, yet traditional methods relying on single microbial strains often fail to effectively degrade complex contaminants such as petroleum hydrocarbons, heavy metals, and pesticides. Engineered microbial consortia offer a promising alternative by leveraging synergistic microbial interactions to enhance pollutant degradation, improve resilience to environmental stressors, and optimize biotransformation pathways. This review explores the design, mechanisms, applications, and challenges of engineered microbial consortia in bioremediation. It discusses key strategies such as co-metabolism, bioaccumulation, and reductive dechlorination, emphasizing their role in breaking down persistent pollutants. Additionally, the study highlights advancements in synthetic biology, artificial intelligence-driven optimization, and regulatory frameworks to enhance the efficacy and scalability of microbial consortia in real-world applications. Despite challenges such as microbial stability, genetic drift, and biosafety concerns, emerging research suggests that engineered microbial consortia hold significant potential for sustainable and large-scale bioremediation.

Key Words: Bioremediation, Engineered Microbial Consortia, Pollution Degradation, Synthetic Biology, Microbial Interactions.

1. Introduction

Traditional bioremediation often relies on single microbial strains for pollutant degradation, but this approach is limited when addressing complex contaminants such as petroleum

hydrocarbons, pesticides, and heavy metals. These pollutants often require diverse metabolic pathways that a single microorganism cannot efficiently perform (Smith *et al.*, 2022). Engineered microbial consortia, composed of multiple strains with complementary capabilities, offer a more effective solution by mimicking natural microbial communities that synergistically degrade complex contaminants (Jiang *et al.*, 2021). These consortia enhance pollutant degradation, improve environmental adaptability, and accelerate remediation by utilizing the unique metabolic functions of each member (Kumar *et al.*, 2023).

Microbial consortia are designed to overcome the limitations of single-strain bioremediation by providing specialized functions, such as pollutant breakdown, co-metabolism, and transformation of toxic intermediates (Wang *et al.*, 2022). The ability to tailor consortia to specific environmental conditions, including varying pH, temperature, and nutrient availability, further enhances their effectiveness in diverse environments (Li *et al.*, 2024). As a result, engineered consortia offer a versatile and sustainable approach for addressing a broad range of environmental pollutants, including emerging contaminants like pharmaceuticals and microplastics (Zhao *et al.*, 2023).

The development and application of engineered microbial consortia in bioremediation offer significant advantages over traditional single-strain approaches, particularly for the degradation of complex and mixed pollutants. As environmental contaminants become increasingly diverse and resistant to conventional methods, consortia-based strategies provide a more adaptable and efficient solution. By harnessing the synergistic interactions between microorganisms, engineered consortia are able to enhance pollutant degradation, improve environmental adaptability, and optimize remediation efficiency. This review underscores the potential of microbial consortia to address a broad spectrum of contaminants, highlighting their ability to tackle both persistent pollutants and emerging contaminants. Moreover, the ability to tailor consortia to specific environmental conditions and pollutants positions them as a promising avenue for sustainable and scalable bioremediation solutions.

2. The Concept of Engineered Microbial Consortia

Engineered microbial consortia are designed combinations of microorganisms, each selected for its specific role in pollutant degradation or biochemical processes. These consortia offer several advantages over single-strain bioremediation, particularly in degrading complex pollutants like petroleum hydrocarbons, pesticides, and heavy metals. By utilizing a range of microorganisms with specialized metabolic pathways, engineered consortia can degrade

pollutants in a more comprehensive and efficient manner. Some species break down pollutants into intermediate products, while others convert these by-products into non-toxic end products, ensuring a complete degradation process (Wang *et al.*, 2022). The ability to tailor consortia for specific environmental conditions, such as fluctuating temperature, pH, or nutrient availability, further enhances their adaptability and efficiency in diverse polluted environments (Jiang *et al.*, 2021 and Li *et al.*, 2024).

In addition to specialization, microbial consortia offer enhanced resilience and synergistic interactions. The diverse species in a consortium can withstand environmental stressors more effectively than individual strains, as each microorganism may be better suited to survive specific conditions (Kumar *et al.*, 2023). Moreover, interactions like cross-feeding or syntrophic relationships between species increase the overall efficiency of pollutant degradation. These interactions enable microorganisms to support one another's metabolic processes, breaking down a broader range of contaminants and accelerating the bioremediation process (Zhao *et al.*, 2023 and Smith *et al.*, 2022). By leveraging such synergistic mechanisms, engineered consortia offer a robust, adaptable, and sustainable solution for addressing complex environmental pollution challenges.

3. Advantages of Engineered Microbial Consortia in Bioremediation

Advantage	Description	References
Specialization in Metabolic Pathways	Engineered consortia combine microorganisms with specialized pathways, allowing for the complete degradation of pollutants.	Wang <i>et al.</i> (2022); Jiang <i>et al.</i> (2021); Kumar <i>et al.</i> (2023)
Resilience to Environmental Stressors	Consortia can withstand environmental stressors (e.g., temperature changes, pH fluctuations) by utilizing a diversity of species with different tolerance levels.	Li <i>et al.</i> (2024); Zhang <i>et al.</i> (2023); Zhao <i>et al.</i> (2023)
Synergistic Interactions	Microbial species interact synergistically (e.g., cross-feeding, syntrophy), enhancing	Zhao <i>et al.</i> (2023); Smith <i>et al.</i> (2022); Wang <i>et al.</i> (2022)

	pollutant degradation efficiency.	
Customizable for Specific Pollutants	Engineered consortia can be tailored to target specific pollutants, such as petroleum hydrocarbons, pesticides, heavy metals, and emerging contaminants.	Zhang <i>et al.</i> (2023); Smith <i>et al.</i> (2022); Kumar <i>et al.</i> (2023)
Sustainability and Long-Term Efficacy	Engineered consortia maintain bioremediation activity over extended periods, ensuring continuous environmental improvement.	Jiang <i>et al.</i> (2021); Li <i>et al.</i> (2024)
Cost-Effective	Consortia offer a more cost-effective bioremediation option compared to single-strain cultures, reducing maintenance and monitoring costs.	Li <i>et al.</i> (2024); Kumar <i>et al.</i> (2023)
Scalability for Large-Scale Applications	Engineered microbial consortia can be scaled up to address large-scale bioremediation challenges in polluted sites or water systems.	Zhao <i>et al.</i> (2023); Wang <i>et al.</i> (2022)

4. Mechanisms of Action in Engineered Microbial Consortia

Co-metabolism and Cross-Feeding: Engineered microbial consortia function through co-metabolism, where one microorganism degrades the primary contaminants and produces by-products that serve as substrates for other species in the consortium. This creates a network of metabolic exchanges that enhances the degradation efficiency of complex pollutants. These consortia may degrade hydrocarbons, pesticides, or pharmaceuticals by utilizing the by-products formed by different species (Zhang *et al.*, 2023; Kumar *et al.*, 2023 and Wang *et al.*, 2022).

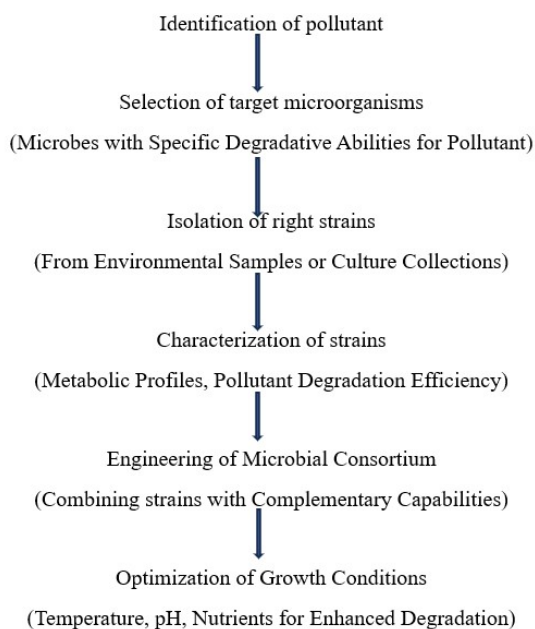
Biotransformation and Bioaccumulation: Certain microorganisms within engineered consortia carry out biotransformation, converting toxic pollutants into intermediate, less

harmful compounds, which are then further degraded by other microorganisms. Additionally, some species have the capacity to bioaccumulate pollutants, such as heavy metals, thus reducing their bioavailability and overall toxicity in the environment (Li *et al.*, 2024; Zhao *et al.*, 2023 and Gupta *et al.*, 2022).

Reductive Dechlorination and Biotransformation of Xenobiotics: Engineered microbial consortia are particularly effective in degrading chlorinated compounds through reductive dechlorination, a biochemical process where chlorine atoms are removed from chlorinated hydrocarbons, such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT). This process renders these compounds less toxic and more biodegradable, aiding in their complete removal from contaminated environments. Furthermore, consortia are also utilized for biotransformation of xenobiotics, non-natural compounds such as pharmaceuticals or industrial chemicals, facilitating their detoxification (Singh *et al.*, 2023; Zhang *et al.*, 2022; Singh *et al.*, 2022; Liu *et al.*, 2023 and Kumar *et al.*, 2023).

5. Engineering Microbial Consortia for Bioremediation

The design of engineered consortia for bioremediation is a complex, multidisciplinary process that integrates principles from microbiology, synthetic biology, and ecology. Successful consortia must be composed of microorganisms with complementary metabolic pathways to facilitate the degradation of a range of pollutants, such as hydrocarbons, pesticides, and heavy metals. Key strategies in engineering microbial consortia include:



6. Applications of Engineered Microbial Consortia in Bioremediation

Applications	Pollutant Type	Microbial Consortia Involved	Mechanisms of Action	References
Petroleum Hydrocarbon Degradation	Crude oil, diesel, benzene, toluene	<i>Pseudomonas sp.</i> , <i>Alcanivorax sp.</i> , <i>Rhodococcus sp.</i>	Co-metabolism and synergistic degradation pathways	Zhang <i>et al.</i> (2023); Li <i>et al.</i> (2024); Khan <i>et al.</i> (2023); Wang <i>et al.</i> (2022)
Heavy Metal Detoxification	Lead (Pb), Mercury (Hg), Cadmium (Cd), Arsenic (As)	<i>Shewanella sp.</i> , <i>Pseudomonas sp.</i> , <i>Bacillus sp.</i>	Bioaccumulation, biosorption, and enzymatic reduction	Zhao <i>et al.</i> (2023); Gupta <i>et al.</i> (2022); Kumar <i>et al.</i> (2023); Liu <i>et al.</i> (2023)
Pesticide and Herbicide Degradation	Organophosphates, atrazine, glyphosate	<i>Sphingomonas sp.</i> , <i>Pseudomonas sp.</i> , <i>Paracoccus sp.</i>	Hydrolysis, oxidation, and microbial mineralization	Chen <i>et al.</i> (2023); Luo <i>et al.</i> (2023); Patel <i>et al.</i> (2023); Singh <i>et al.</i> (2022)
Chlorinated Hydrocarbon Remediation	Trichloroethylene (TCE), Dichloromethane (DCM)	<i>Dehalococcoides sp.</i> , <i>Desulfovibrio sp.</i> , <i>Methanobacterium sp.</i>	Reductive dechlorination	Wang <i>et al.</i> (2022); Zhao <i>et al.</i> (2023); Lee <i>et al.</i> (2023); Brown <i>et al.</i> (2023)
Plastic Waste Biodegradation	Polyethylene (PE), Polyethylene terephthalate (PET), Polystyrene (PS)	<i>Ideonellasakaiensis</i> , <i>Pseudomonas putida</i> , <i>Bacillus subtilis</i>	Enzymatic degradation and microbial assimilation	Singh <i>et al.</i> (2023); Zhang <i>et al.</i> (2023); Kumar <i>et al.</i> (2023); Huang <i>et al.</i> (2024)
Textile Dye Degradation	Azo dyes, Reactive dyes	<i>Aspergillus sp.</i> , <i>Pseudomonas sp.</i> , <i>Bacillus sp.</i>	Enzymatic breakdown via laccases and peroxidases	Chen <i>et al.</i> (2023); Patel <i>et al.</i> (2023); Li <i>et al.</i> (2024); Luo <i>et al.</i> (2023)
Pharmaceutical Waste	Antibiotics, endocrine	<i>Acinetobacter sp.</i> , <i>Pseudomonas sp.</i>	Biotransformation and microbial	Gupta <i>et al.</i> (2022); Zhao <i>et al.</i> (2023);

Decomposition	disruptors	<i>Comamonas sp.</i>	degradation	Singh <i>et al.</i> (2022); Wang <i>et al.</i> (2022)
Polychlorinated Biphenyl (PCB) Degradation	PCB contaminants in soil and water	<i>Burkholderia sp.</i> , <i>Dehalobacter sp.</i> , <i>Pseudomonas sp.</i>	Reductive dechlorination and oxidative metabolism	Brown <i>et al.</i> (2023); Liu <i>et al.</i> (2023); Lee <i>et al.</i> (2023); Huang <i>et al.</i> (2024)
Landfill Leachate Remediation	Ammonia, heavy metals, persistent organic pollutants	<i>Pseudomonas sp.</i> , <i>Acinetobacter sp.</i> , <i>Bacillus sp.</i>	Microbial oxidation, biosorption, and degradation	Lee <i>et al.</i> (2023); Zhang <i>et al.</i> (2023); Patel <i>et al.</i> (2023); Li <i>et al.</i> (2024)

7. Challenges and Limitations of Engineered Microbial Consortia in Bioremediation

Microbial Compatibility and Stability (Wang *et al.*, 2022 and Singh *et al.*, 2023)

- Microbial strains may compete for resources, disrupting the balance of the consortium.
- Ensuring long-term stability of microbial interactions remains a challenge.
- Some microbes may dominate, leading to decreased diversity and efficiency.

Environmental Variability (Gupta *et al.*, 2022 and Luo *et al.*, 2023)

- Fluctuations in temperature, pH, salinity, and oxygen levels affect microbial activity.
- Bioremediation efficiency is often inconsistent across different environmental conditions

Complexity of Pollutant Mixtures (Li *et al.*, 2024 and Huang *et al.*, 2024)

- Some contaminated sites contain diverse pollutants requiring multi-step degradation pathways.
- A single engineered consortium may not efficiently degrade all contaminants.

Scaling Up from Lab to Field Applications (Lee *et al.*, 2023 and Zhang *et al.*, 2023)

- Performance of microbial consortia in controlled laboratory settings does not always translate to real-world environments.

- Large-scale implementation is often unpredictable and less efficient.

Competition with Indigenous Microflora (Singh *et al.*, 2022 and Brown *et al.*, 2023)

- Native microbial communities may outcompete introduced consortia.
- The survival and persistence of engineered microbes in natural environments remain uncertain.

Substrate Availability and Nutrient Limitations (Khan *et al.*, 2023 and Zhao *et al.*, 2023)

- Bioremediation efficiency depends on nutrient availability, which may be limited in contaminated sites.
- Lack of essential electron donors can slow down microbial metabolism.

Cost of Development and Implementation (Gupta *et al.*, 2022 and Kumar *et al.*, 2023)

- High costs associated with strain selection, genetic modification, and process optimization.
- Large-scale deployment and monitoring require significant investment.

Unintended Ecological Consequences (Chen *et al.*, 2023 and Wang *et al.*, 2022)

- Altering microbial populations may disrupt native ecosystems.
- Potential long-term effects on biodiversity and soil microbiota remain uncertain.

8. Future Perspectives in Engineered Microbial Consortia for Bioremediation

The application of engineered microbial consortia in bioremediation is a rapidly advancing field with significant potential for addressing environmental pollution. Future research directions aim to enhance the efficiency, resilience, and scalability of these consortia through innovations in synthetic biology, artificial intelligence (AI)-driven optimization, and eco-friendly deployment strategies.

Advancements in Synthetic Biology for Enhanced Biodegradation (Jiang *et al.*, 2024 and Chen *et al.*, 2023)

- Synthetic biology techniques, such as CRISPR-Cas9 and metabolic pathway engineering, will enable precise genetic modifications to enhance microbial degradation capabilities.

- Biosensors integrated into microbial consortia will facilitate real-time monitoring of contaminant breakdown.

AI and Machine Learning for Consortium Design (Patel *et al.*, 2024 and Luo and Wang, 2023)

- Computational models and AI-driven simulations will aid in predicting microbial interactions, optimizing community composition, and improving degradation pathways.
- Machine learning approaches will enable the rapid identification of the most effective consortia for site-specific pollution challenges.

Adaptive and Self-Regulating Microbial Systems (Singh *et al.*, 2024 and Zhang *et al.*, 2023)

- Engineering microbial consortia that can dynamically adjust to environmental fluctuations, such as pH, temperature, and pollutant concentration, will enhance field applications.
- Self-regulating genetic circuits will ensure the stability and resilience of introduced consortia.

Eco-friendly and Sustainable Bioremediation Strategies (Gupta *et al.*, 2023 and Huang and Li, 2024)

- Future approaches will focus on minimizing ecological disruption by designing consortia that integrate seamlessly with native microbial communities.
- Bioremediation techniques will incorporate bioaugmentation strategies that use plant-microbe interactions (phytoremediation-microbiome integration).

Field-scale Implementation and Regulatory Advances (Kumar *et al.*, 2023 and Wang *et al.*, 2023)

- Translating laboratory-developed consortia into real-world applications requires robust field trials to assess their effectiveness in diverse environmental settings.

- Policy frameworks will need to evolve to address biosafety concerns associated with genetically modified microbial consortia, ensuring ethical and sustainable deployment.

Multi-functional and Multi-pollutant Degradation Consortia (Luo *et al.*, 2024 and Zhao *et al.*, 2023)

- Developing consortia capable of degrading multiple pollutants simultaneously (e.g., hydrocarbons, heavy metals, pesticides) will improve remediation efficiency.
- Engineering microbial communities with complementary enzymatic activities will enable sequential degradation of complex pollutants.

Conclusion

Engineered microbial consortia represent a significant advancement in bioremediation, offering superior pollutant degradation capabilities compared to single-strain approaches. By leveraging microbial diversity, syntrophic interactions, and genetic engineering, these consortia can efficiently degrade a wide range of pollutants, including hydrocarbons, pesticides, heavy metals, and pharmaceuticals. However, challenges such as microbial competition, genetic stability, environmental variability, and regulatory hurdles must be addressed to facilitate their large-scale application. Future research should focus on integrating synthetic biology, machine learning, and biosensor technology to improve microbial stability and adaptability. Additionally, policy frameworks must evolve to ensure the safe and ethical deployment of genetically engineered consortia. With continued advancements, engineered microbial consortia have the potential to revolutionize environmental remediation by providing sustainable, cost-effective, and highly efficient solutions for pollution mitigation.

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Womb of Entrepreneurial Complexities (Some Revised Computations in Choice Estimates)

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Abstract

The Brunswick Lens Model of Choice Making was the first attempt (make use of probabilistic means) towards computational a probabilistic advance to choice making (conduct of human beings) through use of linear regression. The fundamental argument of this mock-up is that a restricted set of signals (use of assortment of indications) can be mapped aligned with a choice objective (ultimate choices) all the way through a weighting design. Aim of this manuscript is to reflect upon heterodoxian and disruption neuroeconomic preferred Brunswik - based choice making process that marks commitment to obdurate intention. The aim is to decipher an interdisciplinary - based optometric peep into dynamics of challenges in neuroeconomic preferred Brunswik - based choice pathways that establish a parallel connecting risk-oriented pattern with preferred Brunswik - based choice-making performing as moderator.

Key Words: Brunswick Lens, Choice Making, Optometrics, Eye Movement and Cognito Entrepreneurial biology

Introduction

Brunswik - based choices are predictable part of personage behavior with daily life being progression of Brunswik - based choices (Goldberg; 1979 and Belton & Dhami; 2021 and Goldstein & Hogarth; 1997). Idiosyncratically, researchers are engrossed in assumptions, beliefs, habits and strategy to make Brunswik - based choices (Goldberg; 1979 and Belton & Dhami; 2021 and Goldstein & Hogarth; 1997). Any iteration of cognito entrepreneurial biology as individual attempt would need elucidation of substrates, techniques in addition to unpredictable effect of poignant maneuver in the lead cognitive utility operative in Brunswik - based choice-making processes relevant and virtual to biological resources (Goldberg; 1979 and Belton & Dhami; 2021 and Goldstein & Hogarth; 1997). Brain considers sources of information before Brunswik - based choice (Goldberg; 1979 and Belton & Dhami; 2021 and Goldstein & Hogarth; 1997). With dissimilar disciplines progressing in course of typically dissimilar techniques and considerable advances, query of how to blueprint Brunswik - based choices has affianced researchers (Goldberg; 1979 and Belton & Dhami; 2021 and Goldstein & Hogarth; 1997). This study investigates optometric basis of Brunswik - based choice inexorableness ('cloud of Brunswik - based choice fog') parameters in cognito entrepreneurial biology of predictable function (Goldberg; 1979 and Belton & Dhami; 2021 and Goldstein & Hogarth; 1997). Cognito compound structure advancement to Brunswik - based choice making, in turn, influence cognito entrepreneurial biology, a perception strappingly entrenched in entrepreneurial psychology and cognito science (Goldberg; 1979 and Belton & Dhami; 2021 and Goldstein & Hogarth; 1997). Amalgamation of these offers stimulating prospective for fabrication of near - precise models of Brunswik - based choice making (Goldberg; 1979 and Belton & Dhami; 2021 and Goldstein & Hogarth; 1997).

Aim and Objective(s)

Scope examines range, dimensions and predictability of biological substrates underlying interdisciplinary modeling attempt to replicate ideology of biology in influence of optometric algorithms in shaping HP-DM with business strategy (Castellan; 1977 and Kowler; 2011). Ocular tracking have been explored towards obtaining deductions in cognito complicated anthropoid capital based business 'drivership' preferred Brunswik - based choices on 05 Participants (Castellan; 1977 and Kowler; 2011). Manuscript addresses new findings to answer issues in business 'drivership' preferred Brunswik - based choice making signature(s) (Castellan; 1977 and Kowler; 2011). Manuscript aims on major planning predicaments and entrepreneurs (practitioners) that make bulk of complicated preferred Brunswik - based choice preferred Brunswik - based choices (Castellan; 1977 and Kowler; 2011). Objective is to screen ideology of ecology in behavioral replicas within Neuro - Probabilistic Functionalism Endoscopy in Brunswik's Lens Preferred Brunswik - based choices (Castellan; 1977 and Kowler; 2011). Purpose is to reject traditional assumptions and evaluate cognitive factors and especially neural - agents have stimulus on actor's preferred Brunswik - based choice (Castellan; 1977 and Kowler; 2011).

Methodology

Cognitive architectures can be really useful as a way "to explore different aspect of cognition" (Koshkin, Shtyrov, Myachykov, and Ossadtchi; 2018 and Zhao, Gersch, Schnitzer, Doshier & Kowler; 2012, Brunswik; 1957, Belton and Dhami; 2021 and Bruce & Friedman; 2002). A few neurocognitive models are quite simple (Koshkin, Shtyrov, Myachykov, and Ossadtchi; 2018 and Zhao, Gersch, Schnitzer, Doshier and Kowler; 2012, Brunswik; 1957 and Belton and Dhami; 2021 and Bruce & Friedman; 2002). Though implemented in spear neurons, it's not clear how organically reasonable they are (Koshkin, Shtyrov, Myachykov, and Ossadtchi; 2018 and Zhao, Gersch, Schnitzer, Doshier and Kowler; 2012, Brunswik; 1957 and Belton & Dhami; 2021 and Bruce & Friedman; 2002). Thalamus-cortical path idea is in fact influential and may in point of fact be basis of a lot (if not all) of cognition (Koshkin, Shtyrov, Myachykov, and Ossadtchi; 2018 and Zhao, Gersch, Schnitzer, Doshier and Kowler; 2012, Brunswik; 1957 and Belton and Dhami; 2021 and Bruce & Friedman; 2002). We apply Koshkin, Shtyrov, Myachykov, and Ossadtchi; 2018 and Zhao, Gersch, Schnitzer, Doshier and Kowler; 2012, Brunswik's Lens apparatus to identify numerous pieces of information and data, within realm of probabilistic functionalism, to synchronize various advancements and up-gradations in designing a 'diagram of cues' based on inter - related signals ;eye (Koshkin, Shtyrov, Myachykov, and Ossadtchi; 2018 and Zhao, Gersch, Schnitzer, Doshier and Kowler; 2012, Brunswik; 1957 and Belton and Dhami; 2021 and Bruce & Friedman; 2002, Koshkin, Shtyrov, Myachykov, and Ossadtchi; 2018 and Zhao, Gersch, Schnitzer, Doshier and Kowler; 2012, Brunswik; 1957 and Belton and Dhami; 2021 and Bruce & Friedman; 2002). Scope examines practitioners' challenges which include (personified, enacted, entrenched and biological) under - defined projects where range, dimensions and predictability of biological substrates underlying cognition processes cannot be reasonably expected (Koshkin, Shtyrov, Myachykov, and Ossadtchi; 2018 and Zhao, Gersch, Schnitzer, Doshier and Kowler; 2012, Brunswik; 1957 and Belton and Dhami; 2021 and Bruce & Friedman; 2002).

Methodology incorporates literature review and conceptual framework of turf research in a Neuro - Probabilistic Functionalism Endoscopy in Koshkin, Shtyrov, Myachykov, and Ossadtchi; 2018 and Zhao, Gersch, Schnitzer, Doshier and Kowler; 2012, Brunswik's Lens Preferred Brunswik - based choices tone (Koshkin, Shtyrov, Myachykov, and Ossadtchi; 2018 and Zhao, Gersch, Schnitzer, Doshier and Kowler; 2012, Brunswik; 1957 and Belton and Dhami; 2021 and Bruce & Friedman; 2002). Methodology include inter-disciplinary modeling attempt with an empirical part (Koshkin, Shtyrov, Myachykov, and Ossadtchi; 2018 and Zhao, Gersch, Schnitzer, Doshier and Kowler; 2012, Brunswik; 1957 and Belton and Dhami;

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Experimental Results and Discussion

Five (N=05) participants were adopted for the eye tracking test. The Tobii eye tracker was used for the purpose. The data obtained are as under;

Subject: 01

Fixation Data

Fp1	Fp2 Pz	F3 Oz	F4 FC1	C3 FC2	C4 CP1	P3 CP2	P4 FC5	O1 FC6	O2 CP5	F7 CP6	F8 TP9	T7 TP10	P7 POz	P8 ECG	Fz	Cz															
0.033682	24.671412	0.172009	7.861377	-6.647998	34.022144	29.998737	21.032007	-18.893045	18.115742	24.508354	45.013084	5.885443	85.155548	-1.324815	60.952770	24.978699	3.106235	36.929749	7.628222	-2.337736	43.489971	26.218437	-6.520770	-16.588787	45.609886	44.029114	27.474224	-7.964358	172.664719	-17.771767	-706.660583
-0.965546	24.244377	0.240662	7.663444	-6.825913	34.585590	28.586773	20.393476	-19.931005	18.381664	23.793961	42.277485	5.586985	84.080780	-2.520565	60.278538	25.012394	2.573836	35.411797	8.175406	-2.853816	43.425816	25.079868	-7.575635	-16.833443	43.755295	42.752907	26.019911	-9.466019	172.288300	-18.951574	-782.008972
-1.369186	25.317146	0.756557	8.054731	-7.456155	35.041466	26.925602	19.680927	-21.251297	18.244781	24.663288	40.227379	5.385910	82.689888	-3.966821	59.303085	25.636478	2.129652	33.852360	8.027685	-3.329039	43.615578	23.928846	-8.803739	-17.097572	42.095875	41.108864	24.413532	-11.050455	171.572906	-20.435474	-854.389221
-0.908024	27.927149	1.910053	8.865606	-8.542793	35.076370	24.873840	18.805042	-22.638500	17.759922	27.531326	38.780323	5.210974	80.784409	-5.649893	58.010658	26.629150	1.748022	32.126411	7.139426	-3.619663	43.715782	22.742901	-10.129781	-17.183048	40.412636	38.945976	22.787754	-12.889858	170.077255	-21.955790	-894.306274
0.345871	31.240946	4.021040	9.722837	-9.627351	34.501442	22.613224	17.850105	-23.458778	17.356770	32.658089	37.305729	5.268284	78.205978	-7.233913	56.546116	27.607836	1.428079	30.128889	5.742206	-3.572217	43.197510	21.538271	-11.515790	-16.705368	38.081085	36.230988	21.156078	-15.055040	167.175323	-23.106047	-881.922180
1.748079	34.126396	7.195946	10.259102	-10.097607	33.564472	20.890440	17.174711	-23.117146	17.761742	39.632782	35.543999	6.068541	75.565506	-8.008397	55.510323	28.401825	1.213889	28.229292	4.439224	-3.076350	42.043938	20.684765	-12.934031	-15.408071	34.922203	33.667793	19.688482	-16.989887	163.979889	-23.512365	-816.111694
2.659975	36.207893	10.989790	10.488289	-9.734606	32.615154	20.251877	17.065634	-21.506618	19.414013	47.000862	33.934566	7.756420	73.717308	-7.565348	55.413280	29.178194	1.171677	26.897717	3.843730	-2.150822	40.758228	20.513548	-14.263574	-13.384700	31.427891	31.992496	18.751745	-17.958521	162.084976	-22.943712	-711.686523
2.538000	36.516834	15.120340	10.223135	-8.190639	31.908207	20.955996	17.724911	-18.533636	22.561132	53.659615	32.085670	10.413119	73.021797	-5.758231	56.451431	29.785465	1.305463	26.260054	4.299258	-0.940505	39.383869	21.033535	-15.494828	-10.684702	27.511765	31.455526	18.314957	-17.811234	161.851807	-21.346828	-595.331726
1.387058	35.285347	19.029484	9.606755	-5.821324	31.486937	22.612251	19.018597	-14.703677	26.858467	58.586983	30.092745	13.511078	73.367645	-3.080188	58.484528	30.255013	1.526863	26.177242	5.711425	0.321672	38.064159	21.984045	-16.636234	-7.731685	23.470196	31.808744	18.338413	-16.759729	163.010834	-18.940825	-498.981476
-0.451645	33.675812	22.078726	9.051246	-3.394385	31.399628	24.606653	20.695448	-10.946718	31.576372	61.256836	28.548794	16.392254	74.567749	-0.299080	61.226593	30.822691	1.769908	26.543003	7.690402	1.393791	37.173683	23.122822	-17.603798	-5.158272	20.016876	32.736702	18.873730	-15.132549	165.424622	-16.164284	-447.597534
-2.375839	33.073280	23.718750	8.964443	-1.779256	31.581955	26.142321	22.356152	-8.315971	35.696140	61.625992	27.991318	18.374371	76.206802	1.682804	64.109924	31.633593	1.938777	27.136835	9.673822	2.051702	36.968861	24.123589	-18.259571	-3.547424	17.839806	33.766716	19.889458	-13.534003	168.535721	-13.556440	-452.394745
-3.886944	34.242176	23.755013	9.502269	-1.425336	31.970818	26.793118	23.730103	-7.297468	38.523067	60.285271	28.547173	19.195225	77.937302	2.418114	66.746010																

Subject: 02

Fixation Data

Fp1	Fp2 Pz	F3 Oz	F4 FC1	C3 FC2	C4 CP1	P3 CP2	P4 FC5	O1 FC6	O2 CP5	F7 CP6	F8 TP9	T7 TP10	T8 POz	P7 ECG	Fz	Cz
32.600426	1.969907	27.795832	11.336749	2.159958	37.456474	24.787504	-18.516167	-3.146668	17.284016	34.668671	21.278065	-12.444899	171.772125	-11.478798	-510.415283	
-4.707321	37.712261	22.149010	10.818339	-2.417769	32.577866	26.708633	24.796896	-7.712485	39.926807	57.858833	30.584190	19.016787	79.875145	2.129310	69.172104	33.737350
1.931717	28.658606	12.822327	1.731556	38.792217	25.241211	-18.258774	-3.936689	18.755081	35.721092	23.180805	-11.652980	175.332840	-9.932147	-604.534241		
-4.962822	42.248116	19.517612	12.390495	-4.076628	33.353649	26.403004	25.602039	-8.822177	40.148647	55.342972	33.404823	18.488216	82.066925	1.509009	71.391235	34.582073
1.795019	29.718044	14.378338	0.868198	40.503376	25.613773	-17.622253	-5.329662	21.700829	37.256569	25.274443	-11.035838	179.158264	-8.832572	-711.862488		
-4.324585	47.930561	16.019833	14.317131	-6.190574	33.896667	25.846462	25.939224	-10.153314	39.215256	52.499069	37.316063	17.654497	84.477890	0.796661	72.977837	35.134190
1.617187	30.823658	16.204552	-0.248204	42.482307	25.824652	-16.480690	-6.821834	26.079367	39.205330	27.678837	-10.373908	182.630219	-7.817923	-809.551697		
-3.153755	53.405991	12.459917	16.149225	-7.873134	34.125572	25.730083	25.976971	-10.793549	37.868240	49.841263	41.897869	17.197735	87.309799	0.830313	74.101440	35.288437
1.506144	32.049801	18.527206	-1.330451	44.391899	26.149452	-15.044860	-7.680905	31.099571	41.833836	30.197449	-9.173668	185.974594	-6.585850	-874.429871		
-2.221516	56.903004	9.994680	17.259504	-8.068768	34.309898	27.171421	26.195749	-9.809548	37.280785	48.621758	46.508629	18.153099	90.926224	2.680079	75.503105	35.076019
1.626926	33.715008	21.401958	-2.035307	45.955109	27.175985	-13.726727	-7.287472	35.692719	45.633465	32.521801	-6.760546	190.297745	-4.996946	-890.309509		
-1.644394	57.812141	9.355522	17.419952	-6.546178	34.386391	30.160423	26.592648	-7.158452	37.762287	49.708504	50.601982	20.627417	94.763275	6.279311	77.147636	34.600914
1.966608	35.568226	24.237803	-2.154970	46.780075	28.887508	-12.842851	-5.478067	38.897442	50.130070	34.253525	-3.409523	195.004318	-3.198366	-852.381042		
-0.823086	56.808079	10.566822	16.838383	-3.997088	33.928726	33.439774	26.619139	-3.768980	38.563568	53.103409	53.744976	23.692272	97.404999	10.333603	78.122154	33.927307
2.302310	36.782883	26.008652	-1.700691	46.428173	30.577475	-12.509440	-2.603588	40.122040	53.934780	34.974625	-0.408797	197.709290	-1.596901	-766.851685		
0.409166	54.504250	13.342524	15.732526	-1.045374	33.024372	36.269035	26.107079	-0.547201	39.148098	58.633568	55.632442	26.773609	98.131851	13.898872	78.084648	33.188400
2.504948	37.047302	26.135775	-0.797658	44.927811	31.882568	-12.746036	0.725537	39.276344	56.351727	34.480965	1.402883	197.474976	-0.653898	-647.515686		
1.647766	51.255310	17.154551	14.264105	1.968316	32.297493	38.756313	25.419264	2.001059	39.504047	65.915451	56.241688	29.856388	97.276596	16.776300	77.488068	32.595177
2.605029	36.749077	24.773191	0.359933	42.836227	32.965759	-13.458945	3.815278	36.836792	57.698746	32.925526	2.045340	195.388855	-0.671099	-513.278076		
2.775709	47.637184	21.119806	12.677211	4.567249	32.061268	40.641121	24.752747	3.517378	39.410839	73.745064	55.604595	32.524078	95.087448	18.612823	76.469429	32.153732
2.580985	36.060772	22.344124	1.471457	40.569710	33.694855	-14.392533	6.044849	33.483040	58.042774	30.607473	1.446070	191.764099	-1.688051	-386.078613		
4.080145	44.392551	24.303185	11.237811	6.178920	32.166008	41.245621	24.023945	3.767185	38.461132	80.519417	53.835133	33.986786	91.545181	18.875286	74.746269	31.679895
2.327224	34.829151	19.297743	2.233265	38.236237	33.637665	-15.225291	7.037329	29.855200	57.038860	27.860573	-0.640367	185.948486	-3.489460	-285.863831		

Subject: 03

Fixation Data

Fp1	Fp2 Pz	F3 Oz	F4 FC1	C3 FC2	C4 CP1	P3 CP2	P4 FC5	O1 FC6	O2 CP5	F7 CP6	F8 TP9	T7 TP10	T8 POz	P7 ECG	Fz	Cz
5.617201	42.092823	26.151585	10.135323	6.552718	32.598957	40.534786	23.364119	2.824293	36.761730	85.136086	51.575886	33.982765	87.275452	17.641703	72.540558	31.194607
1.872854	33.307255	16.232977	2.517769	36.180393	32.835915	-15.713098	6.722445	26.741655	54.926933	25.196623	-3.756504	178.762360	-5.709141	-225.459869		
7.194185	40.951920	26.568741	9.450030	5.820937	33.504448	39.059750	23.091490	1.026213	34.873478	87.230942	49.679047	32.820457	83.316345	15.501627	70.468597	30.728483
1.385561	31.186726	12.043498	1.892082	34.371277	30.458328	-15.187713	3.088733	24.256369	52.409454	23.165524	-6.984310	172.084045	-7.935755	-208.582413		
8.840677	40.991432	25.624208	9.207572	4.133657	34.716278	36.982216	23.206827	-1.371391	33.089157	86.660011	48.562054	30.608608	80.080666	12.738415	68.664871	30.728483
0.953601	31.186726	12.043498	1.892082	34.371277	30.458328	-15.187713	3.088733	24.256369	49.690990	22.028393	-9.948644	166.525436	-9.835916	-231.172668		

10.637733	42.237572	23.391937	9.504749	1.618886	35.975311	34.247101	23.523243	-4.240294	31.449059	83.346085	48.397507	27.376656	77.596443	9.428795	66.986893	30.764618	0.637636	30.621130	11.127242	1.132007	34.651039	28.963881	-14.142152	0.337262	25.019150	46.752350	21.859283	-12.579483	161.983322	-11.226234	-283.723450
12.384542	44.707127	19.976027	10.417871	-1.567922	37.262428	31.083487	23.919323	-7.618146	29.949049	77.475769	49.420307	23.429672	76.086655	5.775784	65.489113	31.171307	0.525504	30.444141	10.884221	0.182893	35.942032	27.429615	-12.711423	-2.858737	27.177685	43.924484	22.637989	-14.641726	159.147629	-12.202335	-353.222656
13.827568	48.204025	15.582355	11.888380	-5.239499	38.530357	27.663963	24.196434	-11.602514	28.398170	69.467781	51.480988	19.113705	75.534439	1.902712	64.108551	32.058392	0.639775	30.593473	11.040105	-0.891231	38.316399	25.949261	-11.141080	-6.441531	30.542889	41.419342	24.088009	-16.138428	158.291565	-13.065566	-424.539246
14.968035	52.214165	10.578348	13.651675	-9.214954	39.421104	23.776535	23.931330	-16.197060	26.349010	59.944832	53.782310	14.521517	75.272400	-2.319603	62.386913	33.200546	0.825261	30.545137	11.128031	-2.066642	41.282864	24.249765	-9.777818	-10.236907	34.381664	38.914768	25.593487	-17.601976	158.216354	-14.147338	-482.127686
15.662201	55.690517	5.744829	15.183475	-13.024736	39.730354	19.597481	22.944338	-21.042688	23.724426	50.235138	55.271980	10.078433	74.777313	-6.644374	60.203323	34.256195	0.920971	29.908216	10.743833	-3.266422	44.145966	22.258387	-9.054708	-13.874002	37.654572	36.267349	26.448816	-19.368973	158.044144	-15.708644	-513.414856
15.664984	57.662693	2.100145	15.985271	-16.080832	39.575958	15.818317	21.443727	-25.575941	20.935059	42.180672	55.369667	6.577950	74.067932	-10.419044	57.971664	35.079277	0.888510	28.743130	9.645804	-4.312737	46.453468	20.328411	-9.299359	-16.901030	39.556213	33.780434	26.234322	-21.231695	158.103821	-17.854240	-512.030212
15.217903	57.881042	0.390503	15.853838	-18.165857	38.889355	12.676817	19.509987	-29.423801	18.233074	37.216408	53.942070	4.333658	72.923355	-13.379643	55.824162	35.565269	0.651569	26.984951	7.577607	-5.046986	47.745712	18.600368	-10.610393	-18.942455	39.557491	31.394430	24.793104	-23.168324	158.048706	-20.498566	-478.898865
14.909740	56.608387	0.842686	14.891191	-19.353716	37.444553	9.918921	17.119257	-32.320431	15.707933	35.875885	51.077461	3.112888	70.876335	-15.663568	53.584679	35.558216	0.132103	24.422268	4.467510	-5.392315	47.552681	16.908920	-12.799684	-19.786270	37.410484	28.673332	22.246201	-25.394096	156.616898	-23.306995	-420.657898

Subject: 04

Fixation Data

Fp1	Fp2 Pz	F3 Oz	F4 FC1	C3 FC2	C4 CP1	P3 CP2	P4 FC5	O1 FC6	O2 CP5	F7 CP6	F8	T7 TP9	T8 TP10	P7 POz	P8 ECG	Fz	Cz														
14.844948	54.003353	3.201937	13.319235	-19.598066	35.478432	7.752479	14.635142	-33.950611	13.777323	37.877945	47.218433	2.862896	68.184341	-17.089485	51.496857	33.076553	-0.566452	21.393818	0.776697	-5.340253	45.946083	15.399893	-15.470441	-19.473246	33.422024	25.778061	19.062206	-27.602051	153.921036	-25.840868	-347.373108
14.645912	50.352108	6.754677	11.529031	-18.822298	33.678337	6.801087	12.733819	-34.134918	13.006613	42.168228	43.282127	3.743200	65.860558	-17.239681	50.177135	34.429825	-1.165027	18.765890	-2.642264	-4.953685	43.675705	14.539332	-18.089798	-18.317879	28.584137	23.501057	15.993487	-28.977898	151.609085	-27.718420	-271.978699
14.030853	45.999947	10.548796	9.863661	-17.166071	32.499702	7.225836	11.817245	-32.974506	13.533024	47.193291	39.884335	5.538595	64.544975	-16.113974	49.882835	33.785561	-1.499867	17.079000	-5.094917	-4.407050	41.349537	14.464084	-20.230167	-16.766941	23.846437	22.243176	13.577333	-29.148090	150.529022	-28.730894	-208.966034
12.991459	41.328506	13.680387	8.541267	-15.003694	31.974266	8.622081	11.873163	-30.802094	15.037268	51.457672	37.185085	7.633377	64.176842	-14.182756	50.413467	33.115334	-1.590687	16.345680	-6.296651	-3.920758	39.178078	14.905128	-21.681274	-15.302432	19.767761	21.800816	11.976070	-28.339470	150.249023	-28.857389	-171.227432
11.624746	36.977364	15.453932	7.731599	-12.887973	31.990196	10.470215	12.675309	-28.237318	16.954256	53.815887	35.438786	9.371031	64.542259	-12.074422	51.443798	32.495644	-1.514911	16.464533	-6.331018	-3.651246	37.416122	15.594552	-22.382011	-14.405293	16.862108	21.904095	11.186992	-26.907013	150.447189	-28.293896	-167.147461
10.494658	34.741745	14.984592	8.154247	-11.881551	32.553513	11.379656	13.551501	-27.642431	17.328112	53.080078	35.413223	9.420041	64.824341	-11.861740	51.752659	32.397720	-1.199589	17.142511	-6.954851	-3.933658	36.808838	16.053902	-22.170948	-14.852556	15.869938	21.392303	10.860415	-26.331623	149.711197	-28.088684	-199.306671
9.431892	33.768002	12.795126	8.797960	-11.726248	32.843292	11.364971	13.944798	-28.236748	16.648617	49.492393	35.811871	7.800888	64.476662	-12.997759	51.254997	32.288383	-0.883142	17.809214	-7.628338	-4.428271	36.674850	16.250751	-21.600033	-16.321276	15.840865	20.412968	10.632318	-26.547859	147.659943	-27.939095	-264.745148
8.585978	33.908276	9.296621	9.682967	-12.496792	33.064419	10.935560	14.149737	-29.424126	15.283388	43.872055	36.826580	5.138754	63.875454	-14.804237	50.416893	32.312298	-0.608793	18.708935	-8.013711	-5.103248	37.209362	16.321362	-20.598526	-18.726870	16.950150	19.515184	10.690075	-26.919624	145.449982	-27.766226	-356.119232
8.003176	35.131584	4.659395	10.718616	-14.162997	33.216888	10.109334	14.113469	-31.200859	13.249218	36.494942	38.290894	1.557497	63.037228	-17.179829	49.223461	32.461445	-0.345630	19.784353	-8.171347	-5.912869	38.480453	16.295280	-19.223381	-22.004448	19.099258	18.795494	11.046748	-27.417259	143.293411	-27.626099	-462.152740

7.873968	37.419441	-0.963032	11.847743	-16.666925	33.122360	8.542229	13.638615	-33.642273	10.353340	27.514030	39.810913	-2.996572
61.741718	-20.285839	47.354507	32.572323	-0.106595	20.681337	-8.280728	-6.859241	40.248974	15.946880	-17.512226	-25.957048	22.003450
17.919525	11.648524	-28.366146	140.483292	-27.561989	-567.933411							
7.980011	40.367222	-7.074478	12.919200	-19.584244	32.971260	6.513258	12.911017	-36.583221	6.885149	17.757534	41.099499	-7.991140
60.360626	-23.750267	45.072414	32.599472	0.190220	21.418491	-8.395761	-7.899053	42.380566	15.440871	-15.591763	-30.216845	25.360605
17.026381	12.497811	-29.615364	137.564621	-27.628033	-656.620972							
7.782772	42.869541	-12.635597	13.539945	-22.031374	33.189194	4.956846	12.457150	-39.370983	3.740202	9.082630	41.699413	-12.254248
59.578880	-26.600880	43.256252										

Subject: 05

Fixation Data

Fp1	Fp2	F3	F4	C3	C4	P3	P4	O1	O2	F7	F8	T7	T8	P7	P8	Fz	Cz
Pz	Oz	FC1	FC2	CP1	CP2	FC5	FC6	CP5	CP6	TP9	TP10	POz	ECG				
32.473778	0.660870	22.278301	-8.437315	-8.899678	44.584942	15.280474	-13.793948	-34.153152	28.540522	16.649946	13.500871	-30.619602					
135.854889	-27.821220	-713.414673															
7.214636	44.057522	-16.603909	13.413147	-23.365828	33.780014	4.302015	12.492071	-41.397190	1.561267	3.323895	41.100033	-14.985250					
59.442242	-28.274549	42.355801	32.001694	1.264351	23.155491	-8.504660	-9.710271	46.199322	15.599391	-12.469054	-37.091576	30.687706					
16.765417	14.421887	-31.272324	135.286346	-28.047604	-729.787109												
6.749836	44.019882	-18.389973	12.660395	-23.565937	34.407192	4.299910	12.828967	-42.538994	0.398466	1.455975	39.377415	-16.068659					
59.517010	-28.866697	42.152042	31.165806	1.842033	23.702351	-8.873572	-10.217629	46.711472	16.162317	-11.763059	-38.617046	31.357323					
16.920357	15.090595	-31.818390	134.870377	-28.224049	-705.360413												
6.673620	43.158890	-17.845253	11.614084	-22.792940	34.967232	4.934798	13.415050	-42.742676	0.332751	3.662975	37.138027	-15.469049					
59.716064	-28.376307	42.538250	30.230505	2.330983	23.954605	-9.537495	-10.326980	46.181290	16.931612	-11.611836	-38.653946	30.681347					
17.106476	15.560621	-32.103264	134.531418	-28.233334	-646.860291												
6.885752	41.477081	-15.262085	10.468474	-21.129532	35.504395	6.423764	14.328649	-41.744411	1.560511	9.269798	34.760761	-13.238760					
60.106354	-26.643871	43.540565	29.386292	2.730173	24.168268	-10.099767	-10.022073	44.860668	17.965700	-11.884500	-37.408073	28.895836					
17.598803	15.896534	-31.790558	134.523712	-27.873371	-566.877747												
7.325804	39.005913	-11.400196	9.354478	-18.851362	35.891998	8.555550	15.423162	-39.511177	3.857786	16.666466	32.334862	-9.926611					
60.450657	-23.938446	44.820572	28.636562	2.989669	24.363903	-10.185161	-9.417390	42.948494	19.053265	-12.411927	-35.312077	26.295774					
18.361666	16.057554	-30.940205	134.308777	-27.022886	-482.977722												
7.930471	36.217339	-7.227463	8.481126	-16.452267	36.089661	10.929431	16.490204	-36.485840	6.640257	23.984459	30.203127	-6.264015					
60.649258	-20.777967	45.953648	28.092066	3.085087	24.567869	-9.666121	-8.698565	40.902599	19.997421	-12.981248	-32.951706	23.539371					
19.321983	16.047821	-29.690002	133.689255	-25.812487	-414.175110												
8.516338	33.803913	-3.536423	8.060617	-14.412318	36.294674	13.349569	17.480087	-33.332333	9.387529	29.969002	28.992636	-2.738863					
60.979671	-17.547482	46.864582	28.020361	3.065105	24.992659	-8.638522	-8.034959	39.396473	20.852222	-13.435214	-30.887323	21.482832					
20.631105	15.985664	-27.977322	133.477417	-24.565538	-374.653992												
9.062540	32.147362	-0.735210	8.069601	-13.055651	36.524582	15.478638	18.279129	-30.624771	11.634604	34.133308	28.839985	0.233027					
61.434708	-14.686073	47.528324	28.394487	2.916087	25.569273	-7.415175	-7.542527	38.625076	21.548805	-13.768931	-29.402565	20.497879					
22.134306	15.882520	-26.069717	133.836029	-23.587116	-368.397705												
9.801317	31.389374	1.076504	8.374111	-12.531925	36.543045	16.748283	18.662949	-28.781200	12.945770	36.472778	29.408628	2.243343					
61.633324	-12.733802	47.744755	28.910452	2.572336	25.908045	-6.404887	-7.264640	38.287079	21.793922	-14.013089	-28.461157	20.448795					
23.277763	15.685777	-24.609371	133.840744	-23.037529	-387.994904												
10.819437	31.658756	1.979042	8.917657	-12.798208	36.337929	16.986757	18.648331	-28.004730	13.247404	37.266937	30.614429	3.261475					
61.641335	-11.847547	47.595119	29.421268	2.066872	25.915127	-5.797943	-7.150151	38.273449	21.549677	-14.110974	-27.887024	21.312180					
23.906321	15.581298	-23.818748	133.445068	-22.917120	-416.527069												
11.837664	33.055550	2.096451	9.797280	-13.566199	36.273510	16.681387	18.637913	-28.056803	13.023064	36.842026	32.744175	3.715284					
62.162834	-11.558748	47.640278	30.078997	1.613812	25.990131	-5.351366	-7.070176	38.877052	21.182667	-13.890180	-27.494261	23.306139					
24.507137	16.054976	-23.184193	133.913116	-22.995258	-431.782776												

SUMMARY

Fixation Data

0.249740 49.604111 36.159378 20.515045 922335 37.701614 42.947479 31.086334	114606 59.848961 36.650101 30.264475 33.252781 37.420448 38.561821	0.256561 15.459188 9.691168 51.969713 31.274460 17.209667 51.910400	0.283406 5.881588 32.778893 45.310707 230804 21.660797 48.675854 10.225013
0.092536 43.525070 36.823029 16.643749 522875 38.591988 47.930969 32.205273 43.667919 6.306565	0.266488 10.762671 2.162234 49.331666 623138 16.386589 52.824471 44.503956 46.127213 23.787205	2.166258 35.052559 43.737629 10.081213 884331 48.788235 11.075224 62.491264 44.818047 35.599926	5.849841 53.446583 46.742943 50.534601 467117 43.586895 54.705036 31.992371 5.045295 44.295555
0.488688 36.636951 37.711143 12.222711 32.358532 39.955627 53.601555	5.981517 9.569544 4.717523 48.883438 347435 16.150452 53.133259 45.854355	1.271610 37.689903 42.307720 13.073258 22.391621 48.974087 12.055657	8.984014 48.517994 25.788338 54.008209 50.271069 33.930779 1504.005005
1.958525 29.677258 38.412350 6.961436 475864 41.372063 58.239376 35.297401	7.614517 13.676004 21.373814 79.709191 569748 38.720882 5.733801 43.144428	2.351521 39.743797 40.463829 15.142411 239353 49.247143 12.761192 47.627869	6.693375 51.576843 28.405563 33.015491 177963 29.662485 4.860037 44.604118
3.913353 24.136766 38.887836 2.296417 31.787346	2.68211 6.338207 7.42.176628 60.791038 35.892723 892084	8.834910 26.136217 30.617464 49.598722 3.931264 23.668926 48.513527	2.338218 15.545036 31.230486 47.322487 104376 22.257351 48.583614 9.755950

The lesson of complicated preferred Brunswik - based choice making and predicament solving has attracted concentration (Brunswik; 1957 and Belton and Dhimi; 2021 and Bruce and Friedman; 2002). Long-drawn-out researches necessitate (model - based experiential) study of behavior and offer locale for basic research on how ill-structured predicament are, and can be, solved (Brunswik; 1957 and Belton and Dhimi; 2021 and Bruce and Friedman; 2002). Clinician neuro - complicated preferred Brunswik - based choice making can be premeditated with great yield using time-honored method of inquisition, especially all the way through concentrated studies (Brunswik; 1957 and Belton and Dhimi; 2021 and Bruce and Friedman; 2002). Neuro - optometric entrepreneurial biology propose elucidation all the way through succession of proportions of eye motion at point of obscure preferred Brunswik - based choice (Brunswik; 1957 and Belton and Dhimi; 2021 and Bruce and Friedman; 2002). It affords conceptual and idealistic framework for perceptive and conducting research at Optometric Science, Entrepreneurial biology and Psychology gamut (Brunswik; 1957 and Belton and Dhimi; 2021 and Bruce and Friedman; 2002).

Neuro - optometric offers ;

- Explanation in the course of set of data obtained via progression of measurements of eye motion at time of complicated preferred Brunswik - based choices (Satpathy, Larsen, Lockhart and Misra ;2023),
- Provides abstract and idealistic scaffold for discerning and conducting neuro - optometric research at junction of Neuroscience, Optometric and Psychology (Satpathy, Larsen, Lockhart and Misra ;2023),
- Describes customary replica for preferred Brunswik - based choice process that links and spans Neurobiological, Psychological, and Optometric levels of analysis (Satpathy, Larsen, Lockhart and Misra ;2023),
- Applies neuroscience to neuro optometric and neo-classical optometric, and ties to biological constraints in how we adjudicate preferred Brunswik - based choices (Satpathy, Larsen, Lockhart and Misra ;2023),
- An important resource from optometric to neuroscience, to progress of inter-disciplinary research (Satpathy, Larsen, Lockhart and Misra ;2023),
- Seen as sub turf of experimental optometric, where neuro data is enriched with eye data (Satpathy, Larsen, Lockhart and Misra ;2023),
- proposes to construct eye-based models capable of predicting observed behaviour (Satpathy, Larsen, Lockhart and Misra ;2023),
- Shed light on causes of behaviour (and of neuro anomalies) and capable of explaining and predicting complicated preferred Brunswik - based choices (Satpathy, Larsen, Lockhart and Misra ;2023),
- Provides information about underlying mechanisms used by the eye for period of preferred Brunswik - based choice processes, In particular, it shows which eye regions are activated when a complicated preferred Brunswik - based choice is made and how these regions interact with each other, This knowledge

can then be used to build a model that represents this particular mechanism (Satpathy, Larsen, Lockhart and Misra ;2023),

- Combining above gives interdisciplinary insight to define fundamentals of neuro - optometric complicated preferred Brunswik - based choice making that has eluded researchers working within each individual turf (Satpathy, Larsen, Lockhart and Misra ;2023 and Kowler; 2011).

Conclusion

Manuscript raises interesting theoretical and practical levels of analysis significant in business strategy (Kowler; 2011). Research efforts conclude with characteristic schemes and presents directions for future research (Kowler; 2011). Manuscript concludes with number of propositions that have been generated from theoretical ‘mosaic’ and presents directions for future research (Satpathy, Larsen, Lockhart and Misra; 2023 and Kowler; 2011).

What are the apparatuses that keep gaze stable with either stationary or moving targets? How does motion of cognitive image on retina affect vision? Where do look - and why - when performing complicated task? How can the world appear clear and stable despite continual movements of eyes? Cognitive processes driving eye movements for the period of complicated preferred Brunswik - based choice making are not in any consequential way different from those in similar tasks (Kowler; 2011 and Satpathy, Majumdar, Mallik, Mahapatra, Warriar, Khatun and Okeyo; 2023). Eye movements in complicated preferred Brunswik - based choice making are partially driven by (complicated) task demands (Kowler; 2011 and Satpathy, Majumdar, Mallik, Mahapatra, Warriar, Khatun and Okeyo; 2023). Eye movements in complicated preferred Brunswik - based choice making are partially driven by stimulus properties that bias information uptake in favor of visually salient stimuli (Kowler; 2011 and Satpathy, Majumdar, Mallik, Mahapatra, Warriar, Khatun and Okeyo; 2023). Eye movements do not have causal effect on Brunswik - based choice formation (Kowler; 2011 and Satpathy, Majumdar, Mallik, Mahapatra, Warriar, Khatun and Okeyo; 2023). However, through properties inherent to visual system, such as stimulus-driven concentration, eye movements do lead to down-stream effects on complicated preferred Brunswik - based choice making (Kowler; 2011 and Satpathy, Majumdar, Mallik, Mahapatra, Warriar, Khatun and Okeyo; 2023). Complicated preferred Brunswik - based choice makers optimize eye movements to reduce demand on memory and reduce number of fixations and length of saccades needed to complete complicated preferred Brunswik - based choice task (Kowler; 2011 and Satpathy, Majumdar, Mallik, Mahapatra, Warriar, Khatun and Okeyo; 2023). Drivers of eye movements in complicated preferred Brunswik - based choice making change dynamically within tasks (Orquin and Loose; 2013; 2011 and Satpathy, Majumdar, Mallik, Mahapatra, Warriar, Khatun and Okeyo; 2023). Concentration should be paid for performing experimental procedures in order to evaluate usability, accuracy and reliability of eye tracking systems (Kowler; 2011 and Satpathy, Majumdar, Mallik, Mahapatra, Warriar, Khatun and Okeyo; 2023). Any model that aims to describe complicated preferred Brunswik - based choice making must reflect that visual information play central role in complicated preferred Brunswik - based choice dynamics (Kowler; 2011 and Satpathy, Majumdar, Mallik, Mahapatra, Warriar, Khatun and Okeyo; 2023).

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Nature's Cure: Bridging Traditional Wisdom and Modern Science

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Abstract: Nature has been a perennial source of inspiration and healing, offering solutions to human health challenges through its diverse flora and fauna. "Nature's Cure" explores the integration of traditional remedies and modern science, emphasizing the potential of natural products in addressing various health issues. This study examines medicinal plants, bioactive compounds, and holistic therapies that have gained recognition for their therapeutic properties. The ancient wisdom embedded in traditional systems such as Ayurveda, Traditional Chinese Medicine, and Siddha is revisited, highlighting their relevance in the contemporary world. Advances in pharmacognosy and biotechnology have enabled the identification, isolation, and synthesis of natural compounds, leading to the development of novel drugs. Furthermore, the growing trend of personalized medicine and wellness underscores the significance of natural cures in preventive healthcare. This research underscores the importance of biodiversity conservation, as many medicinal resources are under threat due to habitat destruction and climate change. Ethical and sustainable harvesting practices are essential to ensure the availability of these resources for future generations. The study also discusses challenges, such as the lack of standardization, rigorous clinical trials, and potential toxicity associated with some natural remedies. The findings aim to foster collaboration between traditional practitioners, researchers, and policymakers to develop integrated approaches for human well-being. By bridging the gap between nature's wisdom and scientific innovation, "Nature's Cure" aspires to contribute to a healthier and more sustainable future.

Keywords: Medicinal plants, natural remedies, bioactive compounds, biodiversity conservation, traditional medicine, pharmacognosy.

Introduction

Nature has long served as a treasure trove for human health, offering remedies that date back thousands of years. Traditional medicine systems, including Ayurveda, Traditional Chinese Medicine (TCM), and Siddha, have utilized natural products for their therapeutic

potential (Kumar *et al.*, 2023). Today, these practices are being re-evaluated through the lens of modern science, uncovering the molecular mechanisms underlying their efficacy. This paper explores the intersection of traditional and modern approaches to natural cures and highlights the significance of biodiversity conservation. Nature's cure is a timeless approach to health and healing that harnesses the therapeutic power of natural elements to promote well-being. Rooted in ancient traditions and supported by modern science, this holistic method emphasizes prevention, balance, and alignment with the natural world. Nature's cure involves the use of plant-based medicines, herbal remedies, mineral-rich substances, and therapies like hydrotherapy, aromatherapy, and natural nutrition to address physical, mental, and emotional health challenges. These natural remedies are not only effective but also eco-friendly and sustainable, aligning with the body's innate ability to heal itself.

For centuries, civilizations across the globe have relied on nature's resources for healing, drawing on the rich biodiversity found in forests, mountains, and oceans. Plants like neem, turmeric, and aloe vera, alongside minerals like clay and salts, have been key components in traditional healing systems such as Ayurveda, Traditional Chinese Medicine, and Native American practices. Today, advancements in science and technology are validating the efficacy of these natural cures, fostering their integration into modern healthcare systems. In an era of rising lifestyle diseases and drug-resistant pathogens, nature's cure offers an alternative path that focuses on addressing root causes rather than merely treating symptoms. It advocates for a preventive approach through proper diet, regular exercise, and mindful living, fostering a deeper connection between individuals and the environment. Moreover, the resurgence of interest in natural remedies has spurred efforts to conserve biodiversity, recognizing its role as a treasure trove of medicinal resources.

Nature's cure represents a harmonious blend of tradition and innovation, encouraging a lifestyle that is healthier for individuals and the planet. By embracing its principles, we can achieve not only personal wellness but also contribute to sustainable development and environmental preservation for future generations.

Traditional Medicine: Ancient Wisdom Rediscovered

Traditional medicine systems have provided holistic healthcare for centuries. For instance, Ayurveda emphasizes balance among body, mind, and spirit, using herbal formulations like Ashwagandha (*Withania somnifera*) and Turmeric (*Curcuma longa*) (Gupta *et al.*, 2022). Similarly, TCM integrates herbal remedies such as Ginseng (*Panax ginseng*) and Astragalus

(*Astragalus membranaceus*) to enhance immunity and vitality (Wang *et al.*, 2021). Despite their efficacy, many of these remedies lack robust clinical validation, posing challenges to their integration into mainstream healthcare.

Traditional medicine, rooted in ancient wisdom, is being rediscovered as a valuable resource for modern healthcare. Passed down through generations, it encompasses practices like herbal remedies, acupuncture, and holistic healing systems such as Ayurveda and Traditional Chinese Medicine. These time-tested methods focus on balancing the body, mind, and spirit, emphasizing prevention and natural remedies. With growing interest in sustainable and integrative healthcare, traditional medicine is gaining recognition for its potential to complement modern treatments. Research and collaboration are unlocking its therapeutic potential, blending ancient knowledge with scientific validation to create innovative solutions for today's health challenges while preserving cultural heritage.

Bioactive Compounds: The Building Blocks of Modern Drugs

Natural products have been instrumental in drug discovery, with bioactive compounds such as alkaloids, flavonoids, and terpenoids serving as pharmacological leads. For example, artemisinin, derived from *Artemisia annua*, revolutionized malaria treatment (Tu, 2015). Similarly, paclitaxel from *Taxus brevifolia* has been pivotal in cancer therapy (Smith *et al.*, 2023). Recent advancements in metabolomics and genomics have further enhanced the identification of bioactive compounds, paving the way for novel therapeutic agents (Jones *et al.*, 2022).

Bioactive compounds are the cornerstone of modern drug development, offering immense potential for treating diverse health conditions. Derived from natural sources such as plants, marine organisms, and microorganisms, these compounds exhibit therapeutic properties like anti-inflammatory, antimicrobial, and anticancer activities. Their unique chemical structures often serve as templates for synthesizing new drugs, bridging the gap between nature and pharmaceutical innovation. From aspirin, inspired by willow bark, to anticancer agents like paclitaxel from yew trees, bioactive compounds have revolutionized medicine. Ongoing research and conservation efforts are essential to explore, protect, and sustainably utilize these natural treasures for advancing healthcare and improving global well-being.

Biodiversity Conservation: Protecting Nature's Pharmacy

Biodiversity is the cornerstone of natural medicine, yet it faces threats from habitat destruction and climate change. The loss of medicinal plants like *Rauwolfia serpentina* and *Hoodia gordonii* underscores the urgency for conservation (Mishra *et al.*, 2023). Sustainable harvesting practices, community involvement, and habitat restoration are crucial for preserving these invaluable resources (Singh and Verma, 2021).

Biodiversity conservation is crucial for protecting nature's pharmacy, as countless medicinal resources are derived from the rich variety of life forms on Earth. Plants, fungi, and microorganisms are the sources of many life-saving drugs, from antibiotics to cancer treatments. By safeguarding ecosystems and preventing habitat destruction, we ensure the survival of species that hold potential for medical discoveries. Conserving biodiversity also supports ecosystem services like pollination and nutrient cycling, essential for sustaining life. Protecting nature's pharmacy requires collaborative global efforts, including sustainable practices, research, and education, to balance development with the preservation of this invaluable natural treasure for future generations.

Integrating Traditional and Modern Approaches

Combining traditional knowledge with modern research methodologies can enhance healthcare outcomes. For instance, integrating TCM's holistic approaches with evidence-based practices has shown promise in treating chronic conditions like diabetes and hypertension (Zhao *et al.*, 2023). Collaborative efforts between ethnobotanists, pharmacologists, and healthcare practitioners can facilitate the development of standardized and safe natural therapies (Li *et al.*, 2022). Integrating traditional and modern approaches creates a synergistic pathway for innovation and holistic progress. Traditional knowledge, rooted in centuries of experience, offers insights into natural remedies, sustainable practices, and cultural wisdom. Modern approaches, driven by scientific advancements and technology, provide precision, scalability, and evidence-based validation. By blending these perspectives, we can develop solutions that are both effective and sustainable, addressing contemporary challenges while preserving heritage. This integration fosters inclusivity, respects diversity, and bridges the gap between ancient practices and contemporary needs, ensuring a balanced approach to progress that honors the past while embracing the future for the benefit of all.

Challenges in Harnessing Nature's Cure Despite its potential, the use of natural remedies faces several challenges:

1. **Standardization and Quality Control:** Variability in bioactive compound concentrations due to environmental factors complicates standardization (Ramesh *et al.*, 2022).
2. **Clinical Validation:** Many natural remedies lack rigorous clinical trials, limiting their acceptance in evidence-based medicine (Patel *et al.*, 2021).
3. **Safety and Toxicity:** Potential adverse effects and drug interactions necessitate thorough safety assessments (Chen *et al.*, 2023).
4. **Intellectual Property Rights:** The commercialization of traditional knowledge raises ethical and legal concerns, particularly regarding benefit-sharing with indigenous communities (Sharma *et al.*, 2023).

Future Directions

The future of natural medicine lies in interdisciplinary collaboration and sustainable practices. Priorities include:

- **Promoting Research:** Encouraging large-scale clinical trials to validate traditional remedies (Reddy *et al.*, 2023). Nature Cure provides a distinct understanding of physiology and pathology that reflects a broad, expansionist, environmental perspective on health rather than a reductive biological perspective on prevention and cure, keeping in mind that integrative medicine in contemporary practice bridges the divide between holism and reductionism in important ways (Aggarwal M, *et al.*, 2017). The way in which a body is said to “heal itself” reflects an understanding of this kind of agency rather than a contrived anthropomorphized—and moralized—form of cultural agency shrouded in the entelechy of biological purpose. This finds expression in a utopian vision of ecological equipoise. Biomedicine, by contrast, is a directional project that imposes the will of culture on nature with the utopian prospect of liberating the body and society from the burden of disease. Both visions have their place in contemporary India.
- **Conservation Efforts:** Strengthening biodiversity conservation initiatives to secure medicinal plant resources (Nair *et al.*, 2023).

- **Policy Frameworks:** Establishing policies that balance innovation with ethical considerations, ensuring equitable benefit-sharing (Khan *et al.*, 2023).

Conclusion

"Nature's Cure" underscores the untapped potential of natural products in modern healthcare. By integrating traditional wisdom with scientific innovation, humanity can address pressing health challenges while fostering environmental sustainability. Collaborative efforts, backed by robust research and ethical practices, are essential to harness nature's pharmacy for the benefit of future generations. Nature's cure offers a holistic approach to health and well-being, leveraging the healing power of natural elements such as plants, herbs, and minerals. It emphasizes prevention, addressing root causes rather than symptoms, and promotes balance in body, mind, and spirit. Integrating traditional knowledge with modern science, nature's remedies are safe, eco-friendly, and sustainable, aligning with the body's natural processes. By fostering a connection with the environment and adopting natural healing methods, we not only improve personal health but also contribute to the preservation of biodiversity and ecological balance. Embracing nature's cure is a step toward a healthier and harmonious life.

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Exploring the Healing Nexus: A Review of Insect-Medicinal Plant Symbiosis

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Abstract: Symbiotic relationships between insects and medicinal plants play a crucial role in ecological balance, biodiversity conservation, and pharmacological advancements. These interactions can be mutualistic, commensalistic, or parasitic, with varying degrees of dependence and benefit. Pollination, seed dispersal, herbivory, and chemical defence mechanisms are among the critical aspects of these relationships. Insects play a key role in ensuring the reproductive success of medicinal plants, while plants provide food and habitat for insect populations. This study explores these dynamic interactions in detail and highlights their significance for both ecological sustainability and pharmacological research. Additionally, we provide a case study focusing on the pollination of *Withaniasomnifera* by honeybees (*Apis mellifera*), which demonstrates how insect-plant symbiosis can directly impact plant phytochemical composition and medicinal efficacy. Understanding these relationships is crucial for conservation efforts and sustainable medicinal plant cultivation, as environmental changes and human activities continue to threaten these natural partnerships.

Keywords: Symbiotic relationships, Insects, Medicinal plants, Ecological balance, Biodiversity conservation, Pharmacological advancements, Pollination, Seed dispersal, Herbivory, Chemical defence mechanisms, Reproductive success

Introduction

Medicinal plants have long been utilized in traditional and modern medicine due to their therapeutic properties. Many of these plants rely on insects for fundamental biological processes, including pollination, seed dispersal, and protection against herbivory. In turn, insects depend on these plants for sustenance, shelter, and breeding sites. The interplay between insects and medicinal plants is an essential aspect of ecological stability, shaping biodiversity and influencing evolutionary pathways. This study seeks to examine how insects and medicinal plants form symbiotic relationships, focusing on their ecological significance

and pharmacological implications. Insects such as bees, butterflies, beetles, ants, and moths interact with medicinal plants in different ways, with mutualistic relationships being the most common. These relationships not only enhance plant reproduction but also contribute to the bioavailability of medicinal compounds. Understanding these relationships can lead to improved conservation strategies and more effective cultivation techniques for medicinal plants, ensuring their availability for future generations. However, threats such as habitat destruction, climate change, and pesticide use disrupt these relationships, making research on insect-plant symbiosis increasingly important.

Types of Symbiotic Relationships

1. **Mutualism** – Both organisms benefit from the interaction. For example, bees pollinate flowers while collecting nectar. This process enhances plant reproduction and ensures a stable food source for the insects. The relationship between the fig tree (*Ficus spp.*) and fig wasps (*Agaonidae*) is another example, where wasps help fertilize the plant while using it as a breeding ground (Bronstein, 1992).
2. **Commensalism** – One species benefits while the other remains unaffected. Certain beetles may use medicinal plants as shelter without harming or benefiting the plant directly. Some ants create colonies within medicinal plant stems without affecting the plant's growth (Hölldobler and Wilson, 1990).
3. **Parasitism** – One organism benefits at the expense of the other. Some insect larvae, such as those of leaf miners and aphids, feed on medicinal plants, weakening them and reducing their medicinal efficacy. Parasitic relationships often lead to plant defense mechanisms, including the production of secondary metabolites that have medicinal properties for humans (Bennett and Wallsgrave, 1994).

Case Study: Pollination of *Withaniasomnifera* by Honeybees (*Apis mellifera*)

Withaniasomnifera, commonly known as ashwagandha, is a medicinal plant known for its adaptogenic and anti-inflammatory properties. Honeybees (*Apis mellifera*) play a critical role in the pollination of ashwagandha, facilitating fruit and seed production. Studies indicate that increased pollination can lead to a higher concentration of bioactive compounds such as withanolides, which enhance the plant's medicinal properties (Mirjalili et al., 2009). The mutualistic relationship between ashwagandha and honeybees is a prime example of how insect interactions influence the phytochemical composition of medicinal plants. Moreover,

environmental factors such as habitat loss and pesticide exposure pose significant threats to honeybee populations, which could subsequently affect the propagation and medicinal potency of *Withaniasomnifera*. Sustainable agricultural practices and pollinator-friendly initiatives are essential for maintaining this beneficial relationship. Further research is needed to explore how pollinator activity directly correlates with phytochemical synthesis, which could help optimize medicinal plant farming techniques.

Additional Symbionts and Their Roles

1. **Butterflies (*Danaus plexippus*) and *Echinacea purpurea*** – Monarch butterflies feed on the nectar of Echinacea, a plant known for its immune-boosting properties, aiding in its cross-pollination. This mutualistic relationship ensures the plant's reproductive success while providing nourishment for the butterflies (Boggs and Ross, 1993).
2. **Ants (*Camponotus spp.*) and *Catharanthus roseus*** – Ants protect the periwinkle plant, which contains anticancer compounds, from herbivorous insects in exchange for nectar. This defense mechanism helps the plant survive in challenging environments, while ants benefit from a constant food source (Blüthgen and Fiedler, 2004).
3. **Beetles (*Scarabaeidae*) and *Aristolochia gigantea*** – These beetles act as pollinators while seeking shelter inside the flower's chamber, ensuring reproduction. The beetles' activity assists in fertilization while providing a safe space for their reproduction cycle (Dafni, 1992).
4. **Moths (*Hyles lineata*) and *Datura stramonium*** – Night-blooming flowers of the sacred datura depend on hawk moths for pollination, while the moths gain access to nectar. This interaction highlights the importance of nocturnal pollinators in maintaining plant populations (Grant and Grant, 1983).
5. **Termites (*Macrotermes spp.*) and Fungal Symbionts in Medicinal Plants** – Termites cultivate fungal gardens that help break down plant material, indirectly supporting the growth of medicinal fungi with therapeutic properties. This complex ecological interaction enhances soil fertility and promotes plant health (Wood and Thomas, 1989).

Ecological and Pharmacological Significance

- **Biodiversity Conservation:** The relationship between medicinal plants and insects supports ecological stability and plant reproduction. Pollinator diversity directly impacts plant genetic variation, enhancing ecosystem resilience (Kearns and Inouye, 1997).
- **Sustainable Medicinal Plant Use:** Insects influence the yield and potency of medicinal compounds. Understanding insect-plant interactions can lead to optimized cultivation techniques, maximizing bioactive compound extraction (Poveda *et al.*, 2003).
- **Threats and Conservation Measures:** Habitat destruction, climate change, and pesticide use threaten these relationships, emphasizing the need for conservation efforts. Sustainable farming, habitat restoration, and reduced pesticide usage are critical strategies for preserving these interactions (Potts *et al.*, 2010).

Conclusion

The intricate interactions between insects and medicinal plants underscore the importance of preserving natural ecosystems. These relationships not only ensure plant survival but also enhance the medicinal efficacy of many plant species. Conservation efforts must prioritize pollinator-friendly environments to sustain biodiversity and optimize medicinal plant production. Further research on these relationships can enhance medicinal plant cultivation and pharmacological applications while promoting biodiversity conservation. Sustainable agricultural practices, combined with awareness campaigns, can help protect these vital ecological partnerships for future generations.

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A Comparative Analysis: Advancements in Nanotechnology for Medical Diagnostics

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Abstract: Nanotechnology has emerged as a transformative force in medical diagnostics, offering unprecedented accuracy, sensitivity, and efficiency in disease detection and monitoring. Smith (2022) explored the role of nanoparticles in enhancing biomarker detection, imaging techniques, and point-of-care (POC) diagnostic devices. This comparative analysis evaluates Smith's findings alongside recent advancements, including the integration of quantum dots, plasmonic nanoparticles, and artificial intelligence (AI) in nanodiagnostics. Recent developments have expanded the applications of nanotechnology to liquid biopsy techniques, theranostic nanoparticles, and wearable nanosensors, enabling real-time health monitoring and personalized medicine. The incorporation of AI and machine learning further optimizes diagnostic accuracy, reducing false positives and enabling early disease prediction. These innovations highlight a paradigm shift in medical diagnostics, promising faster, non-invasive, and highly precise diagnostic solutions. As nanotechnology continues to evolve, its convergence with AI and personalized medicine is expected to drive the future of healthcare, improving patient outcomes and reducing the burden on traditional diagnostic infrastructures.

Keywords: Nanotechnology, medical diagnostics, biosensors, point-of-care testing, liquid biopsy, imaging nanomaterials, theranostics, personalized medicine, AI in diagnostics, nanomedicine.

Introduction

Nanotechnology has revolutionized numerous scientific fields, with healthcare and medical diagnostics being among the most profoundly impacted. This interdisciplinary field focuses on manipulating materials at the nanoscale (1-100 nm), allowing for the creation of highly sensitive, efficient, and targeted diagnostic tools. The application of nanotechnology in medicine,

commonly referred to as nanomedicine, has led to significant breakthroughs in disease detection, treatment, and monitoring. These advancements have enabled the development of innovative diagnostic methods that offer superior precision compared to traditional techniques, thereby improving early disease detection and patient outcomes.

One of the most promising areas of nanotechnology in medical diagnostics is the use of nanoparticles for biomarker detection. Biomarkers—biological molecules that indicate disease presence or progression—can be detected at extremely low concentrations using nanomaterial-based sensors. Gold nanoparticles, quantum dots, and magnetic nanoparticles have been particularly useful in improving the sensitivity and specificity of diagnostic assays. These advancements are especially crucial for detecting diseases such as cancer, infectious diseases, and neurodegenerative disorders at an early stage when treatment is most effective.

In his paper "*Advancements in Nanotechnology for Medical Diagnostics*", Smith (2022) explores the transformative role of nanotechnology in diagnostic applications. His study highlights key areas such as nanoparticle-enhanced biomarker detection, imaging techniques, and the development of point-of-care (POC) devices. These innovations have led to faster and more accurate disease identification, reducing reliance on traditional laboratory-based diagnostic procedures.

Since the publication of Smith's (2022) work, the field has witnessed further advancements. Recent developments in nanodiagnostics have expanded into areas such as liquid biopsy, theranostics, artificial intelligence (AI)-integrated diagnostics, and wearable nanosensors for real-time health monitoring. Liquid biopsy techniques, which rely on the detection of circulating tumor DNA (ctDNA) and extracellular vesicles, have gained prominence as non-invasive alternatives to traditional tissue biopsies. Meanwhile, theranostic nanoparticles—capable of both diagnosing and treating diseases—represent a paradigm shift in personalized medicine. Furthermore, AI-powered algorithms are increasingly being used to analyze nanodiagnostic data, improving accuracy and reducing false positives.

This comparative analysis aims to evaluate the findings of Smith (2022) in light of recent developments in nanotechnology for medical diagnostics. By examining advancements in

biomarker detection, imaging, point-of-care devices, and AI integration, this study provides a comprehensive overview of how nanotechnology is shaping the future of medical diagnostics.

Comparative Analysis

Nanoparticles for Biomarker Detection

Smith (2022) highlights the role of nanoparticles in detecting disease biomarkers with high sensitivity, particularly in cancer, infectious diseases, and cardiovascular conditions. Recent advancements have taken this further with the integration of quantum dots and plasmonic nanoparticles, which provide even greater precision in biomarker detection (Gupta *et al.*, 2023). Gold nanoparticles, for example, have been widely used in lateral flow assays, such as COVID-19 antigen tests, due to their stability and ease of functionalization (Huang and Li, 2023). Additionally, machine learning algorithms are now being incorporated to interpret nanoparticle-based diagnostic data, enhancing detection accuracy and enabling early disease prediction (Kim and Park, 2023).

Nanomaterials in Imaging and Theranostics

While Smith (2022) discusses the use of gold and iron oxide nanoparticles in MRI and CT imaging, the field has since expanded to include multifunctional nanoparticles capable of theranostics—combining diagnosis and therapy in a single system (Johnson and Patel, 2022). For instance, superparamagnetic iron oxide nanoparticles (SPIONs) have been engineered for dual imaging and drug delivery, offering a more comprehensive approach to disease management (Singh and Kumar, 2023). Quantum dots have also gained attention for their high-resolution fluorescence imaging capabilities, allowing for real-time tracking of disease progression (Gupta *et al.*, 2023).

Liquid Biopsy Applications

Smith (2022) primarily discusses biosensors for biomarker detection, but more recent advancements emphasize liquid biopsy techniques, which allow for non-invasive disease monitoring. These methods leverage nanotechnology to detect circulating tumor DNA (ctDNA), microRNAs, and extracellular vesicles from blood samples (Huang and Li, 2023). Gold nanoparticles and magnetic nanostructures have significantly improved the sensitivity of liquid

biopsy assays, making them more viable for early cancer detection and treatment monitoring (Zhao and Lin, 2023).

Point-of-Care (POC) Devices and Wearable Sensors

Smith (2022) highlights the role of nanotechnology in portable diagnostic devices, but the current landscape has expanded with wearable nanosensors that continuously monitor health parameters in real time. Advances in graphene-based biosensors have enabled continuous glucose monitoring for diabetes patients, reducing the need for frequent blood tests (Martinez & Torres, 2023). Nanosensor-integrated smartwatches are also emerging as non-invasive tools for tracking cardiovascular health, including blood pressure and heart rate variability (Williams & Evans, 2023). These innovations align with the broader trend of personalized medicine, allowing for proactive health management.

AI and Machine Learning Integration in Nanodiagnosics

While Smith (2022) focuses on the physical and chemical properties of nanoparticles, recent advancements highlight the role of artificial intelligence (AI) in nanodiagnosics. AI-driven platforms can analyze complex biological signals detected by nanosensors, reducing false positives and improving diagnostic precision (Kim and Park, 2023). Deep learning algorithms have been particularly effective in processing nanoparticle-enhanced imaging data, leading to faster and more reliable diagnostic interpretations (Zhao and Lin, 2023). This integration is paving the way for fully automated, AI-assisted diagnostic tools that require minimal human intervention.

Comparative Table

Aspect	Smith (2022)	Recent Advancements (2023)
Nanoparticles for Biomarker Detection	Gold and iron oxide nanoparticles enhance biomarker detection in cancer and infectious diseases.	Quantum dots and plasmonic nanoparticles provide higher sensitivity; AI integration improves accuracy and early disease prediction (Gupta <i>et al.</i> , 2023; Kim and Park, 2023).
Nanomaterials in Imaging and Theranostics	Gold and iron oxide nanoparticles are used for MRI and CT imaging.	Theranostic nanoparticles combine imaging and drug delivery; SPIONs enable dual-function applications (Johnson and Patel, 2022; Singh & Kumar, 2023).
Liquid Biopsy Applications	Focuses on biosensors detecting biomarkers from bodily fluids.	Nanoparticle-assisted liquid biopsy enhances detection of ctDNA, microRNAs, and extracellular vesicles for non-invasive diagnostics (Huang & Li, 2023; Zhao and Lin, 2023).
Point-of-Care (POC) Devices and Wearable Sensors	Portable nanotechnology-based diagnostic devices provide faster disease detection.	Graphene-based wearable nanosensors enable real-time health monitoring, reducing the need for frequent tests (Martinez and Torres, 2023; Williams and Evans, 2023).
AI and Machine Learning in Nanodiagnostics	Focuses on the physical and chemical properties of nanoparticles in diagnostics.	AI-driven platforms analyze biological signals, enhancing precision and reducing false positives in diagnostic data (Kim and Park, 2023; Zhao and Lin, 2023).

Conclusion

Smith's (2022) study provided a solid foundation for understanding the role of nanotechnology in medical diagnostics, particularly in biomarker detection, imaging techniques, and point-of-care devices. However, the field has evolved significantly, with recent advancements introducing more sophisticated and multifunctional nanotechnologies. The integration of quantum dots, plasmonic nanoparticles, and AI-powered diagnostic tools has not only improved sensitivity and specificity but has also enabled early disease detection with unprecedented accuracy.

Furthermore, theranostic nanoparticles now offer dual functionality by combining diagnosis and treatment, paving the way for personalized medicine. Liquid biopsy techniques, enhanced by nanotechnology, provide a non-invasive alternative to traditional diagnostic methods, making early cancer detection more accessible. Wearable nanosensors and real-time monitoring devices are revolutionizing healthcare by allowing continuous tracking of vital biomarkers, reducing the reliance on hospital-based diagnostics. Additionally, AI and machine learning are enhancing diagnostic precision, optimizing data interpretation, and reducing false positives, marking a significant step toward automation in medical diagnostics.

These innovations highlight the transformative potential of nanotechnology in reshaping healthcare. As research continues, we can expect even greater strides in nanodiagnostics, including enhanced biosensors, more effective AI-driven diagnostic platforms, and widespread adoption of nanotechnology in clinical settings. The ongoing convergence of nanotechnology, artificial intelligence, and personalized medicine will not only improve diagnostic accuracy but also lead to more proactive and preventive healthcare solutions. Ultimately, these advancements will enhance patient outcomes, reduce healthcare costs, and drive the next era of medical diagnostics.

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Animal Apothecary: The Fascinating Secrets of Plant-Based Self-Medication

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Abstract: Zoopharmacognosy or Animal Apothecary, the phenomenon of animals self-medicating with plant-based substances, is a fascinating and complex behaviour. Various species, from primates to insects, utilize the medicinal properties of plants to maintain health and combat ailments. This phenomenon is exemplified in diverse self-meditative behaviours, such as primates consuming bitter leaves to manage intestinal parasites and birds incorporating antibacterial herbs into their nests. By examining these behaviours, we gain insights into the evolutionary advantages and adaptive strategies animals have developed. Through scientific research, we uncover how animals identify, process, and utilize medicinal plants. Sensory perception, learning, and social transmission all play a role in acquiring self-meditative knowledge. The study of zoopharmacognosy has significant implications for conservation biology, veterinary medicine, and human pharmacology. By exploring the medicinal properties of plants used by animals, we may discover new therapeutic compounds. This fascinating phenomenon highlights the dynamic interplay between flora and fauna, underscoring the importance of biodiversity in sustaining health across species. It challenges conventional notions of medicine and encourages a broader understanding of health and healing rooted in nature. By appreciating the ingenuity and resourcefulness of the animal world, we recognize the untapped potential within nature's pharmacy and the wisdom inherent in the animal kingdom.

Keywords: Zoopharmacognosy, Animal Apothecary, veterinary medicine, therapeutic compounds.

Introduction

Birds, bees, lizards, elephants, and chimpanzees all share a survival trait: They self-medicate. These animals eat things that make them feel better, or prevent disease, or kill parasites like flatworms, bacteria, and viruses, or just to aid in digestion. Even creatures with brains the size of pinheads somehow know to ingest certain plants or use them in unusual ways when they need them. Anyone who has seen a dog eat grass during a walk has witnessed self-medication. The dog probably has an upset stomach or a parasite. The grass helps them vomit up the problem or eliminate it with the feces. The science of animal self-medication is called zoopharmacognosy, derived from the roots zoo (“animal”), pharma (“drug”), and gnosy (“knowing”). It’s not clear how much knowing or learning is involved, but many animals seem to have evolved an innate ability to detect the therapeutic constituents in plants. Although the evidence is entirely circumstantial, the examples are plentiful. The practice is spreading across the animal kingdom in sometimes surprising ways.

Most studies of animal self-medication, however, are in the great apes. In the 1960s, the Japanese anthropologist Toshisada Nishida observed chimpanzees in Tanzania eating *aspella* leaves, which had no nutritional value. Harvard primatologist Richard Wrangham saw the same behavior at Jane Goodall’s Gombe reserve, where chimps were swallowing leaves whole. Other scientists noted the same in other chimp colonies. Without chewing, the animals weren’t getting much nutritional benefit. In 1996, biologist Michael Huffman suggested the chimps were self-medicating. Huffman, an American who has worked for years in Japan at the Primate Research Institute at Kyoto University, first saw a parasite-ridden, constipated chimpanzee in Tanzania chew on the leaves of a noxious plant it would normally avoid. By the next day, the chimpanzee was completely recovered (Huffman, 1997). The plants had bristly leaves, rough to the touch. Huffman theorized the chimps were swallowing the plants to take advantage of that roughness, using the leaves and stems to scour their intestines and rid themselves of parasites. Other researchers observed the same practice among other apes across Africa. Huffman established widely used criteria for judging when an animal is self-medication. First, the plant eaten cannot be a regular part of the animal’s diet; it is used as medicine not food. Second, the plant must provide little or no nutritional value to the animal. Third, the plant must be consumed during those times of year for example, the rainy season, when parasites are most likely to cause

infections. Fourth, other animals in the group don't participate (Fruth ,*et al.* 2014 and Tedx Osaka, 2012). If the activity meets these standards, it is safe to assume the animal is self-medicating, Huffman says. Researchers have observed the practice in 25 regions involving 40 different plants.

Coping with Parasites The latest example of self-medication among the great apes comes from the deepest part of the Congo Basin and involves bonobos, sometimes called pygmy chimps. Barbara Fruth and her team from the Max Plank Institute for Evolutionary Anthropology in Leipzig, including her husband Gottfried Hohman, have been studying bonobos since 1990. Researchers sit quietly in the forest watching a particular colony of the animals and searching the ground below their living quarters for facesscience at its least glamorous. The faces hunt, however, has revealed how the bonobos cope with parasites. Fruth's fieldwork took place over several weeks between October 2007 and June 2009. Her team studies a bonobo community on the fringes of Salonga National Park, about 25 kilometers from the nearest village. The researchers follow the bonobos on foot, sometimes crawling under vines or wading through a swamp to follow. They generally stay at least seven meters from the animals, wearing masks to prevent human infections from spreading to the apes. Fruth watches with a good pair of Leica binoculars. One of the plants Fruth saw the bonobos eat was *Manniophytonfulvum*, a shrub used by local humans to make animal traps. Sitting in the forest, watching and recording, even counting bites per minute, Fruth and her colleagues saw the bonobos take a leaf or stem from the *M. fulvum* and rest it flat on their tongues. The animals would layer the leaves on their tongues, produce saliva, and fold the leaves back, forming a ball while avoiding their lips [contact with the skin causes itching and sores. The bonobos eventually swallowed without chewing. The researchers saw it happen 56 times, according to their 2013 report in the online American Journal of Primatology (Fruth,*et al.*, 2014) . The plant is not, Fruth points out, part of the bonobos' regular diet. They ingested the leaves mostly when the weather was prime for parasites. The practice fits four of Huffman's criteria. The bonobos sleep in the trees and defecate in the early morning from their high perches. The morning after they observed the leaf swallowing, the researchers collected 694 piles of faces that had dropped to the ground. Huffman's theory is that apes use the leaves as sandpaper to scour the parasites and speed elimination. The hairs on the outside of the leaves are made of rigid silica, and the apes eliminate it in six hours. Fruth thinks

there's more to it, at least for the bonobos. Her team didn't see elimination speed up. Twenty-four hours later, the apes were still excreting leaves.

It's a reasonable scenario, says Mark Hunter, a professor of ecology and environmental biology at the University of Michigan. Learning these practices can be either innate or behavioral, or both. Apes, being intelligent creatures, certainly pass their knowledge on to their progeny. They are always watching each other and can communicate both vocally and through gestures, so their children watch as they treat themselves: active learning. However, there is innate learning as well. "You should never underestimate the power of natural selection," Hunter says. "It does not take a smart organism to develop an instinctive behavior." There could be a genetic variation that leads the gorilla to taste a plant it does not normally eat, and eating the plant makes it healthier. The gorillas with that genetic background live longer and have more progeny and no real thought goes into it. "That level of learning might operate with other kinds of organisms too," he says. Take the monarch butterfly that lays its eggs on milkweed, which has antiparasite effects. "All we have to do is look at a healthy monarch butterfly and a sick monarch butterfly," says Jacobus de Roode, assistant professor of biology at Emory University, who specializes in the beautiful creatures. "Now, a sick monarch butterfly is really affected by these parasites. The parasites bore little holes in the abdomen, and she will lose some of her bodily fluids and doesn't feel good." The changes in her physiology can change the way she responds to smells of the vegetation around her and she may have a genetic preference for these that would do her good.

At our current level of understanding, health maintenance and self-medicative behaviors can be classified into five levels:

- 'Sick behaviors' (lethargy, depression, anorexia, reduction in grooming, behavioral fever, basking behavior);
- Optimal avoidance or reduction of the possibility for disease transmission (avoidance of feces contaminated food, water, substrates)
- The dietary selection of items with a preventative or health maintenance affect (items eaten routinely in small amounts or on a limited basis)

- Ingestion of a substance for the curative treatment of a disease or the symptoms thereof (use of toxic or biologically active items at low frequency or in small amounts, having little or no nutritional value)
- Application of a substance to the body or a living area for the treatment or control of a disease or condition (fur rubbing, anting, nest lining). [Huffman, 2019].

Antiparasitic behavior occurs in animals ranging from arthropods to primates, and is undoubtedly the product of a long evolutionary process. Ethnographic literature and recent ethnomedicinal research suggest that humans have long been aware of the use of medicinal plants by animals and have looked to them for clues about the medicinal properties of plants.

Animal self-medication and ethnomedicine

The first clear-cut demonstration of self-medication came from research on chimpanzees in western Tanzania. By the intentional ingestion of the extremely bitter juice extracted from the pith of *Vernonia amygdalina* (Asteraceae), chimpanzees suppress strongyle nematode infection and its associated symptoms when sick, bringing about relief of symptoms and a marked decrease in parasite load. Interestingly, chimpanzees use the pith, and the WaTongwe, traditionally living in the same habitat, use the leaves to treat similar symptoms. It was demonstrated that they ingest approximately the same dose of the same major bioactive compound, and both chimpanzees and humans show signs of recovery within 20 hours [Huffman and Seifu (1989) ; Huffman *et al.*, 1993; Huffman, *et al.*, 1996]. However, given the widespread use of *V. amygdalina* across Sub-Saharan Africa by humans, even in areas where chimpanzees are not found, it is likely they discovered the plant's curative properties independently. Pharmacological studies have demonstrated a wide array of biologically active properties in *V. amygdalina* [Erasto, *et al.*, 2006; Yeap, *et al.*, 2010]. The compounds have demonstrated anthelmintic (*Oesophagostomum*, *Toxicara*, *Ancylostoma*, *Schistosoma*, *Entamoeba*), antimalarial (*P. falciparum*) and tumor-growth-inhibition properties [Ohigashi , *et al.*, 1994; Adedapo, *et al.*, 2007; Egharevba, *et al.*, 2014]. This species also has many uses for the treatment of both human and livestock diseases [Oyeyemi, *et al.*, 2018].

Tabernanthe iboga (Apocynaceae) contains several indole alkaloids, and is used as a powerful stimulant and aphrodisiac in many secret religious societies in Gabon. In 1968 Harrison speculated that because of the widespread reports from local people of gorilla, bush pig and porcupine going into wild frenzies after digging up and ingesting the roots, they probably learned about these peculiar properties of the plant from watching the animals' behavior. The most active principle, found in the root, is called ibogaine and is shown to affect the central nervous system and the cardiovascular system. Two other known similarly active compounds in the plant are tabernanthine and iboluteine. The stimulating effects are similar to caffeine. The sloth bear and local people of central India are noted to become intoxicated from eating the fermented madhuca flowers. Reindeer and the indigenous Lapps consume fly agaric mushrooms known for their intoxicating effects.

Table 1 Case studies of ethnomedicinal plants in folklore and ethnographical studies purportedly derived from observed instances of animal self-medication.

Plant species	Animal species (Mode of self-medication)	Ethnomedicinal uses	Observed animal behavior leading to its use by humans
<i>Aeschynomene cristata</i> (Fabaceae)	Crested porcupine (<i>Hystrix africaeaustralis</i>)	Antibiotic properties suggested. Root concoction used to treat patients with sexually transmitted disease and other illnesses with symptoms	Porcupine dug up and ingested the toxic root. It subsequently recovered from dysentery-like symptoms (bloody stools)

		resembling dysentery in East Africa. Used as a treatment for secondary infections in AIDS patients.	
<i>Amanita muscaria</i> (Amanitaceae)	Reindeer, Caribou (<i>Rangifer tarandus</i>) Brown bear	Hallucinogenic effect used to communicate with the spirit world and to meet powerful spirits with ‘courage’; induces a temporary sense of euphoria. Used to increase strength for hunting, walking long distances, and enduring cold temperatures.	After eating the mushrooms, reindeer move around as if intoxicated. In the mating season, bears that ingest them are said to act with ‘courage’, losing the inhibition to confront others.
<i>Banisteriopsiscaapi</i> (Malpighiaceae)	Jaguar (<i>Panthera onca</i>) (stimulant/therapeutic treatment)	Used in ceremonies for its hallucinogenic properties, to	After chewing on the bark and roots, leopards roll on the ground

		heighten alertness and night vision ('jaguar' eyes) while hunting. Used as an anti-parasitic treatment	in an intoxicated state like cats with catnip
<i>Catha edulis</i> (Celastraceae)	Domestic goats (<i>Capra</i> spp.) (stimulant, tonic food)	Used as a mild euphoric stimulant, to minimize fatigue, improve work and mental performance, and to stay awake. Improves mental performance and is thought to be beneficial for head aches, colds, body pains, fevers, arthritis, and depression	After chewing on the leaves, goats friskily run around at faster than normal speeds seemingly filled with energy.
<i>Coffea arabica</i> (Rubiaceae)	Domestic goats (<i>Capra</i> spp.) (stimulant, tonic food)	First consumed as a beverage for mental clarity, and to counteract	After feeding on the red berries of the wild coffee bush, goats

		fatigue in the highland region of present-day Ethiopia.	become more alert and move about friskily
<i>Piliostigmathonningii</i> (Caesapilioideae)	African elephant (<i>Loxodonta africana</i>) (therapeutic treatment)	Treatment for dysentery, intestinal upsets, diarrhea, flatulence, malaria, fever, respiratory infection, snakebite, hookworm, and skin diseases, among others.	Elephants suffering from gastrointestinal disturbance chew on the leaves and temporarily store them in their esophageal pouch together with water. After leaving to soak for a while, leaves are discarded and the water is swallowed.
<i>Ligusticumporteri</i> (Apiaceae)	Black bear (<i>Ursusamericanus</i>), Brown bear (<i>Ursus arctos</i>), Kodiak bear (<i>Ursusarctos middendorffi</i>) (therapeutic treatment)	Roots chewed to increase endurance. Possess antiviral, antibacterial, antifungal and anti-inflammatory	Bears consume the roots after emerging from winter hibernation in a weakened state, to restore strength, or when wounded

		activities. Roots support healing of respiratory conditions, indigestion, headaches, dizziness, sinusitis, and arthritis. Taken to ease breathing at higher altitudes, and for long-distance hiking	or sick. Roots are chewed, and then, mixed with saliva, rubbed into their fur to treat skin infection and possibly wounds.
<i>Terminalia kaiseriana</i> (Combretaceae)	Bush pig (<i>Phacochoerus africanus</i>) (therapeutic treatment)	A treatment for cholera(extreme diarrhea).	Wild boar with diarrhea dig up and ingest the roots. They subsequently recover.
<i>Trema orientalis</i> (Ulmaceae)	Chimpanzee(<i>Pantroglodytesschweinfurthi</i>) (therapeutic treatment)	A decoction of the leaves is used as an anthelmintic medicine for roundworms and hookworm. Also used to treat diarrhea.	Chimpanzees suffering from heavy strongyle nematode burden, fold, and swallow leaves whole, expelling adult worms within 6 hours.

Conclusion

The scientists researching zoopharmacognosy are convinced that humans can learn from the animals, particularly in finding new medications. Much of folk medicine, particularly in the undeveloped world, likely came from medicine men watching animals self-medicate, and in the case of the plant used by the bonobos, what they saw works. Huffman knows cases of medicine men deliberately studying animals for clues for treating their patients. The monarch's milkweed, for example, appears to have antiparasite powers, and may be worth further study. Researchers have shown that the butterflies use milkweed chemicals called cardenolides to fend off a parasite called *Ophryocystis elektroscirrha* [Lefèvre *et al.*, 2010]. Cardenolides are already used to relieve pain and as an aid to treating asthma [Nicholson, 2010]. Animals use other plants that can combat schistosomiasis, plasmodium, and leishmania, all terrible human afflictions, and some medicines have been derived from them [Allegretti, *et al.*, 2014].

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An Overview on *Cyamopsis tetragonaloba* Pharmacological Activity

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Abstract: Cluster bean is a very familiar produce in the Indian vegetable markets. Eighty per cent of the world's cluster bean is cultivated in our country, as the crop can tolerate both frequent rainfalls and arid conditions. The crop is mainly grown in the dry habitats of Rajasthan, Haryana, Gujarat and Punjab and to a limited extent in Uttar Pradesh, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka and Kerala. It has possess anti-diabetic, hypo-cholesteremic, anti-ulcer, cyto-protective, anticholinergic, anticoagulant, haemolytic, antimicrobial, anti-asthmatic, anti-inflammatory, reversible antifertility, anthelmintic and wound healing activity etc. This review gives a view mainly on the phytochemical characteristics, traditional uses, phytochemistry and pharmacological actions of *Cyamopsis tetragonaloba*.

Key Words: *Cyamopsis tetragonaloba*, Anti-inflammatory, Antidiabetic, Antimicrobial, cyto-protective, anti-asthmatic,

Introduction

Cluster bean or guar (*Cyamopsis tetragonaloba* rooted, summer annual legume. It belongs to the tribe Galagae (Indigoferae) of the Leguminosae family. The crop is mainly grown in the dry habitats of Rajasthan, Haryana, Gujarat and Punjab and to a limited extent in Uttar Pradesh, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka and Kerala (Pabal 2013). It is generally 50-100 cm tall and bear branches. However, non-branch type-varieties have main stem only, which is heavily clustered with pods. Root nodules contain nitrogen-fixing bacteria and incorporation of crop residues improves soil fertility and productivity. Traditionally, guar plant was mainly used as a green manure and soil conserving cover crop. It is consumed as a vegetable and snacks by human beings (Saini, 2014). In India, cluster

bean occupies an area of 2.20 million hectares with a production of 0.60 million tonnes. It is cultivated under larger area in Rajasthan (18.18 lakh ha) followed by Gujarat (2.27 lakh ha), Haryana (1.27 lakh ha) and Punjab (0.14 lakh ha). In north India it is mainly cultivated for gum present in its endosperm called as guar gum, whereas in South India it is being cultivated for vegetable purpose in a quite negligible contribution (Bagenia, PS *et al.*, 2018, Kumar D *et al.*, 2002). Seeds are rich in protein, vitamins and minerals like Ca, Cu, Fe, K, Mg, Mn, P and Zn and also excellent source of complex carbohydrates (Reddy *et al.*, 2019). The cluster bean, also known as guar, is a drought-tolerant crop of the legume family. It is grown in India for vegetable, green manure and seed production. It is also recognized as a medicinal plant and possesses a high quantity of phytochemicals (Hassan S M *et al.*, 2010 & Sharma P *et al.*, 2019). It is commonly used to cure various diseases like ulcer, secretion, hyperglycemia, and cathartic. Cluster bean is known as folklore medicine, a highly potent source of phytochemicals or natural compounds (Daniel, 1989; Morris and Wang, 2007; Badr *et al.*, 2013; Kaushik *et al.*, 2020), and cultivated for its multiple uses in tropical Africa and Asia (Ashraf *et al.*, 2002; Gresta *et al.*, 2013; Mubarak *et al.*, 2015). It is an important leguminous herb, highly adapted to arid and semi-arid parts of the world requiring low inputs and care. The seeds are highly valued for industrial gum. It is cultivated mainly in rainy season as a rain fed crop in arid zones of India and various other parts of the world. 3. Guar is also used as a home remedy for the treatment of gastric disturbances and abdominal discomfort. In recent years, guar gum—a galactomannan, prepared from *C. tetragonoloba* has been shown to be a potent hypocholesterolemic agent in both normal and hypercholesterolemic animals. The methanolic extract of cluster bean was reported beneficial for having maximum anti-oxidants properties linked directly to its phenolic contents (Moteriya *et al.*, 2015; Babbar *et al.*, 2014). In the present study, the review on the pharmacological activity of *C. tetragonoloba* was reported and recognised the different *in-vivo* and *in-vitro* pharmacological properties of *C. tetragonoloba*.

Pharmacological activities

Antidiabetic effect

In alloxan and normal diabetic rats, blood glucose level was significantly reduced when an aqueous extract of guar plant pods at 250 mg/kg body weight dose was directed. On

total lipid serum, esterified and free cholesterol, phospholipids and triglycerides effects observed under guar feeding (Srivastava *et al.*,1987).

Hyper-Cholesterolemic potential

Hyper-cholesterolemic potential examined due to guar gum reactions obtained from seed endosperm on indication of protein involvement. Instead of these facts, blood lipids increased due to diabetes and guar gum reduces cholesterol triglycerides serum. A considerable high level of HDL/LDL cholesterol was observed with an increased level in HDL-cholesterol. Diabetic rats can be raised and improved by guar gum protein utilization and absorption (Saeed S, *et al.*,2012 and DerMarderosian A *et al.*,2002)

Anticoagulant activity

The anticoagulant effects was examined in the seeds of guar, sulphate derivatives with less molecular weight of galactomannan. For this purpose, depolymerization of galactomannan was done by using enzymes. Different ranges of molecular weight were obtained (12.6 –245.6K). Sulfate derivatives prepared. Results obtained concluded that all assemblage expressed anticoagulant activities and activity of anti-thrombin was up to 65-87U/MG and it was independent of sulfate derivatives (Hassan *et al.*,2010).

Antiulcer effect

Hypothermic restraint stress caused gastric lesions and at 500 mg/kg rate, guar extract reduced indomethacin significantly (Rafatullah *et al.*,1994).

Antimicrobial activity

C. tetragonoloba methanol extracts of different concentrations (20%, 60%, 100%) observed against *S. typhimurium*, *E. coli* and *S. aureus*, *Lactobacillus* spp. Results showed that 20% and 60% methanol extracts had antibacterial observations with *Lactobacillus* spp. 3.13 and 0.78 fraction/ml value of MIC observed for methanol extract in counter to *Lactobacillus* spp. and *S. aureus*. Same results of MIC obtained when *S. typhimurium* and *E. coli* treated with 100% methanol extract (Hassan *et al.*,2010)

Anti-Helmintic activity

From aerial parts (stem, leaf and fruit) of *C. tetragonoloba* extracts (aqueous and ethanol) with 12.5, 25, 50 and 100 mg/ml concentrations are prepared to analyze anthelmintic possibilities against test worms that were *Pheretima posthuma*. Significant results obtained for fruit and leaf extracts but stem extracts showed non-significant results. As an anthelmintic agent, leaf and fruit extracts hold up for positive results (Singh *et al.*,2015).

Wound healing activity

Fruits and leaves of *C. tetragonoloba* are used to prepare hydrogels to check the mitigate results on incision and excision wounds on Swiss Albino mice. Two types of hydrogels prepared: one with 5% w/w fruit ethanol and other with 5% w/w leaf ethanol. Both gels showed different but quite similar results. In excision wound model, 99% healing results obtained with fruit extract gel and 90% results observed by leaf extract gel. Results compared with control and conventional metro gel (1% w/w) on twelfth day of wound. Tensile strength significantly increased in incision wound representation ($p < 0.001$) and results showed that gel has capability to cure wounds (Singh *et al.*,2015).

Conclusion

Ethno-medicinal studies received much attention as this brings to light the numerous little known and unknown medicinal virtue especially of plant origin which needs evaluation on modern scientific lines such as phytochemical analysis, Pharmacological screening and clinical trials. In the recent review, the *Cyamopsis tetragonoloba* possess various pharmacological activities was discussed. However, it is imperative that more clinical, phytochemical and pharmacological studies should be showed to scrutinize the available potential of this plant.

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Nutritional Survey on Adult's Nutritional Status and Correlates of Obesity in Tiruchirappalli District

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Abstract: Obesity is a complex chronic disease characterized by excessive body fat accumulation, significantly increasing the risk of various health issues like type 2 diabetes, heart disease, certain cancers, and musculoskeletal problems, primarily caused by an imbalance between calorie intake and expenditure, influenced by factors like genetics, diet, physical activity levels, and environmental cues; early intervention and lifestyle modifications including dietary changes and regular exercise are crucial for managing obesity and mitigating associated health risks. As obesity is multi factorial disease it can be caused by various reasons such as poor eating habit, lack of physical activity, genetics and various factors such as socioeconomic factors, metabolic factors and behavioral factors. The desire to eat, desire to choose food, digestibility of food consumed, the absorption and metabolism of nutrients and physical generally determines the body weight. The aim of the study is to analyze and understand the factors to correlates on obesity. 70 samples were selected across Tiruchirappalli district using random sampling method and socio-economic status of samples were analyzed using questionnaire method. A questionnaire was formulated and information was collected on background details such as age, gender, education, occupation, type of family, family size, birth order, income level and monthly expenditure on food and other details regarding the adults. Dietary and lifestyle information was collected regarding dietary pattern, food habits, hydration status, physical activity, sleeping pattern and medical history using the questionnaire. Socio economic and anthropometric parameters were assessed in the entire adult's population. The collected data were statistically analyzed and interpreted.

Keywords: Obesity, adult, nutritional status, lifestyle pattern and anthropometric parameters.

Introduction

Obesity is now considered as a global issue and its occurrence in population has been elevated dramatically in past few decades. According to 2016 report of World Health Organization, about 1.9 billion of total population were consider as overweight and about 650

million peoples were considered as obese. A survey conducted in 2020 states that there are 39 million children aged 0-5 years were obese and this childhood obesity occurs due to various reasons (WHO.,2016). Obesity is defined as the accumulation of excessive fat in the body that impairs health and results with the risk of development of certain diseases such as diabetes mellitus, hyperlipidemia, hypertension and other cardiovascular diseases. Obesity is a disease with multi-factorial etiology and it is the second most common cause of death after smoking (Kozlov.,2019). Balanced diet is an important role player in maintaining body weight and it also plays a significant role in prevention of obesity and other cardiovascular diseases (CDC., 2004). Proper diet intake with good physical activity, environment and lifestyle changes helps in maintaining good health throughout the adulthood (Brown.,2011). Adults generally require 0.66g / kg/ day of protein, 10 - 20 grams of fat per day and require 40 grams / 2000 kcals per day of dietary fibre to achieve their proper nutrient requirement (Srilakshmi,2019).

The present study was carried out with the following objectives:

- To assess the socio-demographic characteristics of adults with obesity.
- To determine the lifestyle pattern and work conditions of the adult's population.
- To assess the nutritional and hydration status of adults.

Review of Literature

Obesity is now considered as a global issue and its occurrence in population has been elevated dramatically in past few decades. According to 2016 report of World Health Organization, about 1.9 billion of total population were consider as overweight and about 650 million peoples were considered as obese. A survey conducted in 2020 states that there are 39 million children aged 0-5 years were obese and this childhood obesity occurs due to various reasons (WHO,2016). There are numerous studies which explains the correlation of obesity and the association of obesity with numerous diseases such as diabetes mellitus, hypertension, hyperlipidemia, stroke, metabolic disorder and cancer.

It also confirms that obesity decreases the quality and lifespan of humans and elevates the chances of occurrence of various psychological issues such as anxiety and depression (Haslam & James, 2005). Obesity usually risks the person's life and may reduce the lifespan of the individual by the occurrence of various types of chronic and cardiovascular diseases. Excess accumulation of body fat can lead to the elevated cholesterol level and may lead to hypertension and dyslipidemia. The life span of the obese patients will be 2- 20 years shorter than the healthy individuals and the decrease in lifespan can be estimated on the basis of type of obesity (Blucher,2019).

Materials and Methods

1. Selection of Area and Samples

The area selected for the study was Tiruchirappalli District. Seventy adult samples, with the ages of 20 to 34 years females were selected from the village of Vayalur in Tiruchirappalli District. The excellent co-operation rendered by subjects and concerned authorities of the respective village was the main reason for the successful conduct of the study.

2. Collection of Background Information

A questionnaire is a series of questions asked to individuals to obtain statistically useful information about a given topic. Computerized questionnaire was formulated and validated by the statistician. It was helpful to collect the data on socio-demographic profile, dietary and lifestyle patterns to correlation of obesity. The social standing or class of an individual or group is referred to as socioeconomic status. They are employed globally to assess information on a population for demographic and economic status. The socioeconomic status was evaluated by profession, marital status, nature of family, family monthly income, highest education level, number of family members and background information of family.

3. Assessment of Nutritional Status

The nutritional status of an individual or a population indicates their current physical condition as it directly relates to the consumption and utilization of foods and nutrients in it. The nutritional status of the samples was assessed by nutritional anthropometry, dietary and lifestyle patterns.

Nutritional Anthropometry

Nutritional anthropometry is the important method of assessment of gross composition and physical dimensions of human body at measuring various body measurements such as individual's weight, height, waist circumference and hip circumference and indices such as Body Mass Index (BMI) and Waist Hip Ratio (WHR) were calculated.

Dietary Patterns

The dietary pattern defined the ratio, quantity of combination of different foods with beverages and snacking habit. The dietary pattern often links with the education, economic status, culture, religious belief and preferences. Food frequency questionnaire was used to assess the frequency of consumption of selected food such as cereals, pulses, green leafy vegetables and other vegetables, roots and tubers, fruits, milk and milk products, egg, fish and flesh foods, sugar and jaggery, oil, butter and ghee by all the subjects.

Lifestyle Patterns

The lifestyle habits and healthy practices determines the well-being of individuals which includes physical activity, dietary pattern, screen time, sleep pattern, alcohol consumption and snacking pattern.

4. Statistical Analysis and Interpretation of Data

The data was consolidated and tabulated. The responses of subjects were expressed in frequency and percentages. Mean and standard deviation were computed.

Results and Discussion

1.Socioeconomic Status of Samples

70 samples were selected through random sampling method and all the samples were females. The socioeconomic status of all the 70 female samples were analyzed by collecting primary data on different categories namely gender, marital, age, education level, profession, type of family, body mass index, frequency of food consumption, frequency of soft drink consumption, frequency of alcohol and smoking habit and it also includes the assessment of lifestyle habits of samples by assessing the duration of sleep, frequency of sleeping pattern, frequency of physical activity, regulation of periods cycle and medical history of all the samples.

Gender and Marital Status

The samples selected for the survey was females who were 70 in numbers (n=70) selected through random sampling method. Among them, 50 females were unmarried and 20 female samples were married.

Education Level

Highest level of education of the samples were identified and analyzed. Among 70 females, 61 have completed their Undergraduate and Post graduate in their respected field and 8 females have completed diploma as their major study and one female have completed their mid-level education.

Profession

The professions of all the females samples were collected as a part of socioeconomic data in which 52.9% (n=37) females were working while 40% (n=28) were students and 4.3% female were homemaker.

Type of Family

The nature of family of all the samples were collected to know the basic type of family they accommodate. Among 70 females, 91.4% (n=64) belongs to nuclear family and remaining 8.6% (n=6) belongs to joint family.

2. Anthropometric Assessment

Body Mass Index

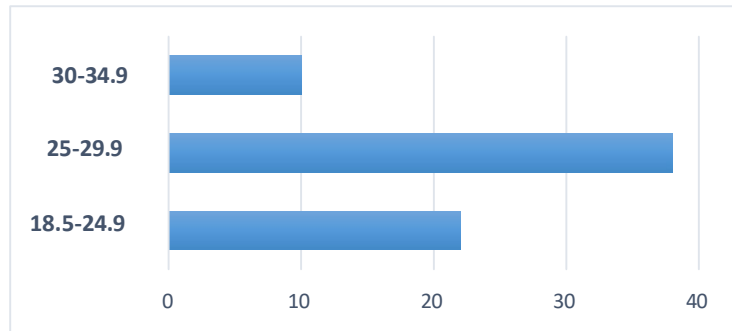
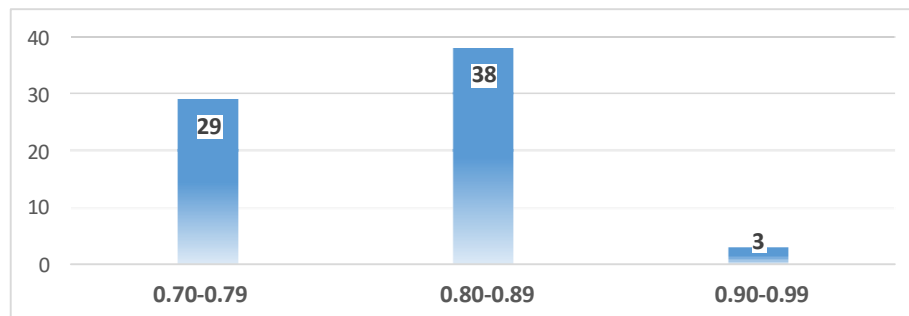


Figure 1 – Body Mass Index

The Body Mass Index (BMI) value helps in classifying samples under different categories such as underweight, normal, overweight and obesity. On analyzing the Body mass index, 37 samples belongs to normal BMI whereas 32.9% peoples (n=23) belongs to overweight category and 14.3% (n=10) belongs to obese first class whose BMI were between 30 - 34.9 kg/m².



Waist Hip Ratio

Figure 2 – Waist Hip Ratio

Waist hip ratio (WHR) is one of the body assessment method used to assess the distribution of fat in the body. Body measurements mainly hip and waist measurement is used to assess the body fat. Waist hip ratio lesser than 0.80 is considered as normal range. The above pictorial representation explains that 29 samples falls between the WHR of 0.70 - 0.79, 38 samples lies between 0.80-0.89 range and 3 samples lies between 0.90 - 0.99. According to the normal range of waist hip ratio 35 member among the 70 samples are considered to have central obesity.

3. Dietary Assessment

Nature of Food Consumption(Type of Diet)

Understanding the Type of diet helps in proper monitoring and designing of proper

dietary modification on considering their likes and dislikes. 62.9% were non- vegetarians, 10% were pescatarian, 21.4% were vegetarian and remaining were ova vegetarian.

Number of Meals Taken Per Day

The number of meals consumed per day and the quantity of meal consumption per day determines the nature of body type and it also helps in identifying the nature of meal consumption of samples. 92.9% samples prefer 3 meals a day where as nearly 4.3% prefer 2 meals and 2.9 % peoples prefer 4 meal consumption per day.

Skipping of Meals and Frequency of Skipping Meals

77.1% samples (n=54) skip their meals at different pattern from skipping meals from daily, weekly once, weekly twice to occasionally. Among them, 22.9% peoples slightly prefer low counts of skipping meals.

Snacking Pattern

The assessment of snaking pattern pays an important role in understanding the nature of food frequency assessment of the samples. 90%(n=63) prefers consumption of snacks at different frequencies from daily to weekly once and twice.

Food Frequency

The assessment of food choices of samples is performed by using Food Frequency pattern method. In food frequency pattern method in order to understand the consumption of different foods, all the samples are instructed to select the pattern of consumption of various food group namely cereals, pulses, green leafy vegetables, other vegetables, roots and tubers, fruits, milk products, eggs, fish, flesh foods, sugars, jaggery, oil, butter, nuts and oilseeds.

Soft Drink Consumption and Frequency

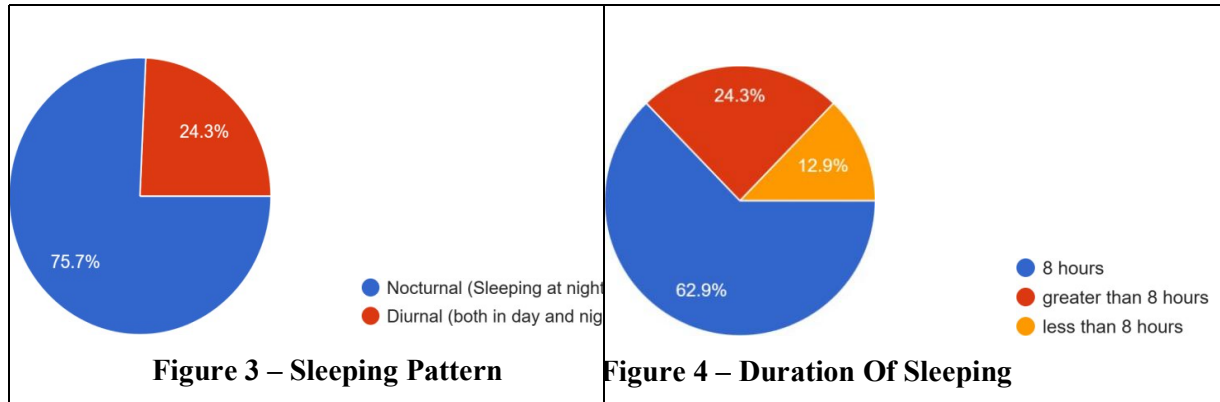
Nearly 77.1% females refer soft drink consumption in which nearly 1.4% females' daily consumption of any type of beverages, 25.7% prefers consumption of soft drinks once a week whereas 65.7% prefers occasional consumption of soft drinks.

Outside Meals and Frequency of Consumption

Consumption of meals from outside has becomes a trend and most peoples started preferring consumption of food from restaurants and hotels. Among 70 samples, nearly 87% peoples prefer foods from hotels and restaurants. Majority of the samples were consumed breakfast as daily consumption in outsides and most of the samples were consumed dinner for weekly once.

4.Lifestyle Pattern Assessment

Sleeping Pattern and Duration of Sleeping



Quality sleep always indicates the quality of health status of individuals. Every individuals requires nearly 7 to 8 hours for active performance in following day. Among 70 samples, nearly 62.9% peoples prefers 8 hours of sleep and nearly 12.9% peoples prefers less than 8 hours of sleep time; 75.7% follows nocturnal sleeping pattern and 24.3% follows diurnal sleeping pattern who prefers sleeping at both day and night time.

Disturbed Sleep

Disturbed sleep is one of the factors to be considered in obesity. Disturbed sleep during night may trigger excess sleeping pattern in day time and may lead to weight gain. From the 70 samples nearly 27% peoples experience disturbed sleep.

Regulation of Periods Cycle

The 70 samples were females with different marital status faces different periods cycle based on their hormonal change, weight, diet and sleeping pattern. Among 70 samples, 56 persons (80%) have normal periods cycle whereas nearly 10% faces irregular periods cycles and nearly 7.1% peoples faces period cycle with the duration of 40 - 45 days.

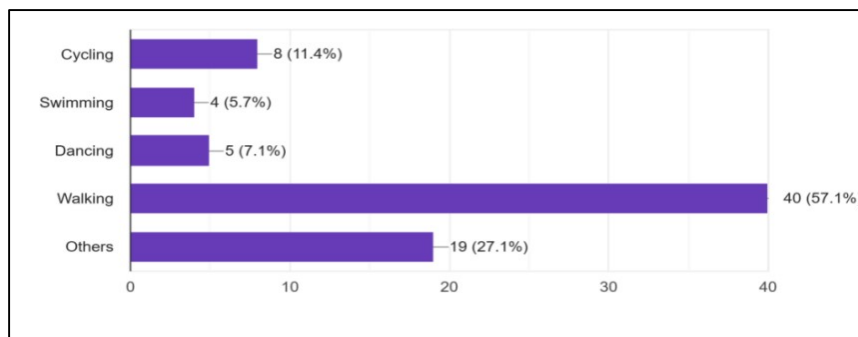


Figure 5 –Physical Activity

Physical Activity

Performing Physical activity helps in maintaining physical and psychological health of the individuals. Performing any form of physical activity helps in retaining freshness throughout and helps in improving the overall flexibility. 57.1% prefers walking as their whereas 11.4% prefer cycling, 5.7% prefers swimming and 7.1% prefers dancing such as zumba dancing as their regular physical activity.

Alcohol and Smoking Consumption

Alcohol consumption and smoking habit affect human health and may leads to several diseases such as fatty liver, cancer, lung infection, weight gain and may cause severe breathing problems. All the samples were non-alcoholic and non- smokers.

Medical History

Understanding and analyzing the medical history of a person helps in identifying the root cause of risk of diseases. Medical history of all the samples were collected in which nearly 78.6% does not have any past medical history whereas 12.9% have irregular periods cycle issue, 4.3% have PCOD, 1.4% people suffer from cardiovascular diseases and nearly 2.9% have hypertension.

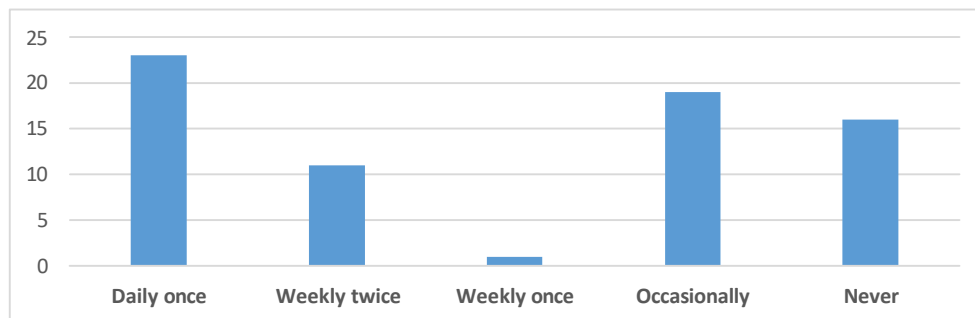


Figure 6 –Frequency of Exercise

Frequency of Exercise

Performing Physical activity helps in maintaining physical and psychological health of the individuals. Performing any form of physical activity helps in retaining freshness throughout and helps in improving the overall flexibility. Type of exercise and duration of exercise are the two major component which helps in attaining the entire benefit of exercises. Nearly 32.9% peoples exercise daily whereas 15.7% prefers exercising twice a week and 27.1% were occasional exercisers. Among 70 samples 22.9% were non-exercisers.

Conclusion

The adulthood age range is commonly considered to begin at the age of 20 or 21 years. During the lifespan of 20 and 64 years, there will be a major change in diet, physical activity, bodyweight, lifestyle and sleeping patterns which strongly influence the future well-being. These changes result in development of certain disease conditions such as obesity, diabetes mellitus, hypertension and other cardiovascular diseases. Obesity is one of the global issue and it is mandatory to take significant measures for prevent and manage obesity. Creating awareness and monitoring children and younger adults pays a way to control obesity. From the research findings, Total number of samples were 70 in numbers from which 32 samples were overweight and obesity category on the basis of their BMI and Waist hip ratio.

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Integrating Indian Knowledge Systems in Food Science and Nutrition: A Statistical Approach to Traditional Dietary Practices and Modern Nutritional Science

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Abstract: This research paper examines the integration of Indian Knowledge Systems (IKS) into contemporary food science and nutrition, focusing on the statistical validation of traditional dietary practices. India's vast repository of food wisdom, encompassing Ayurveda, Siddha, and regional culinary traditions, provides valuable insights that align with modern nutritional science. By employing statistical methodologies, this study evaluates the nutritional composition, health benefits, and sustainability of traditional Indian diets. The findings reveal that Indian dietary practices, characterized by plant-based nutrition, seasonal eating, and holistic well-being, offer effective solutions to modern dietary challenges such as malnutrition, lifestyle disorders, and food sustainability. The study further explores the role of traditional fermentation, herbal formulations, and functional foods in promoting gut health and disease prevention. Statistical analysis demonstrates the efficacy of these dietary principles in enhancing metabolic health, immunity, and longevity. Additionally, the research underscores the environmental sustainability of traditional food systems, advocating for their integration into contemporary nutritional policies. By bridging the gap between traditional wisdom and scientific validation, this study reinforces the relevance of IKS in addressing global health challenges and advancing sustainable food security.

Keywords: Indian Knowledge Systems, Ayurveda, Nutrition, Sustainable Diets, Statistical Analysis, Public Health, Traditional Food Practices, Functional Foods.

Introduction

Food science and nutrition have undergone significant transformations over the years, driven by advances in scientific research and technological innovation. While modern nutrition focuses on

macronutrients, micronutrients, and dietary guidelines, traditional knowledge systems provide a holistic perspective that integrates food with overall well-being. Indian Knowledge Systems (IKS) offer a rich repository of wisdom that dates back thousands of years, encompassing Ayurveda, Siddha, and regional dietary traditions. These systems emphasize the interplay between diet, health, and the environment, advocating for natural, seasonal, and personalized nutrition to achieve optimal well-being.

Indian traditional food practices are deeply rooted in cultural heritage and scientific rationale. The Ayurveda system, for example, classifies food based on its intrinsic qualities (gunas), taste (rasa), and post-digestive effects (vipaka). It highlights the importance of consuming fresh, locally sourced, and balanced meals to maintain harmony between the three doshas—Vata, Pitta, and Kapha. Similarly, Siddha medicine, which originated in Tamil Nadu, focuses on the medicinal properties of foods and their role in disease prevention. These principles are increasingly being validated by contemporary research, which highlights the benefits of traditional Indian diets in managing lifestyle disorders such as diabetes, cardiovascular diseases, and obesity.

With the rising global interest in functional foods, probiotics, and plant-based diets, there is an urgent need to bridge traditional knowledge with modern scientific frameworks. Many indigenous food practices align with the current trends in nutrition science, such as fermented foods for gut health, spice-based therapeutics, and plant-based proteins. However, scientific validation of these practices remains a challenge due to the lack of empirical data, standardized methodologies, and comprehensive studies.

Objectives of the Study

This study aims to explore the intersection of Indian Knowledge Systems and modern food science, focusing on the statistical validation of traditional dietary practices. The key objectives include:

- **Nutritional Composition Analysis:** To assess the macro and micronutrient profiles of traditional Indian diets and compare them with modern dietary recommendations.

- **Health Benefits Assessment:** To examine the impact of traditional Indian diets on various health parameters, including metabolic health, immunity, and gut microbiota.
- **Sustainability and Environmental Impact:** To evaluate the ecological footprint of traditional Indian food practices and their role in promoting sustainable diets.
- **Challenges and Opportunities:** To identify the barriers in integrating IKS-based food practices into contemporary nutrition science and suggest strategies for their mainstream adoption.
- **Statistical Correlation and Evidence-based Analysis:** To employ data-driven methodologies, such as meta-analysis and nutritional epidemiology, to establish a scientific foundation for traditional dietary wisdom.

Significance of the Study

The findings of this research hold immense significance in multiple domains, including public health, policy formulation, and sustainable food systems. With rising concerns about ultra-processed foods and non-communicable diseases, rediscovering and scientifically validating traditional dietary patterns can offer viable solutions for better health outcomes. Additionally, promoting IKS-based food practices can contribute to food security, biodiversity conservation, and climate resilience by encouraging sustainable agricultural practices.

As globalization continues to shape food choices and eating habits, the integration of IKS with modern nutrition science can provide a balanced approach that respects cultural heritage while embracing evidence-based dietary recommendations. Through this study, we aim to bridge the gap between tradition and modernity, ensuring that ancient wisdom is not only preserved but also adapted for contemporary nutritional needs.

Literature Review

Traditional Indian Diets and Nutritional Composition Traditional Indian diets are predominantly plant-based, emphasizing whole grains, legumes, vegetables, fruits, nuts, and seeds. Dairy products, including milk, yogurt, and ghee, play an essential role in Indian cuisine. Spices and herbs such as turmeric, ginger, and fenugreek are not only used for flavor enhancement but also for their medicinal properties.

Several studies have highlighted the nutritional benefits of Indian diets. Whole grains and legumes provide high fiber content, promoting digestive health. Antioxidant-rich spices contribute to disease prevention by reducing oxidative stress. However, comprehensive statistical analyses are required to quantify these benefits and compare them with modern dietary guidelines.

Ayurveda and Functional Foods Ayurveda classifies food based on its taste (rasa), energy (virya), and post-digestive effect (vipaka). This classification framework offers insights into the functional properties of food and its health impacts. For instance, turmeric, a staple in Indian cuisine, has been extensively studied for its anti-inflammatory and antioxidant properties. Fenugreek seeds exhibit hypoglycemic effects, making them beneficial for diabetes management.

Sustainability of Traditional Indian Food Systems Traditional Indian food systems promote sustainability by relying on locally sourced, seasonal ingredients and minimizing food waste. The practice of utilizing every part of a plant or animal, such as banana stems or fish heads, reflects a deep respect for natural resources. In contrast, modern food systems often result in high environmental costs, including greenhouse gas emissions, water pollution, and deforestation.

Challenges in Integrating IKS into Modern Nutrition Despite its potential, integrating IKS into modern nutrition presents challenges, including the lack of standardized methodologies for evaluating traditional practices, limited awareness among healthcare professionals, and the influence of Western dietary trends. Addressing these challenges necessitates a collaborative approach involving researchers, policymakers, and practitioners.

Findings

Nutritional Composition of Traditional Indian Diets

The statistical analysis of traditional Indian diets revealed that they are exceptionally rich in dietary fiber, vitamins, and minerals. A comprehensive examination of various regional diets, such as the South Indian diet, demonstrated an impressive average daily fiber intake of 25-30 grams. This is notably higher than the recommended daily intake of 25 grams of fiber for adults, underscoring the dietary benefits of consuming plant-based foods prevalent in Indian cuisine.

Key components such as whole grains, legumes, fruits, and vegetables in traditional Indian meals contributed to this higher fiber intake.

Moreover, the consumption of a wide range of vegetables, pulses, and leafy greens in traditional Indian dishes provided an abundant source of essential vitamins and minerals. For example, traditional foods like spinach, fenugreek, and other leafy vegetables are rich in folate, iron, and calcium. Additionally, regional variations incorporating ingredients such as amla (Indian gooseberry), which is high in Vitamin C, and jaggery, a form of unrefined sugar, contributed to vitamin and mineral intake. Furthermore, the use of spices, including turmeric, cumin, and coriander, contributed not only to the flavor profile of traditional Indian diets but also enhanced the antioxidant capacity. The active compounds in these spices, particularly curcumin in turmeric, are known for their anti-inflammatory and antioxidant properties, making them a valuable component in maintaining overall health.

Health Benefits of IKS-Based Dietary Practices

Indian Knowledge Systems (IKS) have long advocated for food practices that are not only culturally significant but also have various health benefits. One of the key aspects of traditional Indian diets is the incorporation of fermented foods, such as idli, dosa, and curd. These foods are rich in probiotics, which play a vital role in enhancing gut health. Studies indicate that fermented foods contribute to the growth of beneficial bacteria in the intestines, improving digestion, boosting the immune system, and preventing gastrointestinal issues. The probiotic strains in these fermented foods can enhance the microbiota balance in the gut, which is associated with overall better health and well-being.

Another beneficial aspect of IKS-based dietary practices is the use of ghee (clarified butter) as a cooking medium. Unlike modern vegetable oils, ghee is known for its high content of short-chain and medium-chain fatty acids that enhance the bioavailability of fat-soluble vitamins such as Vitamins A, D, E, and K. These vitamins are essential for a range of physiological functions, including vision, bone health, and immunity. Moreover, ghee's stable fat structure makes it less prone to oxidation during cooking, making it a healthier option compared to other oils. The

practice of using ghee in moderation, combined with its digestive properties, has been an integral part of Indian culinary traditions, contributing to long-term health benefits.

Sustainability of Traditional Indian Food Systems

The sustainability of traditional Indian food systems is a significant area of focus, particularly in the context of modern environmental concerns. The analysis revealed that traditional Indian food practices, which primarily rely on plant-based ingredients, have a significantly lower environmental impact compared to Western diets that are heavily reliant on animal-based products. The incorporation of grains, legumes, vegetables, and fruits into daily meals not only provides high nutritional value but also ensures a lower carbon footprint. Plant-based foods require fewer resources in terms of water, land, and energy when compared to livestock farming, which is one of the leading contributors to greenhouse gas emissions globally.

In addition to the dietary structure, the sustainability of traditional food systems is also reflected in agricultural practices. The methods of crop rotation, intercropping, and organic farming that are inherent in traditional Indian agriculture have been key to maintaining soil fertility and biodiversity. These practices avoid the overuse of chemical fertilizers and pesticides, promoting a healthier ecosystem. For example, the combination of pulses and cereals in crop rotation prevents soil depletion and encourages natural nitrogen fixation, enhancing soil health and reducing the need for synthetic fertilizers. Moreover, the consumption of locally grown, seasonal foods minimizes the environmental cost of transportation and storage, contributing to a more sustainable food system.

Traditional Indian diets also embrace minimal processing, a practice that not only preserves the nutritional integrity of foods but also reduces the energy required for food production. With the rise of industrialized food systems, traditional food practices offer a model for sustainable living by promoting foods that are closer to their natural form, requiring fewer resources and creating less waste.

Discussions

Bridging Traditional Wisdom and Modern Science

The integration of Indigenous Knowledge Systems (IKS) with modern scientific approaches offers a unique opportunity to address contemporary nutritional challenges. Traditional diets, often built on centuries of experience, contain essential nutrients and offer sustainable practices that are highly compatible with current health goals. Modern scientific methods can validate and enhance the effectiveness of these traditional practices by providing empirical evidence to support their use in global health strategies. The combination of traditional wisdom and modern science could potentially shape new, evidence-based dietary guidelines, optimizing health and promoting sustainability in food systems worldwide.

Implications for Public Health

The application of IKS in public health interventions has significant potential in the fight against diet-related diseases such as obesity, diabetes, and cardiovascular conditions. Many traditional foods and spices contain bioactive compounds that have demonstrated therapeutic effects. For instance, the inclusion of turmeric, ginger, and garlic in traditional diets has been linked to anti-inflammatory and antioxidant properties that can help prevent chronic diseases. Reintroducing these natural alternatives in modern diets could reduce reliance on synthetic additives and preservatives, promoting healthier and more sustainable food practices. Integrating IKS into public health strategies could contribute to improved nutritional outcomes, offering a cost-effective approach to combating global health challenges.

Policy Recommendations

To fully realize the potential of IKS in modern nutrition, policymakers must prioritize research funding to explore and validate traditional dietary practices. Establishing standardized methodologies for documenting and testing IKS is essential to ensure its credibility and scientific acceptance. Public health campaigns should also be launched to raise awareness among healthcare professionals about the value of traditional knowledge in nutrition. Furthermore, educational programs can be developed to teach the public about the health benefits of traditional

foods, ensuring that these practices are passed on and widely adopted for long-term societal benefit.

Conclusions

This research underscores the significant potential of Indian Knowledge Systems (IKS) in advancing the fields of food science and nutrition. The study demonstrates that integrating traditional Indian dietary practices, supported by statistical validation, can effectively bridge the divide between ancient wisdom and modern scientific approaches. The findings provide compelling evidence of the nutritional, health, and environmental advantages inherent in IKS-based diets. These benefits make a strong case for the inclusion of IKS principles in global nutritional frameworks, contributing to the promotion of health and sustainability on a global scale.

Traditional Indian dietary practices, grounded in holistic and sustainable food systems, emphasize the importance of local, seasonal ingredients and mindful consumption. These practices have not only stood the test of time but also align with contemporary scientific research advocating for plant-based, nutrient-dense, and environmentally conscious diets. By validating these practices statistically, this research highlights their relevance and potential impact on addressing global nutritional challenges, such as malnutrition, obesity, and climate change.

Looking ahead, future research should focus on expanding the scope of statistical analyses to further strengthen the evidence base for IKS-based diets. Additionally, exploring the therapeutic properties of traditional ingredients could uncover new approaches to disease prevention and treatment. There is also immense potential for innovation by developing modern food products that incorporate the principles of IKS, combining tradition with technology to meet contemporary demands.

In conclusion, embracing the richness of Indian knowledge systems offers a promising pathway to achieving a healthier, more sustainable future. The integration of IKS into modern nutrition and food science could play a pivotal role in fostering global well-being while preserving cultural heritage.

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Photocatalytic Degradation of Azo Dye by Visible Light using Sodium Sulphide (Na₂S) as Catalyst: A Colorimetric Study

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Abstract: Water pollution is the contamination of water bodies by harmful substances, such as chemicals, pollutants, and dyes that can harm humans, animals, and the environment. Water pollution due to waste effluents of the textile industry is seriously causing various health problems in humans. Among the chemical pollutants, organic dyes due to toxic nature, are one of the major contaminants of industrial wastewater. Sodium sulfide (Na₂S) is a highly alkaline, water-soluble compound commonly used in paper production, and textile manufacturing and chemical industries. It is also a reducing agent or in the production of dyes and pigments. Azo dye is one of the more detrimental classes because it is highly persistent in the aquatic environment, due to its chemical compositions, involving aromatic rings, azoic linkages and amino groups which produces stable azo bonds, resistance in biodegradation, persistence in environment. Dye degradation is the breakdown or transformation of dye molecules into simpler, less toxic, and more biodegradable compounds, often through chemical, biological, or photocatalytic degradation processes. Photocatalytic degradation uses light energy and a catalyst to break down pollutants, such as azo dyes, into harmless compounds. In this study, the photocatalytic degradation of azo dye was done using Na₂S as catalyst which showed effective degradation.

Keywords: Water pollution, Catalyst, Photocatalytic degradation, Azo dye, Environment.

Introduction

Water pollution has become a global problem. Nowadays ongoing evaluation of water resource policy is needed to counter this problem. The presence of dye in water stream leads to unexceptional effects on living life. As dyes are consumed globally from small-scale to large-scale industries inculcating tanneries, food, cosmetic, textile, medicinal sectors with the production of 1,000,000 tons all over the world. Majorly, the textile sector plays a pertinent role in dye emissions into the ecosystem ^[1]. Out of several natural and synthetic dyes, azo group proliferation has been highly carcinogenic due to amine and benzidine emissions. Besides this, the fact of being non-biodegradable makes the dye molecules last longer in the environment producing hazards. Henceforth, eradication of dye molecules from wastewater is thus needed before discharging them stream into the environment with long-term and short-term effects. The severe implications have been reported for the aquatic life due to direct contact. Whereas to human life, there were observations from skin irritations to cancer-like disease. Several approaches were reported for the treatment of dye-containing streams but the exploration for the best available technique is still ongoing. Azo dye plays a crucial role in the governance of the dye and printing market. These dyes are synthesized from a simple method of diazotization and coupling ^[2]. Different routes and modifications are made to obtain the desired color properties, yield and particle size of the dye for improved dispersibility. Azo dyes are the most used dyes and account for more than 60 % of total dyes ^[3]. Azo dye compounds are characterized by the functional group (-N=N-) uniting two symmetrical and/or asymmetrical identical or non-azo alkyl or aryl radicals. Most azo dyes are synthesized by diazotization of an aromatic primary amine, followed by coupling with one or more electron-rich nucleophiles such as amino and hydroxyl groups ^[4]. The azo group may be bonded to benzene rings, naphthalenes, aromatic heterocycles or to enolizable aliphatic groups. These are essential to give the color of the dye, with their shades of different intensities ^[5]. Photocatalytic degradation is a process where a photocatalyst, typically a semiconductor material, absorbs light energy and uses it to break down organic pollutants into harmless compounds. Photocatalytic Degradation is used in light adsorption, charge separation, redox reaction, pollutant degradation in dye degradation ^[6]. Sodium sulfide (anhydrous) is a sulfide salt with formula Na₂S. The pentahydrate and (particularly) the nonahydrate are also known. In gel form, sodium sulfide is used to soften toenails to assist in trimming (and so relieve pain) of ingrowing toenails. It is a sulfide salt and an inorganic sodium

salt. Sodium sulfide is a chemical compound with a chemical formula Na_2S . Sodium sulfide is used in the pulp and paper industry, water treatment, textile industry, and various chemical manufacturing processes including the production of rubber chemicals, sulfur dyes and oil recovery. The sodium sulphide itself has the dye degrading properties. It has been explored as a catalyst for dye degradation in water. Because they are known for their redox reactions, sulphide ion interaction and catalytic activity in photocatalytic dye degradation. It exhibits catalytic activity in various dyes, including azo, anthraquinone, triphenylmethane dyes. It facilitates hydrolysis, oxidation and free radical formation to degrade the dye in water^[7].

Materials and Method:

- Sodium sulphide
- Azo dye
- Magnetic stirrer
- Visible light
- Colorimeter

Azo Dye Preparation:

Solution 1:

About 2ml of aniline was added in beaker and placed it in ice bath and 5ml of HCl was added to the solution. Then 10ml of water was added and shaken well. About 10ml of sodium nitrate solution and stirred well.

Solution 2:

About 30ml of NaOH and 2g of 2-Naphthol was added in beaker, placed in ice bath and shaken well. Then both the solution 1 and 2 were mixed to get azo orange dye and kept at rest for 10 minutes. The temperature was set as 5°C. The solution was filtered using Whatman filter paper and stored.^[8]

Photocatalytic Degradation of Azo Dye using Na_2S :

About 0.50g and 1g of sodium sulphide were dissolved in 10 ml of distilled water in two beakers and added in each separate beaker containing 100ml of azo dye. Then the solution was placed in

magnetic stirrer for 2 hours along with visible light source. The readings were taken in the regular interval using colorimeter. Here, about 4 readings were taken at an interval of 30 minutes. ^[9-10]

Result and Discussion:

The sodium sulphide (Na_2S) of both concentration (0.50g, 1.00g) degraded the azo dye effectively. But the 1.00g concentration degraded the dye efficiently than 0.50g finally. In 30 mins incubation period, the dye degraded better in 0.50g (22.2%) than 1.00g (11.1%). In 60 mins incubation period, the 0.50g (38.8%) again degraded effectively than 1.00g (22.2%). In 90 mins incubation period, both concentration degraded the dye in same percentage (50%). In 120 minutes incubation period, the 1.00g (66.6%) showed better result than 0.50g (72.2%). Both concentration showed more or less same reading in the degradation of azo dye but comparatively in the final reading the 1.00g concentration of Na_2S solution degraded azo dye at 72.2% while the 0.50g concentration degraded only 66.6% from total 100% of azo dye.

Time (2hrs) in minutes	Initial Value=0.18 at 485nm	0.50g in Na_2S at 485nm	1.00g in Na_2S at 485nm
30	100%	22.2%	11.1%
60	100%	38.8%	22.2%
90	100%	50%	50%
120	100%	66.6%	72.2%

Table 1

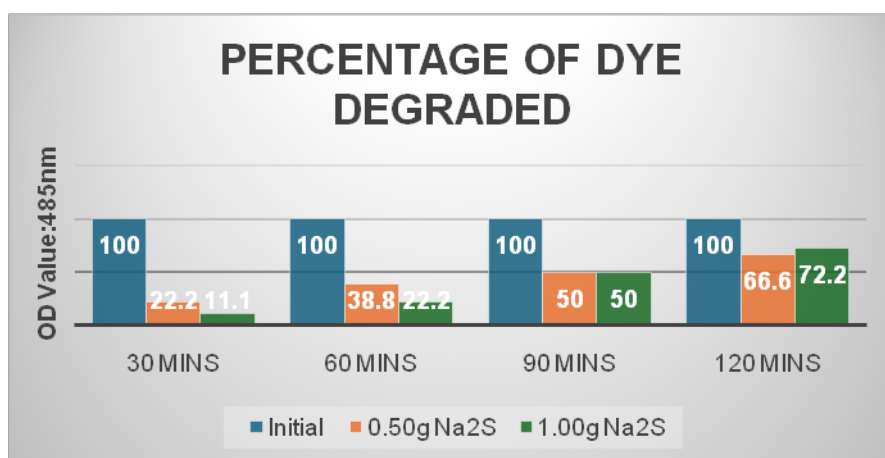


Figure 1.1

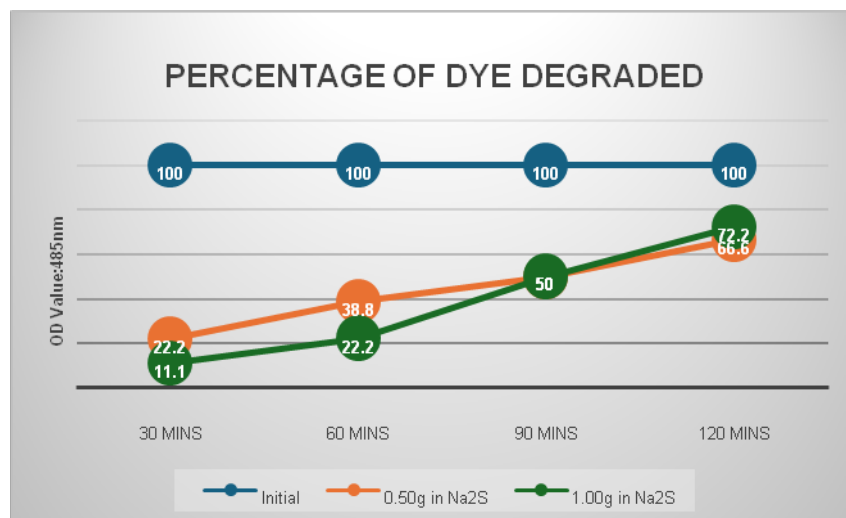


Figure 1.2

Conclusion:

This study demonstrated the effectiveness of sodium sulfide (Na_2S) as a catalyst for the photocatalytic degradation of azo dye under visible light irradiation. The results showed that Na_2S significantly enhanced the degradation rate and efficiency of azo dye degradation, making it a promising catalyst for wastewater treatment applications. Future studies can build on these findings to explore the scalability and applicability of Na_2S -catalyzed photocatalytic degradation for real-world wastewater treatment scenarios.

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Life Cycle and General Morphology of Millipedes (Myriapodes: Diplopoda)

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Abstract: Millipedes are frequently found in large swarms under the cover of decaying litter layers in agricultural and horticultural land areas, as well as forests on humid soils in India. Because of burrowing, litter breakdown, and the mixing of organic and inorganic substances in their digestive system, these ancient soil invertebrates have a significant impact on the soil, that it contributes to nutrient flux and plays an important role in the food chain. *Anoplodesmus tanjoricus*, it measures about 4-5 cm (1.7-2 in) long and is a member of the Order Polydesmida. When mature, the animal's upper surface is usually black, with yellow splotches along the sides. It has twenty body segments, with 31 pairs of legs on females and 30 pairs on males, with one pair modified to form gonopods in males. Ozopores are chemical defence gland openings that run the length of the body. Müllerian mimicry rings could form. *Anoplodesmus tanjoricus* has a relatively short life cycle, 100-400 eggs are laid in earthen nests constructed by the females 5-10 cm deep in the soil, 20-25 days after copulation. During her life, a female can lay 2-4 egg masses. In Stadium VIII, it takes seven moultings to mature from egg to adult. To overcome dry periods, larvae in the sixth and seventh stadia can go into long diapauses. As a result, adults appear in large numbers on the surface after the rainy season begins. *Anoplodesmus tanjoricus* needs 3-4 months to complete its life cycle, depending on temperature, moisture, and food supply. Adults can be seen on the soil surface and very active, mate at any time of day, and live for 4-6 weeks, visible and easy to identify.

Key Words: Diplopods, Millipede, Morphology and life cycle

Introduction:

The Diplopoda (millipedes) are the third largest class of terrestrial arthropods, after insects and arachnids, with over 12 000 species described in 145 families and 16 orders (Adis 2002, Shelley 2002, Sierwald and Bond 2007, Shelley 2007), and an estimated global fauna of more

than 80 000 species. They are ecologically significant as detritivores and are a significant component of terrestrial ecosystems in all temperate, subtropical, and tropical regions of the world. Millipedes are among the earliest examples of a terrestrial animal, and fossils from the early Carboniferous Period suggest that they were also among the earliest prey. Millipedes are frequently found in large swarms under the cover of decaying litter layers in agricultural and horticultural land areas, as well as forests on humid soils in India. Millipedes are generally detritivores and prefer to eat decaying plant material rather than living vegetation. These ancient soil invertebrates have a significant impact on the soil due to burrowing, litter breakdown, and the mixing of organic and inorganic substances in their digestive system which are allocated to different soil layers (Hopkin and Read, 1992). The species feeds on various kinds of leaf litter, rotten vegetables, tree stems, wood, decaying fish, and cow dung. (Bano & Krishnamoorthy, 1981a, 1981b; Prasad *et al.*, 1981; Dutta and Joy, 1992, 1993). This species, like millipedes in general, plays an important role in the litter breakdown of the ecosystems in which it lives. Populations of millipede which occur often in high densities. The richness of biodiversity depends on the climatic condition and area of region. *Anoplodesmus tanjoricus* secretes hydrogen cyanide from the repugnatorial glands for defence against potential predators. These aggregations are probably induced by rhythmical secretion of pheromones. This *Anoplodesmus saussurii* species is most active at twilight hours and aggregate at midnight and during midday (Bano *et al.*, 1976; Bano, 1978; Bano and Krishnamoorthy, 1979).

Materials And Methods

Study Area: Extensive study was conducted in Monsoon seasons between July 2022 to October 2022. We selected some different farms in Amravati region and observe the occurrence of different species of millipedes. Most species of millipedes commonly found in dark moist areas near water. Moist places beside the road. Forest roads and farms are excellent areas for millipede collection.

Sample Collection: Millipede samples are collected for photography by hand picking method. Samples can be taken from rotten woods, under stone, uppermost soil strata and all kind of plant debris. Millipede were collected from rotten woods, under stone, uppermost soil strata and all kind of plant debris in Amravati region, they were brought to the laboratory in the glass container with moist soil and feed cucumber, apple, vegetables and rotten leaves with plenty of humus

Identification of species was carried out by Virginia Museum of Natural History, USA.

Observations

Habits

The millipede species are found under logs and stones in moist soil under leaf litter. These millipedes inhabit cool, moist localities under shrubs, bushes, woods and plants, herbivorous animals. They usually avoid light, attracted to dark, cool, moist environment. usually going unnoticed in the summer due to their nocturnal habits and tendency to disperse. They feed on living and decomposing vegetation and occasionally on dead snails, earthworm and insects. They can occur on soft stemmed plants in garden and greenhouses. They cannot tolerate water saturated soils. Which forces them to the surface and higher ground. Likewise dry drought condition can stimulate migration.

Morphology

Anoplodesmus tanjoricus, in the Order Polydesmida (black with yellow spotted millipede), it is about 4-5 cm in length, long, cylindrical, worm-like bodies with many segments. Millipedes lack a waxy layer on their epicuticle and are subject to rapid desiccation in environments with low relative humidity. They do not bite (biting jaws are absent), but can cause chemical irritant contact dermatitis. When mature, the upper surface of its body is usually black, with yellow spotted along the sides. It has twenty body segments with 31 pairs of legs on females, and 30 pairs of legs on males; the difference in number is because in males, one pair is modified to form gonopods. Ozopores, the openings of chemical defense glands, occurring along the length of the body. The secretions of these glands may consist of topical irritants, repellents, antifeedants, or, in the case of the large and widespread Order Polydesmida, hydrogen cyanide (HCN) gas that can be fatal to other arthropods or even small vertebrates in a confined environment. Müllerian mimicry rings may develop in which unrelated species of millipedes that co-occur closely resemble one another, the ability to roll into a smooth, resistant sphere or coil, and still others have spikey projections that may deter soft-mouthed predators.

Behaviour and Feeding

The bright spots on the yellow-spotted millipede are an example of aposematic (warning) coloration that wards off potential predators. The millipede also protects itself by curling into a ball which resembles a snail. It also releases cyanide which is toxic to small predators. This millipede is not dangerous to humans as the amount of cyanide is small and the millipede does

not bite; nonetheless, its secretions can irritate human eyes and skin, and sometimes stain skin. While this millipede has few predator. Mature millipedes feed on a variety of dead leaves and decayed wood including Douglas-fir, true fir, cedar, and several deciduous species. Immature millipedes feed on humus. The chewing increases leaf surface area, giving microbial communities increased access to nutrients. Most of the litter that the millipede consumes is passed through the gut and excreted as fecal pellets. The pellets are an important food source for smaller invertebrates.

Breeding and Reproduction

Spring is typically when millipedes mate; They frequently mate in a single location and form groups. Typically under a log, females lay several hundred eggs in a moist, secure location. Because pupae cannot move, they lay eggs wherever there is readily available food. After at least three weeks of incubation, the offspring emerge. Millipedes that are still in their infancy have fewer segments and a pale color. They gain body segments, become darker in color, and have brighter spots as they molt. The life cycle of *Anoplodesmus tanjoricus* is quite short for a millipede of this size (21–33 mm). 20–25 days after copulation, 100–400 eggs are laid in earthen nests constructed by the females 5–10 cm deep in the soil. One female can lay 2–4 egg masses during its life time. From egg to adult it takes seven moultings to reach maturity in stadium VIII. In the sixth and seventh stadia the larvae can undergo long diapauses to overcome dry periods. Therefore, adults appear in large numbers on the surface after the onset of the rainy season. Depending on temperature, moisture and food supply, *Anoplodesmus tanjoricus* needs 3–4 months to complete its life cycle. The adults are active on the surface, mate even during daylight hours, and live for 4–6 weeks. There are two generations per year, which overlap one another. The life cycle of *Anoplodesmus saussurii* was studied in detail by Bano & Krishnamoorthy (1985) and Prasad *et al.* (1981). A very interesting fact of *Anoplodesmus saussurii*, besides its short life cycle and high reproduction rate, is its sociability with aggregations of up to nearly 200 individuals per square metre.

Conclusion

The significant loss of diplopod specimens per stump after stump harvesting may be linked to a corresponding loss in function, particularly the mechanical breakdown of woody substrates. The results indicate that a reduction of habitats for stump species with low dispersal ability can both reduce their abundance per unit substrate and potentially also change the stump decomposition rate compared to sites with higher stump densities. Ecologically, they are important

because they break up leaf litter, thereby aiding bacterial breakdown, which helps retain nutrients in forest soils

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Molecular identification of hydrocarbon degrading bacteria in textile industry effluents

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Abstract: Textile industry is always an expanding and emerging sector due to the ever-increasing usage of textile products. Apart from dyes, textile effluent also containing hazardous hydrocarbon in the form of oily waste water generated by the usage of crude oils and grease for the fabric processing. Textile effluents are extremely difficult to treat due to their high content of pollutants such as polycyclic aromatic hydrocarbons and aliphatic hydrocarbons. Untreated textile effluent disposal practices leads to harsh environmental pollution by hydrocarbon contamination to the soil, underground water and coastal waterways. Moreover these hydrocarbons deplete the ozone, reduce photosynthetic ability of plants, and increase occurrences of cancer, neurodegenerative and respiratory disorders in humans. Physical and chemical means of crude oil degradation are clearly not affordable in developing countries due to the financial burden. In recent years, bioremediation i.e., the use of bacteria to remediate hydrocarbon pollutants has become a promising technology because of its low cost and eco-friendly nature. Array of bacteria were identified in the hydrocarbon biodegradation, its time to identify indigenous distinct bacterial strains with hydrocarbon degrading properties.

Keywords:Textile effluent, Hydrocarbon contamination, Bioremediation, Bacterial degradation, Environmental pollution

Introduction

The textile industry is a continuously expanding and evolving sector due to the ever-growing demand for textile products. However, the discharge of untreated effluents into water bodies and the environment has become a significant concern, contributing to severe environmental pollution (Mengetal *et al.*, 2016). Textile effluents not only contain dyes but also hazardous hydrocarbons in the form of oily wastewater, generated from the use of crude oils and grease in fabric processing. These effluents are highly challenging to treat due to their complex composition, which includes polycyclic aromatic hydrocarbons (PAHs) and

aliphatic hydrocarbons (Bayat *et al.*, 2016). The improper disposal of textile effluents has resulted in the contamination of soil, groundwater, and coastal waterways, exacerbating environmental degradation (Deng *et al.*, 2014).

Petroleum hydrocarbon pollution (PHP) is increasingly severe due to the rising demand for oil and its derivatives. This form of pollution has been linked to ozone depletion, reduced photosynthetic efficiency in plants, and increased occurrences of cancer, cardiovascular diseases, neurodegenerative disorders, and respiratory illnesses in humans (Jin *et al.*, 2017). Various industrial activities, including the use of cooling and lubricating agents, compressor condensates, metal processing, oil exploitation, and transportation, contribute significantly to PHP (Ghanavati *et al.*, 2008). Oil-polluted effluents typically contain toxic compounds such as PAHs, petroleum hydrocarbons, and phenols, which are difficult to degrade and have adverse effects on ecosystems (Yakimov *et al.*, 2007).

Several remediation approaches have been explored to treat oily wastewater, including physical, chemical, and biological methods. Physical methods such as adsorption, air flotation, and membrane filtration are widely used, whereas chemical methods include electrocoagulation, wet oxidation, electrolytic oxidation, and ozone catalytic oxidation (Das & Chandran, 2011). Bioremediation has emerged as a promising alternative due to its eco-friendly approach and efficiency in degrading petroleum hydrocarbons (Xue *et al.*, 2015). Microbial degradation of hydrocarbons involves various bacterial species each capable of breaking down specific hydrocarbon molecules. Notable hydrocarbon-degrading bacteria include *Acinetobacter*, *Bacillus*, and *Pseudomonas*, which have been reported to utilize saturated hydrocarbons (n-alkanes) during petroleum oil degradation (Asghar *et al.*, 2016). Bioremediation has been widely recognized as an effective and environmentally sustainable approach for mitigating the adverse effects of hydrocarbon contamination in soil and water. It relies on the metabolic capabilities of microorganisms to utilize hydrocarbons as a carbon and energy source, ultimately converting pollutants into biomass and harmless byproducts (Das & Chandran, 2011). The 16S rRNA gene sequencing technique has been widely employed to study bacterial phylogeny and taxonomy, facilitating the identification of hydrocarbon-degrading bacterial strains (Xu *et al.*, 2018). This technique is advantageous due to its universal presence in bacteria, functional stability over evolutionary time, and sufficient sequence length for bioinformatics analysis (Leahy and Colwell, 1990). The use of microbial consortia, rather than individual strains, has been demonstrated to improve petroleum

hydrocarbon degradation due to their broader enzymatic capabilities and synergistic interactions (Wu *et al.*, 2017).

Despite significant progress, research on petroleum hydrocarbon remediation in water bodies remains limited, necessitating further studies to optimize bioremediation strategies for contaminated marine and freshwater environments (Varjani, 2017). Overall the effective bioremediation of petroleum hydrocarbons relies on the synergistic action of diverse microbial communities. Even though extensive research on hydrocarbon bioremediation, no reports are available on the isolation of indigenous bacteria from textile industrial effluents specifically for crude oil degradation.

Material and Methods

Collection of textile industry effluent

In the present study, samples of textile industrial effluents were collected from two distinct locations in Baddi, Solan district of Himachal Pradesh, India. The selected sampling site is located in the main textile industrial area of Baddi and effluent samples collected from wastewater discharge outlets. The following were the coordinates of sample locations: Site 1, Latitude 30.9348; Longitude 76.8038; Altitude 433m and Site 2, Latitude 30.9535; Longitude 76.8253; Altitude 476m. Effluent samples were collected by using sterile Duran bottles, transported to the laboratory, and stored at 4°C until further analysis. Crude oil used in the biodegradation study was procured from Indian Oil Corporation Ltd, Panipat Refinery & Petrochemical Complex, Panipat, Haryana.

The biodegradation experiments were carried out in Minimal Salt Medium (MSM; pH 7.0 ± 0.2) composed of Na₂HPO₄ (33.90 g/L), NaCl (2.50 g/L), KH₂PO₄ (15 g/L), NH₄Cl (5 g/L), and 0.1% solution of trace elements containing the following: ZnSO₄·7H₂O (0.525 g/L), MnSO₄·4H₂O (0.2 g/L), CuSO₄·5H₂O (0.7 g/L), Na₂MoO₄·7H₂O (0.015 g/L), CoCl₂·6H₂O (0.2 g/L), H₃BO₄ (0.015 g/L) and NiSO₄·6H₂O (0.027 g/L). Carbon sources were added separately. Both the nutrient medium and Minimal Salt Medium (MSM) were autoclaved at 120°C for 20 min before use.

Isolation of bacterial strains from effluent samples

To isolate bacterial colonies, 10 mL of textile effluent sample was shaken with 90 mL of autoclaved normal saline solution (NSS) for 30 min. The mixture was then diluted further (10–10,000 times) with autoclaved NSS and plated on nutrient agar (NA) plates. On the basis

of distinct colour and morphology, bacterial colonies were isolated, purified by further quadrant streaking in Nutrient Agar plates to get single colonies. Simultaneously, purified bacterial colonies were streaked on nutrient agar slants and kept at 4°C until further analysis.

Screening of potent crude oil degrading bacteria

Twelve bacterial colonies were selected based on distinct morphology. Single colony of each bacteria was inoculated in 20 mL of Nutrient broth (NB) and incubated at 30°C for 24 hrs (Fig. 2). 2 mL of each bacterial culture was inoculated in 50 mL of MSM containing heavy crude oil (1% w/v) as a sole carbon source. Erlenmeyer flasks were transferred to an orbital shaker at 30°C and 150 rpm for 7 days. Optical density (OD) was measured on 3rd day at 600nm. One potent bacterial strains were selected based on the effective crude oil biodegradation with highest OD. These three bacterial strains effectively utilized crude oil as the sole carbon and energy source, labeled as HDB27.

Identification and molecular characterization of bacterial isolates

Extraction of genomic DNA from bacterial strains

Single colony of each bacterial strain was inoculated and grown overnight in 100 mL of LB (Luria Bertani) medium and centrifuged at 7,000 rpm for 10 min at room temperature. The supernatant was decanted completely and pellets were frozen at -20°C for 2 h and thawed for 3 min at 37°C. The suspension in 10 mL lysis buffer (0.15 M NaCl, 0.05 M sodium citrate buffer) was incubated with 200 µl of lysozyme (10 mg/mL) at 37°C for 60 min. The cells were further lysed with 500 µl of 20% SDS by gentle shaking for 5 min. Thereafter 15 mL of extraction buffer was added [2.5 mL of 5 M sodium perchlorate and 12.5 mL of chloroform: isoamylalcohol mixture (24:1)]. Preparations were incubated at -20°C for 2 h and allowed to thaw at room temperature by gentle shaking for 30 min and subsequently centrifuged at 5,000 rpm for 10 min. Supernatant was precipitated with an equal volume of isopropanol, washed with 70% ethanol. DNA was air dried and dissolved in 1 mL TE buffer (Chandrashekara *et al.*, 2011).

PCR amplification of 16S rRNA

The 16S rRNA gene was amplified from the bacterial isolates with the Forward primer (5'-GAGTTTGATCCTGGCTCAG-3') and Reverse primer (5'-AGAAAGGAGGTGATCCAGCC-3'). Amplification was performed in a total volume of 25

μl containing 100 ng of genomic DNA, 1μl of forward and reverse primer (40p mol/μl), 200 mM of each dNTP (Fermentas), 1 units of Taq DNA polymerase and 1X Taq buffer (10mM Tris-HCl (pH 8.8), 50 mM KCl, and 0.08% Nonidet P40 and 2.5mM MgCl₂ : Fermentas). The contents was mixed thoroughly and centrifuged for 5 sec at 5000 rpm. The PCR reaction mixtures, after incubation at 95°C for 5 min as an initial denaturation, were cycled 30 times through the following temperature profile: denaturation for 30 sec at 94°C; annealing for 15 sec at 56°C; and extension for 1.5 min at 72°C with final extension for 5 min at 72°C, after which 5 μl of each PCR amplification mixture was mixed with 2 μl of Blue/Orange 6x loading dye and analysed by 1.2% agarose/EtBr gel electrophoresis.

Sequencing and BLAST analysis

The purified PCR products were sequenced with automated DNA sequencer with 16S gene specific primers at Barcode Biosciences, (Bangalore, Karnataka). Sequences were edited and aligned using BioEdit 7.0. The BLASTn program (<http://www.ncbi.nlm.nih.gov/blast/>) was used to identify the similarities between the sequences obtained and its homologues in public database.

Results

Isolation and screening of crude oil degrading bacteria

On the basis of distinct colour and morphology, around twenty eight bacterial colonies were isolated, purified by further quadrant streaking in Nutrient Agar plates. Simultaneously, purified bacterial colonies were streaked on nutrient agar slants and kept at 4°C until further analysis. Among twenty eight strains, HDB 27 strain was selected for further studies as it showed the potential crude oil degradation in periodic time viz., 0th day, 3rd day and 7rd day. Among three bacterial strains, HDB27 showed visible utilization and degradation of heavy crude oil when compared to other bacterial strains.

Identification of the bacterial isolates

HDB27 culture is opaque, smooth and white colonies, smooth margin, flat colonies, sticky, non-motile and no pigment production.

Bacterial DNA isolation & 16S rRNA gene sequence analysis

Genomic DNA extracted from the bacterial strain HDB-27 was noticed to be of good quality (Fig. 7). 16S rRNA sequencing technology was used for the molecular

characterization and identification of the selected bacterial isolate. Standardization of PCR conditions for 16S rRNA amplification by variation in the annealing temperature (50-60°C) using gradient PCR technique. Desirable amplification has been achieved at the annealing of 56°C. The band of a 1.5 Kb fragment corresponding to 16S rRNA region has been amplified under standardized PCR conditions. Amplification of the PCR products was confirmed in 1.2 % agarose gel using standard 1 Kb DNA ladder (Fig. 8) and excised from the gel, purified and samples were under sequencing process.

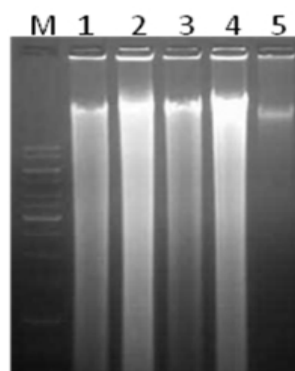


Figure. 7 Genomic DNA isolated from different bacteria isolated from textile industry effluents. Lane M = 1 Kb DNA ladder; lane 1-3: Genomic DNA from HDB-5, 11 & 14 respectively

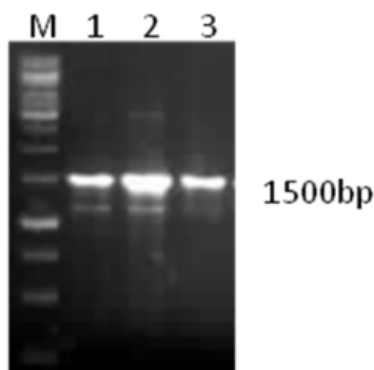


Figure. 4 PCR amplification of 16S rRNA gene

Lane M = 1 Kb DNA ladder; lane 1-3: 16S rRNA gene (1.5 Kb) amplification of HDB-5, 11 & 14 respectively

Consensus sequence of 16S rRNA

GCTCAGATTGAACGCTGGCGGCAGGCTTAACACATGCAAGTCGAGCGGAGCGAGGGTGCTTG
CACCTTAG

CTTAGCGGCGGACGGGTGAGTAATGCTTAGGAATCTGCCTATTAGTGGGGGACAACATTCCG
AAAGGAAT
GCTAATACCGCATACGCCCTACGGGGGAAAGCAGGGGATCTTCGGACCTTGCGCTAATAGAT
GAGCCTAA
GTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCTGTAGCGGGTCTGAG
AGGATGAT
CCGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
GACAATGG
GCGGAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCTTTTGGTTGTAAAGCACTTT
AAGCGAGG
AGGAGGCTACTTAGATTAATACTCTAGGATAGTGGACGTTACTCGCAGAATAAGCACCGGCT
AACTCTGT
GCCAGCAGCCGCGGTAATACAGAGGGTGCGAGCGTTAATCGGATTTACTGGGCGTAAAGCGT
GCGTAGGC
GGCTTTTTAAGTCGGATGTGAAATCCCCGAGCTTAACTTGGGAATTGCATTGCATACTGGGA
AGCTAGAG
TATGGGAGAGGATGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATA
CCGATGGC
GAAGGCAGCCATCTGGCCTAATACTGACGCTGAGGTACGAAAGCATGGGGAGCAAACAGGAT
TAGATACC
CTGGTAGTCCATGCCGTAAACGATGTCTACTAGCCGTTGGGGTCTTTGAGGCTTTAGTGGCG
CAGCTAAC
GCGATAAGTAGACCGCCTGGGGAGTACGGTCGCAAGACTAAACTCAAATGAATTGACGGGG
GCCCCGAC
AAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATA
CAGAGAAC
TTTCCAGAGATGGATTGGTGCCTTCGGGAACCTGTGATACAGGTGCTGCATGGCTGTCGTCAG
CTCGTGTC
GTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCTTTTCCTTATTTGCCAGCACTTCG
GGTGGGAA
CTTTAAGGATACTGCCAGTGACAACTGGAGGAAGGCGGGGACGACGTCAAGTCATCATGGC
CCTTACGA
CCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCTACCTCGCGAGAGGATGCT
AATCTCAA

AAAGCCGATCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGT
AATCGCGG
ATCAGAATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGA
GTTTGTTG
CACCAGAAGTAGCTAGCCTAACTGCAAAGAGGGCGGTTACCACGGTGTGGCCGATGACTGGG
G

BLAST RESULTS

Based on the BLAST analysis and high percentage identity the potent crude oil degrading bacteria HDB-27 was identified as *Acinetobacter tandoii*.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Acinetobacter tandoii strain INA2	2697	2697	100%	0	99.93%	MH917916.1
Acinetobacter sp. PS-1	2697	2697	100%	0	99.93%	MW114828.1
Acinetobacter sp. SC3T	2553	2553	94%	0	99.93%	KU353548.1
Acinetobacter sp. strain LE10	2687	2687	99%	0	99.86%	MT279472.1
Acinetobacter sp. 409	2645	2645	98%	0	99.86%	EU841482.1
Acinetobacter sp. SC36	2560	2560	95%	0	99.86%	KU353550.1
Acinetobacter sp. enrichment culture clone Y11-5	2545	2545	94%	0	99.78%	KR110100.1
Acinetobacter sp. YE10	2542	2542	94%	0	99.78%	KU353561.1
Acinetobacter sp. RB21	2551	2551	95%	0	99.71%	KF688133.1
Acinetobacter junii strain WAB1940	2675	2675	100%	0	99.66%	AM184279.1

Discussion

The textile industry is a significant contributor to environmental pollution, primarily due to the discharge of untreated effluents containing various pollutants, including hydrocarbons. These hydrocarbons, often derived from oils and greases used in fabric processing, pose substantial environmental and health risks. Addressing this issue necessitates the identification and utilization of hydrocarbon-degrading bacteria present in textile effluents. Molecular techniques, particularly those involving 16S rRNA gene sequencing,

have become indispensable tools in identifying and characterizing these microorganisms (Abid *et al.*, 2025).

Hydrocarbon-degrading bacteria, also known as hydrocarbonoclastic bacteria, have evolved mechanisms to utilize hydrocarbons as carbon and energy sources. These bacteria produce biosurfactants that enhance the emulsification and subsequent degradation of hydrocarbons. Genera such as *Pseudomonas*, *Acinetobacter*, *Rhodococcus*, and *Alcanivorax* are well-documented for their hydrocarbon-degrading capabilities. For instance, *Alcanivorax* species are known to dominate in oil-contaminated marine environments due to their efficiency in degrading alkanes (Xu *et al.*, 2018).

Varjani *et al.* (2015) constructed a halotolerant Hydrocarbon Utilizing Bacterial Consortium (HUBC) consisting of the bacterial isolates *Ochrobactrum* sp., *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* that was found to be good at degrading crude oil (3% v/v), with a degradation percentage as high as 83.49%. Tao *et al.* (2017) utilized a defined co-culture of an indigenous bacterial consortium and exogenous *Bacillus subtilis* to effectively accelerate the degradation of crude oil. Wang C. *et al.* (2018) found that an aboriginal bacterial consortium based on the Penglai 19-3 oil spill accident (China) had higher oil degradation efficiency compared to individual bacteria and demonstrated that this indigenous consortium had the potential for bioremediating crude oil dispersed in the marine ecosystem. A field study showed that bioaugmentation with an artificial consortium containing *Aeromonas hydrophila*, *Alcaligenes xylosoxidans*, *Gordonia* sp., *Pseudomonas fluorescens*, *Pseudomonas putida*, *Rhodococcusequi*, *S. maltophilia*, and *Xanthomonas* sp. contributed to high biodegradation efficiency (89%) in a 365-day treatment of diesel oil-contaminated soil (Szulc *et al.*, 2014). Taken together, these studies indicate that improving the biodegradation potential via the application of bacterial consortia possessing multiple catabolic genes is a reasonable and feasible strategy for accelerating the removal efficiency of petroleum hydrocarbons from contaminated environments.

Accurate identification of hydrocarbon-degrading bacteria is crucial for effective bioremediation strategies. Molecular techniques, particularly 16S rRNA gene sequencing, have revolutionized microbial identification. The 16S rRNA gene is highly conserved among bacteria, yet it contains hypervariable regions that provide species-specific signature sequences (Subathra *et al.*, 2013). By amplifying and sequencing this gene, researchers can

compare the obtained sequences with databases to identify and classify bacteria. This method offers higher resolution and accuracy compared to traditional phenotypic identification methods.

Conclusion

Crude oil is the major raw material for many industries viz., pharmaceuticals, solvents, fertilizers, pesticides, synthetic fragrances, and plastics. Cleaning up of petroleum hydrocarbons in the subsurface environment is a real-world problem by noticing the negative impact of hydrocarbon. A better understanding of the mechanism of biodegradation has a high ecological significance that depends on the identification of indigenous novel microorganisms with hydrocarbon clastic activity. This is possible because microorganisms have enzyme systems to degrade and utilize different hydrocarbons as a source of carbon and energy. In this study, single bacteria showed effective crude oil degradation when compared to remaining bacteria. Finally, the potent crude oil degrading bacteria HDB-27 was identified as *Acinetobacter tandoii*. This study initiated in view of toxic hydrocarbon degradation using environment friendly microorganism to maintain ecological and human health.

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Correction of Sickle Cell Disease Mutation in Hematopoietic Stem Cells Using CRISPR-Cas9

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Abstract: Sickle cell disease (SCD) is a severe hereditary blood disorder caused by a single point mutation in the **β -globin gene (HBB)**, leading to the production of abnormal haemoglobin S (HbS). This results in red blood cell sickling, vaso-occlusive crises, and chronic complications. Traditional treatments, such as hydroxyurea and blood transfusions, provide symptomatic relief but do not offer a permanent cure. Sickle cell disease (SCD) is the most common monogenic blood disorder marked by severe pain, end-organ damage, and early mortality. Treatment options for SCD remain very limited. CRISPR-Cas9 gene-editing technology has emerged as a revolutionary approach to correct the genetic defect in **hematopoietic stem and progenitor cells (HSPCs)**. By precisely targeting the HBB gene mutation or modifying regulatory elements to induce fetal hemoglobin (HbF) production, CRISPR-based therapies can restore normal hemoglobin function. Preclinical and clinical studies, including the **exa-cel (CTX001) therapy**, have demonstrated promising results, showing significant reductions in vaso-occlusive crises and long-term therapeutic benefits. Despite its potential, challenges such as **off-target effects, immune responses, and high treatment costs** remain barriers to widespread clinical application. Ongoing research focuses on improving gene-editing efficiency, minimizing risks, and making therapies more accessible. This review discusses the mechanism, clinical advancements, challenges, and future prospects of CRISPR-Cas9 gene editing in treating sickle cell disease through hematopoietic stem cell modification.

Keywords: CRISPR-Cas9, sickle cell disease, hematopoietic stem cells, gene therapy, foetal haemoglobin, genome editing.

Introduction

Stem cells possess unique capabilities for self-renewal and differentiation, making them ideal candidates for regenerative medicine. However, their full potential can be harnessed only when precise genetic modifications can be introduced to correct mutations or enhance desirable traits. Traditional gene modification techniques were inefficient and time-

consuming, but recent advances in genome editing technologies, such as CRISPR-Cas9 and TALENs, have significantly improved precision and efficiency. These technologies allow targeted genetic modifications, paving the way for personalized medicine and novel therapeutic approaches.

Sickle Cell Disease (SCD) is a group of inherited blood disorders characterized by the presence of abnormally shaped red blood cells (RBCs) that resemble a crescent or sickle. These sickle-shaped cells have a reduced ability to carry oxygen and tend to clump together, leading to blockages in blood vessels, severe pain, and organ damage. It is caused by a single-point mutation in the β -globin gene (HBB) that leads to the production of abnormal haemoglobin (HbS). It is caused by a mutation in the HBB gene on chromosome 11, which encodes the β -globin subunit of haemoglobin. It is a single nucleotide substitution mutation (GAG $\rightarrow$ GTG) at codon 6 of the HBB gene, leading to the production of abnormal haemoglobin S (HbS) instead of normal haemoglobin A (HbA). The disease process is driven by the polymerization of deoxygenated HbS, leading to sickle-shaped RBCs. Under low oxygen conditions, HbS polymerizes, forming rigid structures inside RBCs and causes cells to take a sickle shape.

Sickled RBCs have a shortened lifespan (10-20 days vs. 120 days for normal RBCs) and leads to chronic haemolytic anaemia. They also stick to blood vessel walls, leading to obstruction of blood flow, causes ischemia, pain crises, and organ damage. Repeated vaso-occlusion triggers inflammation and oxidative stress. Endothelial cells become activated, increasing thrombosis risk.

Symptoms of Sickle Cell Disease

- **Vaso-Occlusive Crisis (Pain Crisis):** Severe pain in bones, chest, abdomen, and joints due to blocked blood flow.
- **Acute Chest Syndrome (ACS):** Life-threatening complication with symptoms of chest pain, fever, and lung infiltration (similar to pneumonia).
- **Splenic Sequestration Crisis:** Rapid enlargement of the spleen due to trapped sickled cells, leading to severe anaemia and shock.
- **Stroke:** Occlusion of cerebral blood vessels can cause ischemic stroke.
- **Chronic Anaemia:** Fatigue, jaundice, and delayed growth in children.
- **Organ Damage:**
 - **Kidney (Sickle Cell Nephropathy):** Proteinuria, kidney failure.
 - **Heart (Cardiomyopathy):** Increased risk of heart failure.
 - **Liver (Hepatopathy):** Iron overload, gallstones.

- **Infections:** Increased susceptibility due to **functional asplenia** (damaged spleen).
- **Leg Ulcers:** Poor circulation causes non-healing wounds.

Diagnosis of Sickle Cell Disease

- Newborn Screening identifies SCD at birth using haemoglobin electrophoresis or HPLC.
- Hemoglobin Electrophoresis differentiates HbS from other haemoglobin variants.
- High-Performance Liquid Chromatography (HPLC) provides a detailed haemoglobin profile.
- Genetic Testing detects mutations in the HBB gene.
- Peripheral Blood Smear shows sickle-shaped RBCs.

Treatment & Management

Currently, SCD has no universal cure, but treatment focuses on symptom management and prevention of complications. There are Disease-Modifying Therapies like **L-Glutamine (Endari)** that reduces oxidative stress and sickling events, **Voxelotor** which increases haemoglobin's oxygen affinity, reducing sickling and through Monoclonal antibodies preventing vaso-occlusion. Curative Therapies Bone Marrow Transplant (BMT) is important. At present **Gene Therapy Approaches like CRISPR-Cas9** are used that corrects **HBB** mutation or reactivates foetal haemoglobin (HbF). **Exa-cel (exagamglogene autotemcel)** is a CRISPR-based therapy in clinical trials.

Literature Review

Recent research has demonstrated the effectiveness of CRISPR and TALENs in modifying stem cell genomes. CRISPR-Cas9, derived from bacterial immune systems, enables targeted DNA cleavage, allowing for gene knockout, insertion, or correction. TALENs, which rely on customized DNA-binding proteins fused to nucleases, offer similar editing capabilities with high specificity. Studies have successfully applied these technologies to correct mutations in induced pluripotent stem cells (iPSCs) for diseases such as sickle cell anaemia and cystic fibrosis. However, concerns regarding off-target effects and genomic stability remain key areas of ongoing research.

Sickle cell disease (SCD) is a monogenic disorder caused by a single nucleotide mutation (GAG to GTG) in the beta-globin gene (HBB), leading to the production of abnormal hemoglobin S (HbS). This mutation results in erythrocyte deformation, vaso-occlusion, and a cascade of pathophysiological complications. The advent of genome editing technologies, particularly CRISPR-Cas9, has revolutionized the potential for precise

correction of this mutation in hematopoietic stem and progenitor cells (HSPCs), offering a promising curative approach.

Early studies demonstrated the feasibility of CRISPR-Cas9 for SCD correction in patient-derived induced pluripotent stem cells (iPSCs) and HSPCs. Efficient in vivo and ex vivo delivery of CRISPR components remains a critical challenge. Viral vectors, electroporation, and lipid nanoparticles are among the explored delivery methods. Despite high specificity, CRISPR-Cas9 can introduce unintended genetic modifications, necessitating thorough screening and optimization. The bacterial origin of Cas9 can elicit immune responses, potentially reducing therapeutic efficacy.

Methodology

Mechanism of CRISPR-Cas9 in Gene Editing

CRISPR-Cas9, derived from the adaptive immune system of bacteria, employs a guide RNA (gRNA) to direct the Cas9 nuclease to a specific genomic locus, enabling site-specific double-strand breaks (DSBs). The cellular repair mechanisms, either homology-directed repair (HDR) or non-homologous end joining (NHEJ), determine the final genetic outcome. HDR enables precise correction of the sickle cell mutation by providing a donor DNA template.

CRISPR-Cas9 is a gene-editing system derived from bacteria that allows scientists to cut and edit DNA with high precision. The system is composed of:

1. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats):

It is a natural bacterial immune system that recognizes and destroys viral DNA. It stores short viral DNA sequences (spacers) to recognize and fight future infections.

2. Cas9 (CRISPR-associated protein 9): It is an enzyme that acts like molecular scissors to cut DNA at specific sites. It is guided by a small RNA sequence to target the correct DNA location. The steps involved in the process are

Step 1: Isolation of Hematopoietic Stem Cells (HSCs)

- **Source:** CD34+ HSCs isolated from the bone marrow or peripheral blood of an SCD patient.
- **Purification:** Magnetic-activated cell sorting (MACS) or fluorescence-activated cell sorting (FACS) used to purify HSCs.
- A synthetic guide RNA (**gRNA**) is created to match the DNA sequence to be edited.
- The gRNA directs Cas9 to the precise location in the genome.

Step 2: CRISPR-Cas9 Gene Editing

- **Guide RNA (gRNA) Design:** A specific gRNA is designed to target the SCD-causing mutation in the HBB gene.
- **Cas9 Delivery:** The CRISPR-Cas9 system is introduced into HSCs using electroporation or viral vectors (AAV, lentivirus).
- **Homology-Directed Repair (HDR):** A donor DNA template with the correct HBB sequence is provided to enable precise repair of the mutation.
- The gRNA directs Cas9 to the precise location in the genome.
- The gRNA binds to the complementary DNA sequence.
- Cas9 scans the genome and identifies the target site using a PAM sequence.
- Cas9 makes a double-strand break (DSB) at the target site.
- Once the DNA is cut, the cell repairs it using one of two pathways:
 - a. Non-Homologous End Joining (NHEJ):** The cell re-joins the broken DNA without a template. Often introduces small insertions or deletions (indels), leading to gene knockouts.
 - b. Homology-Directed Repair (HDR):** A donor DNA template is provided to correct the mutation. It is used for precise gene corrections or insertions.

Step 3: Verification of Gene Editing

- **Sanger Sequencing / NGS:** Confirms correction of the mutation.
- **Off-Target Analysis:** Bioinformatics tools and whole-genome sequencing (WGS) are used to ensure no unintended edits.

Step 4: Functional Testing

- **Haemoglobin Production:** Edited HSCs are differentiated into erythroid cells in vitro, and haemoglobin levels are analysed using HPLC or ELISA.
- **Engraftment in Mice:** Gene-edited HSCs are transplanted into immunodeficient mice to test their ability to engraft and produce normal red blood cells.
- The outcome is corrected HSCs produce normal haemoglobin (**HbA**) instead of sickle haemoglobin (**HbS**).
- Reduced sickling of red blood cells.
- Successful engraftment and long-term expression of the corrected gene.

This approach has been used in clinical trials, such as the **exagamglogeneautotemcel (exa-cel)** therapy, where CRISPR-Cas9 editing in HSCs has led to successful treatment of SCD patients.

Results

Studies show that CRISPR-Cas9 achieves higher efficiency in gene editing compared to TALENs, with faster editing cycles and simpler design requirements. However, TALENs exhibit superior specificity with fewer off-target effects. Experimental findings indicate successful gene correction in iPSCs and mesenchymal stem cells, demonstrating potential for treating monogenic disorders. Despite the high success rates, challenges such as incomplete editing, mosaicism, and immune responses to Cas9 proteins were noted.

CRISPR-Cas9 has shown promising results in treating **sickle cell anemia (SCA)** by correcting the genetic mutation responsible for the disease.

Exa-cel (ExagamglogeneAutotemcel, formerly CTX001): A CRISPR-based therapy developed by CRISPR Therapeutics and Vertex Pharmaceuticals has successfully treated patients with sickle cell anaemia.

In clinical trials, patients who received this treatment showed elimination or significant reduction in vaso-occlusive crises (pain episodes) associated with the disease. CRISPR-Cas9 edits the patient's hematopoietic stem cells (HSCs) to increase foetalhaemoglobin (HbF) levels, compensating for the faulty adult haemoglobin (HbS). This prevents red blood cells from sickling and improves oxygen transport. Many patients remain symptom-free after treatment, with sustained high levels of foetalhaemoglobin. This therapy offers a potential one-time cure compared to lifelong treatments like blood transfusions and hydroxyurea.

It is an expensive treatment and the costs remain high, limiting accessibility. Though rare, unintended genetic changes could have unknown consequences. Some patients may experience immune reactions to the edited cells.

Discussion:

The application of CRISPR-Cas9 in treating sickle cell anemia (**SCA**) represents a ground-breaking advancement in gene therapy. The results from clinical trials, particularly those involving **Exa-cel (CTX001)**, demonstrate the potential of this technology to provide a long-term or even permanent cure. However, the therapy also presents certain challenges and ethical considerations.

The success of CRISPR-based treatments in eliminating or significantly reducing vaso-occlusive crises (VOCs) is highly promising. Patients who previously suffered from frequent, severe pain episodes due to sickled red blood cells blocking blood flow have experienced significant relief. Additionally, the treatment's ability to increase fetalhemoglobin (HbF) production has helped restore normal red blood cell function. Patients treated with CRISPR-Cas9 have shown long-term benefits, with some remaining symptom-free for years after treatment. Unlike traditional therapies, such as frequent blood transfusions or hydroxyurea, CRISPR therapy offers a one-time intervention with lasting effects.

While the results are promising, CRISPR-based therapy is not without risks. Though rare, unintended genetic modifications could lead to unforeseen health issues, such as increased cancer risk or immune responses. There is a possibility that the body might reject the edited stem cells, leading to complications. The long-term consequences of gene editing are still uncertain, raising ethical questions about germline editing and accessibility.

One of the biggest challenges in implementing CRISPR therapy for sickle cell anemia is its high cost. Current estimates suggest that Exa-cel could cost several million dollars per patient, making it inaccessible for many, particularly in low-income and high-prevalence regions like sub-Saharan Africa. Efforts are needed to reduce costs and improve accessibility through government support and insurance coverage.

Despite these challenges, CRISPR-Cas9 remains a game-changer in genetic medicine.

Conclusion

Gene editing technologies such as CRISPR-Cas9 have significantly advanced the field of stem cell research, offering promising avenues for regenerative medicine. Though this technology has its advantages and limitations, ongoing research aims to refine its precision and applicability. Ethical considerations and regulatory frameworks must evolve alongside technological advancements to ensure safe and responsible use of gene-edited stem cells in clinical settings. Future directions include integrating artificial intelligence for guide RNA design, optimizing delivery methods, and exploring epigenetic editing for greater control over stem cell behaviour.

The results of CRISPR-Cas9 in treating sickle cell anaemia demonstrate a major step toward a potential cure. While current clinical trials show great success in reducing symptoms and improving patient outcomes, cost and long-term safety remain key barriers. Addressing these challenges will be crucial in making gene editing a widely available solution for sickle cell anaemia and other genetic disorders.

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Molecular docking studies of phytochemical from *Ficus amplissima* as cure for Diabetes Mellitus

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Abstract: The Indian system of medicine relies greatly on medicinal plants to treat several disorders. Before the introduction of chemical medicines, man relied on the healing properties of medicinal plants. *Ficus amplissima* which is found in many parts of southern and Western Ghats is used for different ailments by the tribal people in many areas. The knowledge on phytochemical analysis in various parts of the plant *Ficus amplissima* helps us to know about the medicine and toxicology effects present in them. Therefore, to explore the potential use of this plant, the present study was aimed to use computational approaches and rationale for identifying and designing pharmacologically active natural molecules that can target proteins of interest. The natural compounds obtained from plants can be repurposed by computational approaches to prove their anti cancer potential. The compounds were selected for the in silico analysis based on the literature review and biological activity of *Ficus amplissima*. Acarbose was used as standard control in the docking experiment. Analysis of docking resulted that the compound Lupeol was bound to amino acids such as ASP with binding energy of -8.6. The compound used as control Acarbose was bounded to amino acids with binding energy of -8.2. The resulted compound showed higher efficacy than controlled compound, this indicates the plants potential to be an effective drug compound. Lupeol have the best binding affinity and follow all the parameters of toxicity. Lupeol has a lot of potential for the treatment of diabetes, and this hypothesis can be further validated by in vitro and in vivo experiment.

Keywords : Phytochemicals, medicine, Computational approaches, biological activity, Docking, ASP, Lupeol, Diabetes.

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural sources, many of these isolations were based on the uses of the agents in traditional medicine (Omonkhelin et al., 2007). The World Health Organization (WHO) defines traditional medicine as the “diverse health practices, approaches, knowledge and beliefs incorporating plant, animal-and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose, or prevent illness”. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and disturbances of carbohydrate, protein and fat metabolisms, secondary to an absolute or relative lack of insulin (Fatima et al., 2010).

Antioxidants play a major role in protection against molecular oxidative damage (Evans, 2007). In integration to oxidative stress, insulin action is also impaired in diabetes, which leads to increased hepatic glucose production. Due to the side effects of the existing synthetic drugs, plant derived food drugs are in great demand in industrially developed countries as alternative approach to treat diabetes. The pharmacological agents currently used for the treatment of type II diabetes include sulfonylureas, biguanide, thiazolidinedione and α -glucosidase inhibitor. These agents, however, have restricted use due to several undesirable side effects including haematological effects, coma and disturbances of the liver and kidney. In addition, they are not suitable for use during pregnancy (Pari and Uma, 1999).

The genus *Ficus* (Moraceae) constitutes one of the largest genera of angiosperms includes with more than 800 species and 2000 varieties of *Ficus* genus, occur in the most tropical and subtropical forests and this plant is commonly known as fig (Hamed, 2011). *Ficus amplissima* Smith. commonly known as kal-itchchi, is endemic to India and commonly occurs in foothills of Himalayas, Assam, Sikkim, Kerala, Tamil Nadu, Andhra Pradesh and Maharashtra. The ethnobotanical views on *F. amplissima* suggest that fruits are chewed for mouth ulcers whereas leaf juice is applied externally for old chronic wounds. Bark is used against diabetes, which is reported in indigenous herbal formulations in India. In addition to that, *F. amplissima* bark has been used for the ailments of throat—haws up cheesy laumps (Pankaj Oudhia, 2012).

Moreover, bark of this plant is boiled with salt and boiled rice water in equal proportion and blue warm solution is given in colic (Pulliah, 2002) and also leaves were used for jaundice, fruit is used to cure mental deranged and the latex is applied on the wound (Singh and Himadri, 2005). Even though many *Ficus* spp. have been studied extensively regarding their ethnopharmacological relevances, but there are no reports on the phytochemical and pharmacological evaluation of *F. amplissima*.

Using *In silico* approaches now can simultaneously screen out millions of phytocompounds and drugs against any diseases. Furthermore, molecular dynamics-based screening helps to hypothesize the efficacy, stability and toxicity of the important drug candidates or phytocompounds and to design experiments for their *in vivo* testing. Therefore, the present study was undertaken to propose potential phytoconstituents of *F. amplissima* using *in silico* approaches for developing novel therapeutics for the management of diabetes mellitus with the following objectives

- ☐ To Identify the active compounds from *F. amplissima* by literature survey.
- ☐ To predicts biological activity, including pharmacological effects using PASSOnline Tool.
- ☐ To analysis ADMET (absorption, distribution, metabolism, excretion, andtoxicity) properties of natural compounds.
- ☐ To evaluate *F. amplissima* phyto compounds as potential natural compounds totarget protein by using molecular docking computational approaches.

Plant Description (*Ficus amplissima* J. E. Smith).

Systematic position

(Bentham and Hooker's system of classification, 1862-1883)

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Super division	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Hamamelididae
Order	:	Urticales
Family	:	Moraceae
Genus	:	Ficus L.
Species	:	Ficus amplissima Smith



Vernacular names

Tamil	:	Kal - ichchi
Malayalam	:	Chela; Koyil

Characters

- Habitat : Deciduous and evergreen forests.
- Habit : 18 m tall tree.
- Root : Tap root system.
- Stem : Woody, Bark greenish-grey; branchlets, glabrescent or puberulous with annular scars, latex white. Young shoots are whitish puberulous to vinous.
- Leaves : Alternate, petiolate, ovate to ovate-oblong, globrous, entire acute..
- Inflorescence : Monoecious, sessile, old leaf scars, globular or obovoid, glabrous, broadly ovate, 2-25 mm long, glabrous to puberulous basal bracts, internal bristles absent.
- Male Flowers : few flowers, disperse, pedicellate; sepals 2-3; stamen single.

- Female Flower: Sessile; sepsis 3; ovary white, reniform, style long, subterminal.
- Fruit : Figs depressed-globose to pyriform, pink to purple.
- Flowering and Fruiting: Flowering – Dec., to February, fruiting season March to May.
- Distribution : Occasionally found in Central and South India, Sri Lanka and Maldiv Islands (Corner, 1960; Santapau and Janardhana, 1966; Pulliah, 2002; Chaudhary et al., 2012).

Materials and Methods

Selection of plant and identification of compound

The *Ficus amplissima* were selected by literature survey with high medicinal value. The compound were selected based in literature review and biological activity, Acarbose was used as standard control in the docking experiment.

Selection and preparation of Ligands

The phytochemical structure of *Ficus amplissima* has been retrived as SDF file format from PUBCHEM database. SDF format was converted to PDB format using OPENBABEL. OPENBABEL software was used for file conversion (Li and Shah, 2017).

Identification of active sites of target proteins

The target for the inflammation inhibitory effect has been identified. The target provides the site for ligand binding. The 3D structure of the Tyrosine kinase inhibitors target (PDB ID 1IR3) has been obtained from protein data bank (PDB) (Santa and Prakasam, 2017).

Protein data bank

The Protein Data Bank (PDB) is crystallographic database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The PDB is a key resource in the area of structural biology, such as structural genomics. The 3D structure was retrieved to PDB format (Dikshit, 2012).

PUBCHEM

It consists of three dynamically growing primary database. This data base includes a chemical structure, name fragments, chemical formula, molecular weight, XLogP, and hydrogen bond donor and acceptor count.

ADME Toxicity

The ADME/Tox for the compounds were predicted using FAF-DRUGS 2 program from MobyLe portal (López, et al., 2011).

Pass Online

The biological activity of the compounds was predicted using PASS ONLINE, a webserver that predicts pharmacological and biochemical properties on basis of structural formula or 15 structure-specific SMILES. This provides the results in the form of appropriate probability values, i.e., probable activity and probable inactivity.

Molecular docking and studies

MGL tools with AutoGrid and AutoDock vina were used to set up and perform blind docking calculations between the Ligands and Protein. Crystallized 3-dimensional structure was obtained from the Protein Data Bank (PDB). Receptor (protein) and ligand (complex) files were prepared using PyRx (0.8) Tools. The output from AutoDock was rendered with PyMol (Meenambiga et al., 2018).

PyMOL

The PyMOL can produce a high quality of 3D images with a small molecules and biological macromolecules, such as proteins, PyMOL is one of a few open source visualization tools which is used in a structural biology (DeLano, 2002).

Results and Discussion

In silico docking studies on identified compounds from *Ficus amplissima*

Selection and preparation of ligands

Acarbose was used as standard control in the docking experiment. Acarbose is a complex oligosaccharide that acts as an inhibitor of several enzymes responsible for the breakdown of complex carbohydrates in the intestines. It inhibits both pancreatic alpha-amylase and membrane-bound alpha-glucosidases. By inhibiting the activity of these enzymes, acarbose limits the absorption of dietary carbohydrates and the subsequent postprandial increase in blood

glucose and insulin levels. The 2D structures of selected phytochemicals with their molecular weight, molecular formula and pubchem CID were downloaded from PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) and were presented in Table 1.

Table 1: Bioactive compounds of *Ficus amplissima*

NAME OF THE COMPOUND	PUBCHEM CID	MOLECULAR FORMULA	MOLECULAR WEIGHT
Lupeol	259846	C ₃₀ H ₅₀ O	426.7g/mol

Protein Data Bank (PDB)

The target protein, which insulin receptor tyrosine kinase in complex, three-dimensional (3D) structure from (www.rcsb.pdb). The PDB ID of is 1IR3. Tyrosine kinase inhibitors (TKIs) exert antihyperglycemic effects that can reverse or prevent type 1 and 2 diabetes mellitus by correcting insulin resistance and β cell dysfunction. The signaling pathways are precisely regulated, and perturbations in them may lead to insulin resistance.

ADMET Properties of selective compounds

The drug absorption, distribution, metabolism, excretion and toxicity (ADMET) analysis of selected compounds were predicted by using Swiss ADMET. The in-silico prediction results of absorption, distribution, metabolism, and excretion (ADME) of the compounds studied are presented. The hydrogen bonding potential and bioavailability of molecules are closely correlated to the TPSA value. Thus, the TPSA value of the studied compounds was noticed in the range of 20–37° A and is well below the limit of 160° A.

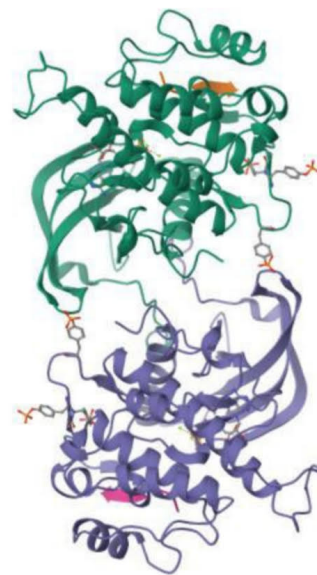


TABLE 2. ADMET properties of the ligands

Name of the ligand	Molecular mass less than 500 Dalton	High lipophilicity (expressed as LogP less than 5)	Less than 5 hydrogen bond donors	Less than 10 hydrogen bond acceptors	Molar refractivity should be between 40-130
Lupeol	338.00	4.72	1	1	13.14

TABLE 3. Drug-likeness prediction of compounds computed by Swiss ADME

Name of the ligand	NHD	NHA	NRB	TPSA	Lipinski' rule of five violation
Lupeol	1	1	1	20.23	1

Table 4: Pharmacokinetics predictions of compounds computes by Swiss ADME

Name of the ligand	GI Absorption	BBB Permeability	Inhibitor Interaction					
			P-gp Substrate	CYP1 A2	CYP2C 19	CYP2 C9	CYP2 D6	CYP3 A4
Lupeol	No	No	No	No	No	No	No	No

PASS ONLINE

The biological activity of the plant molecules were analyzed using the pass online tool which predicts the pharmacological and biochemical properties. Lupeol has the Anti-inflammatory activity.

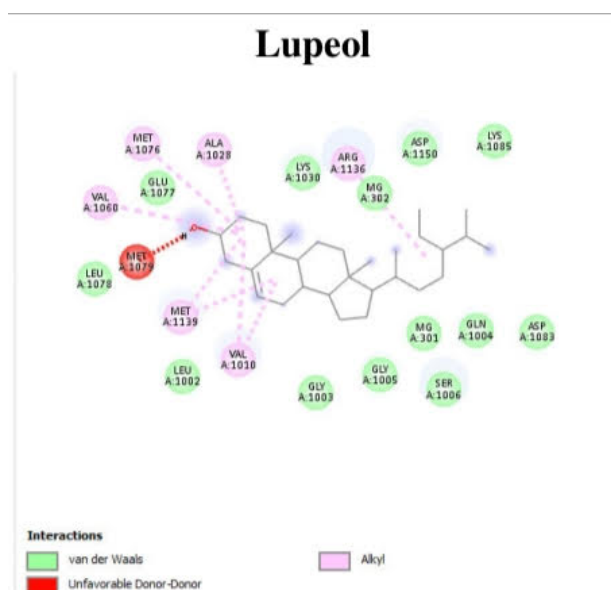
Molecular docking Analysis

The compound Lupeol was bound to amino acids such as ASP with binding energy of -8.6.

Table 5: Interaction of compounds with Insulin receptor tyrosine kinase in complex

Name of the ligand	Binding Affinity	rmsd/ub	rmsd/lb
Lupeol	-8.6	7.179	2.318

Interaction of compounds with Insulin receptor tyrosine kinase in complex protein



Insulin receptor is a tyrosine protein kinase that undergoes autophosphorylation upon insulin binding. The activated receptor phosphorylates substrate proteins on multiple Tyrosidues to propagate insulin signal transduction and insulin action. Three important structural regions are identified in the intracellular part of the beta subunit. They are juxtamembrane (JM) region, the kinase region, and the carboxyl-terminal (CT) region. The JM region (Arg940-) contains one autophosphorylation site; the CT region (Leu1245- Ser1343) contains two autophosphorylation sites. The intracellular portion of the insulin receptor, contain functional groups that bind to the effector molecules. Insulin receptor substrates (IRS-1, -2, -3 and -4) are the targets for insulin receptor kinase. The JM region serves as a binding site for IRS-1. Phosphorylation of the juxtamembrane of the insulin receptor mediates insulin's

metabolic functions. The catalytic loop is formed by residues 1130-1137 and the activation loop comprises of residues 1150-1171. Extensive studies on activated and inactive IR tyrosine kinases indicate that in the activated state the activation loop is tris-phosphorylated and moves out of the active site. The phosphorylated tyrosines become hydrogen bonded to residues in the activation loop. Insulin receptor is a tyrosine protein kinase that undergoes autophosphorylation upon insulin binding. The activated receptor phosphorylates substrate proteins on multiple Tyr residues to propagate insulin signal transduction and insulin action. Three important structural regions are identified in the intracellular part of the beta subunit. They are juxtamembrane (JM) region, the kinase region, and the carboxyl-terminal (CT) region. The JM region (Arg940-Leu987) contains one autophosphorylation site; the CT region (Leu1245-Ser1343) contains two autophosphorylation sites. The intracellular portion of the insulin receptor, contain functional groups that bind to the effector molecules. Insulin receptor substrates (IRS-1, -2, -3 and -4) are the targets for insulin receptor kinase. The JM region serves as a binding site for IRS-1. Phosphorylation of the juxtamembrane of the insulin receptor mediates insulin's metabolic functions. The catalytic loop is formed by residues 1130-1137 and the activation loop comprises of residues 1150-1171. Extensive studies on activated and inactive IR tyrosine kinases indicate that in the activated state the activation loop is tris-phosphorylated and moves out of the active site. The phosphorylated tyrosines become hydrogen bonded to residues in the activation loop. Lupeol has a lot of potential for the treatment of diabetes, and this hypothesis can be further validated by in vitro and in vivo experiments.

Conclusion

Ficus amplissima were screened for anti diabetes by molecular docking with the Insulin receptor tyrosine kinase in complex protein protein. Among all of the selected phytoconstituents, Lupeol have the best binding affinity and follow all the parameters of toxicity. Lupeol has a lot of potential for the treatment of diabetes, and this hypothesis can be further validated by in vitro and in vivo experiments.

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A Review of Plant Genome Sequencing in Crop Production in *Oryza Sativa*, L.

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Abstract: A plant genome sequence, which details the complete genetic makeup of a plant. The field of plant genome sequencing has grown rapidly in the past 20 years, *Arabidopsis thaliana* genome was the first plant genome to be sequenced in 2000 by a team of scientists at the Institute for Genomic Research (TIGR) in Rockville, Maryland. It leading to increases in the quantity and quality of publicly available genomic resources. It is a critical tool in crop production, enabling researchers to identify genes responsible for important agronomic traits, allowing for targeted manipulation to develop improved crop varieties with enhanced yield, stress tolerance, and nutritional value, significantly advancing crop breeding strategies through marker-assisted selection and genetic engineering. With the ability to sequence the genomes of various crops, livestock, and microbes, researchers can gain valuable insights into the genetic makeup of these organisms, which can help improve their productivity, disease resistance, and nutritional value. This review comprehensively examines *Oryza sativa* in increasing the crop production and it was the best example of a plant genome sequence used in crop production, as it was the first crop plant to have its genome sequenced, making it a key model for studying and improving other cereal crops. Rice genome sequencing is constructed by using bacterial artificial chromosomes (BACs) constructing them by using Bio-informatics tool. Complete sequencing of the rice genome will provide valuable information on the effect of repetitive elements on genome organization and evolution in plants and make disease resistance and it gave high nutritional value.

Keywords

1st Crop plant, Genome sequenced, Bacterial artificial chromosomes (BACs), Quality and Quantity, Increased Crop productions, Agronomic traits, Organization and evolution, Stress tolerance, High nutritional value, Improve in productivity, Disease resistance.

Introduction

Rice (*Oryza sativa*) is one of the most important crops in the world and itself is the principal food of half of the world's population mainly in Asia, Africa, and South America. It contains all of the amino acids essential for humans except lysine. A larger and more affluent population means, on the one hand, demand for greater production and better-quality rice, and on the other hand, the availability of less land, water and labour to produce the crop (Nancy A Eckardt, 2018). In short, there will be great demands on biotechnology to improve rice production. More importantly, the rice genome sequence has become the most powerful tool in agriculture enhancing the ability of breeders to develop new cultivars with highly desirable traits such as high yield, resistance to biotic/abiotic stress, good eating quality, and cultivars that could adapt to an ever-changing cultivation environment brought about by global warming. It is expected that subsequent sequencing of a wide array of rice germplasm throughout the world will be the platform for propelling the next green revolution to increase productivity under more sustainable conditions (Baltazar Antonio, 2016).

Rice is a model species for the cereals and a good candidate for DNA sequencing. It has a genome size of 400–430 million base pairs (Mb), the smallest of the major cereals but three times that of *Arabidopsis thaliana*. The rice molecular map, which has over 6000 markers, has already been useful in helping to align physical chromosome maps. Over 40,000 expressed sequence tags (ESTs) have been reported and many are mapped (Benjamin Burr, 2000).

It is now clear that the next wave of progress in genetics will hinge on genome molecular physical mapping. Recent development of large-DNA fragment (>100 kb) manipulation and cloning technologies, such as pulsed-field gel electrophoresis (PFGE), and yeast artificial chromosome (YAC) and bacterial artificial chromosome (BAC) cloning, has provided the powerful tools needed to generate molecular physical maps for higher-organism genomes (HB Zhang, 1997).

A yeast artificial chromosome (YAC) library that has been fingerprinted and ordered with mapped markers currently covers 60% of the rice genome. Several bacterial artificial chromosome (BAC) libraries have also been described (Takuji Sasaki, 2000). In this review about “Plant Genome Sequence in crop production in *Oryza sativa*, L.”

Plant Genome Sequencing of *Oryza Sativa*, L.in Crop Production:

The Rice genome was first sequenced in 2005. Plant genome sequencing of rice is a laboratory process that determines the genetic makeup of rice. The rice genome (*Oryza sativa*; AA genome) is composed of 12 chromosomes ($2n = 24$) and has a total length of 430 Mb (mega base, a nucleotide length of 1000 000 base pairs) corresponding to about 1500 cM (centimorgan, a genetic unit of length measured by the crossing-over frequency in genetic recombination's at meiosis) (Ken-Ichi Nonomura, 2002). Almost the whole genome of rice has been sequenced by the Syngenta group (Goff *et al.*, 2002) and the Chinese sequencing group. Analysis of those draft sequences for the number and functional categories of predicted genes indicates that rice has 40 000–50 000 functional genes with about 15 000 distinct gene families (YuJet al,2002). In Plant genome sequence contain 2 major process (i.e.) Extraction and Arrangement of sequence and Construction of(BCA) Bacterial Artificial Chromosome Vector.

The Extraction of plant genome sequence which involved following methodologies:

- (i) Sample collection
- (ii) DNA Extraction
- (iii)DNA Fragmentation
- (iv)Sequencing of DNA
- (v) Sequence Assembly
- (vi)Genome Annotation

(a) Sample collection:

To sequence the genome of a rice plant, collect samples of the plant's DNA. The samples can be Leaves (Young leaves can be collected from the plant and cut into small pieces), Seeds (Mature seeds can be used as a starting tissue for cell culture), Other plant tissue, such as zygotes or immature embryos, can be used for cell culture (Nancy A Eckardt, 2000).

(b) DNA Extraction:

DNA extraction from rice for genome sequencing involves breaking down the rice cells to release the DNA, and then purifying it. The process can be done using physical method which can separate DNA from other cellular components using solid-phase extraction,

Chemical method which use organic and inorganic chemicals to separate DNA from proteins and cell membranes. Some examples of chemical methods include the phenol-chloroform method, the alkaline extraction method, and the salting-out method, The enzymatic method for extracting DNA from rice involves grinding the rice, adding an enzyme, and then isolating the DNA. The extracted DNA can then be used as a template for PCR amplification (Palika Krittanai, 2009).

(c) DNA Fragmentation:

The DNA is broken into smaller manageable species, which can occur naturally due to various factors like repetitive DNA sequences, transposable elements, or environmental stresses, and can also be induced in laboratory settings for genomic analysis; this fragmentation can sometimes lead to gaps in the assembled rice genome when sequencing, impacting the accuracy of gene identification and analysis (Kaijun Yuan, 2023).

(d) Sequencing of DNA:

Before the sequencing it involved in Library preparation, the DNA fragments are then prepared into a sequencing library by adding adapters that allow them to be attached to the sequencing platform. In Sequencing platform, the sequencing library is loaded onto a next-generation sequencing machine where the nucleotide sequence of each fragment is read (M La Rota, 2003). The DNA is then sequenced using various methods, including Hi-C libraries (i.e.) Cross-linked DNA is extracted and digested with a restriction enzyme. The sticky ends of the digested fragments are biotinylated and ligated to form ligation junctions. The biotin-labelled DNA fragments are then sequenced (Yan Tong, 2020).

(e) Sequence Assembly:

Computational tools used for sequencing assembly in rice genomes include, NaS: A hybrid method that uses the Min-ION to sequence microbial genomes. Cerulean: A hybrid assembler that uses short and long reads to assemble microbial genomes. DBG2LOC: A tool that can be used for eukaryotic-sized genomes (Siddarth Singh, 2016). Polymerase chain reaction (PCR) amplification is a technique that can be used to amplify specific regions of the rice genome (J. Yashitola, 2004).

(f) Genome Annotation:

Rice genome annotation is the process of identifying and describing the genes, their structure, and the proteins they encode in the rice genome. The goal of rice genome annotation is to provide accurate and timely information about the rice genome for the scientific community. In that large fragments of rice genome sequence is identified and isolated for further process of developing vector (Baltazar Antonio, 2016).

Construction of (BCA) Bacterial Artificial Chromosome Vector:

Bacterial artificial chromosomes (BACs) are circular DNA molecules that can carry large fragments of foreign DNA. They contain element necessary for replication and maintenance in bacteria and used to construct large insert genomic libraries for sequencing the rice genome. (Hong-Bin Zhang, 2001).

The process of constructing BACs involves the following steps (Rafał Woycicki, 2008).

- (i) BAC Vector preparation
- (ii) Ligation
- (iii) Transformation
- (iv) Library construction
- (v) Screening and Selection
- (a) BAC Vector preparation:

Linearize the BAC vector by cutting it with a restriction enzyme that is compatible with the ends of the size- fractionated genomic DNA fragments.

(b) Ligation:

Mix the size- fractionated genomic DNA fragments with the linearized BAC vector. Add DNA Ligase, an enzyme that joins the genomic DNA fragments to the linearized BAC vector, creating recombinant BAC vectors.

(c) Transformation:

Introduce the recombinant BAC vectors into E.Coli cells using a technique called electroporation. Electroporation creates temporary pores in the bacterial cell membrane, allowing the DNA to enter.

(d) Library Construction:

Grow the transformed bacteria on a selective medium containing an antibiotic that the BAC vector confers resistance to, only bacteria containing the BAC vector (with the inserted genomic DNA) will survive and form colonies. Collect and store the bacterial colonies, which collectively represent a BAC library of the rice genome. Each colony contains a different fragment of the rice genome.

(e) Screening and Selection:

Screen the BAC library to identify clones containing specific genes or DNA sequences of interest and it can be done through PCR Amplification or hybridization with labelled probes. Select the desired BAC clones for further analysis or use in crop improvement applications.

Result and Discussion

The sequencing of the rice genome has yielded remarkable results that have significantly impacted crop production. The rice genome sequence has helped improve rice breeding and genetics research. It plays a significant role in developing abundant and nutrient-dense food crops. An alternative to traditional breeding approach, it has some major outcomes are (i) Comprehensive Genome sequence, (ii) Gene identification and characterization, (iii) MAS – Marker Assisted Selection, (iv) Gene cloning and transformation. The rice genome sequence has helped researchers understand how repetitive DNA elements affect genome organization and evolution. The rice genome sequence has helped researchers understand how genetic background and ecological conditions affect gene function. The sequencing of the rice genome has had a significant impact on rice breeding and genetics research. It has helped identify genes and molecular markers that can improve rice varieties. The rice genome sequence has helped researchers understand how to engineer rice varieties that can meet the demands of global agriculture.

Conclusion

The rice genome sequence has helped in Understand the evolution and domestication of rice, identify genes and molecular markers that improve rice varieties, provide information on genome structure and organization, provide information on the effect of repetitive elements on genome organization and evolution in plant, Accelerate the breeding process. The Rice Genome Project is a comprehensive initiative aimed at mapping the rice genome to enhance agricultural practices and improve rice quality and yield. Through international collaboration

and detailed genomic analysis, the project seeks to address global food security challenges. The future of plant genome sequencing in rice is likely to focus on high-throughput sequencing of diverse rice varieties to understand genetic variation.

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Exploring the Key Factors Affecting Premenopausal Women's Health in the Context of Hysterectomy

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Abstract:Background: Hysterectomy ranks as one of the most common surgical procedures performed during the reproductive years, following caesarean section in many countries. This study aimed to identify factors influencing premenopausal women's health before and after hysterectomy.**Objective:** This study explores the health impacts of premenopausal hysterectomy, its underlying causes, and the factors influencing women's quality of life before and after the procedure.**Methods:** This follow-up study recruited 20 women who underwent hysterectomy for benign conditions. Data were collected through questionnaires designed to gather information on participants' demographic profiles, health and dietary habits, and reasons for undergoing hysterectomy before menopause. The collected data were then analyzed.**Results and Discussions:** From the selected 20 women, the questionnaires were given and the data were collected. The data indicates that hysterectomies are often performed to alleviate pain or treat serious uterine conditions like fibroids or persistent heavy bleeding. It also highlights that, post-hysterectomy individuals may experience changes in their diet, lifestyle, physiological and psychological habits.**Conclusion:** Hysterectomy is associated with an earlier onset of menopause. Based on the collected data, the next phase will involve a 3-month follow-up diet plan for accepted participants to support weight management.

Key Words: Hysterectomy, uterine fibroids, heavy bleeding, Lifestyle, menopause.

Introduction:

In recent years, the prevalence of uterine diseases such as fibroids, adenomyoma, and endometrial carcinoma has been increasing, with younger women being increasingly affected. Hysterectomy remains one of the most common surgical interventions for these conditions,

particularly among women aged 40–45 years, with approximately 37–39% undergoing the procedure by age 65. A 2018 study in India reported a hysterectomy prevalence of 17 per 1000 ever-married women, varying across states from 2 to 63 per 1000 (Desai *et al.*, 2023). It is also the most frequently performed major surgery among women, primarily for nonmalignant conditions such as menstrual pain, menorrhagia, unexplained uterine bleeding, and chronic pelvic pain. Moreover, factors like age, marital age, literacy, and socioeconomic status significantly influence the likelihood of undergoing this procedure. However, despite its widespread practice, post-hysterectomy conditions often lack adequate attention regarding their physiological and psychological impacts, which can affect overall well-being and quality of life (Hongmei Zhang *et al.*, 2018).

Aims and Objective:

The study analyzes the socio-demographic profile, health impacts, and quality of life of post-hysterectomy women, including their physiological and psychological challenges, through data evaluation.

Methods:

This study recruited 20 women who had undergone hysterectomy before menopause. Data were collected using structured questionnaires to assess demographic details, health and dietary habits, and reasons for the procedure. Additionally, follow-up data on their post-surgery health and well-being will be analyzed using SPSS software and presented further.

Data Collection:

Data on socio-demographic profiles, reasons for the hysterectomy, physical activity levels, dietary habits, and stress experienced post-surgery were collected and analyzed.

Review of Literature

Epidemiology and Global Prevalence of Hysterectomy:

In 2011-2020 and 2015-16 Indian study, as reported by Sapna Desai *et al.* (2023), found that fibroids and heavy menstrual flow were the main reasons for hysterectomies in women 50 and older, with Andhra Pradesh and Telangana possibly accounting for up to 5 out of 10 cases. Angad

Singh et al. (2021) reported an overall hysterectomy rate of 3.2 in India, with Andhra Pradesh having the highest rate at 8.9, and a greater prevalence in rural areas. Further research by Sapna Desai et al. (2023) showed the highest rates in Andhra Pradesh (23.1) and Punjab (21.2), and the lowest in Northeast India.

Yelena Gorina et al. (2024), using 2021 [National Health Interview Survey] data, found that 14.6% of US women aged 18 and older reported having had a hysterectomy. The rate increased significantly with age, from 2.8% in women aged 18-44 to 41.8% in women 75 and older. They also found disparities based on race and ethnicity: 6.1% for Asian non-Hispanic women, 15.6% for White non-Hispanic women, 12.5% for Hispanic women, and 16.3% for Black non-Hispanic women.

Underlying Causes and Medical Indications for Undergoing A Hysterectomy:

Andrea Aguirre, MD et al.,2023 [Hysterectomy signs and causes] If antibiotics are not enough to treat severe pelvic infections that cause significant harm, a hysterectomy may be necessary. Surgery may also be necessary for conditions like hyperplasia, in which the lining of the uterus thickens abnormally, particularly if hormone therapy proves futile. In general, the severity of the ailment and the particular requirements of the patient determine whether a hysterectomy should be performed

Clinical Signs and Symptoms Leading to the Decision for Hysterectomy:

A 2006 study by Ya-Ling Yang and colleagues found that the most common reasons women have hysterectomies are non-cancerous issues like painful periods, very heavy menstrual bleeding (menorrhagia), and pelvic pain. The same study also showed that greater feelings of depression after a hysterectomy are linked to weight gain, feeling less feminine, and fears about aging.

Impact Of Hysterectomy On Chronic Diseases And Long-Term Health Outcomes

A 2022 systematic review found a connection between hysterectomies and several long-term health issues in women, including depression, metabolic problems, cancer, cardiovascular events, and even dementia. This link was seen regardless of whether or not the ovaries were also removed.

Research by Dr. Bonnet, as reported by Corrie Pelc on September 27, 2022, suggests a complex relationship between hysterectomy, oophorectomy (ovary removal), and diabetes. Dr. Bonnet's work indicates that women who have a hysterectomy but keep their ovaries may actually have a 13% *lower* risk of diabetes. However, if both ovaries are removed during the hysterectomy, the risk of developing type 2 diabetes increases by 26%, likely due to the sudden loss of estrogen production.

Results and Discussion:

Results are tabulated in following tables where n = no. of cases recruited.

Demographic information of the respondents:

Demographic data collected included age, region, religion, educational levels, and other socioeconomic factors, providing a comprehensive understanding of the respondents' backgrounds.

Table 1: Demographic information of the respondents

Demographic information		Number (N=100)	Percentage (%)
Age	Less than 45years	4	20
	46 to 48 yrs	10	50
	49 and above	6	30
	Total	20	100
Region	Rural	11	55
	Urban	9	45
	Total	20	100
Religion	Christian	1	5
	Hindu	16	80
	Muslim	3	15
	Total	20	100
Mother Tongue	Malayalam	3	15
	Others	2	10

	Tamil	15	75
	Total	20	100
Educational level	Graduate and above	1	5
	Higher secondary	2	10
	Illiterate	11	55
	Primary	6	30
	Total	20	100
Occupation	Business	1	5
	Daily wages	16	80
	Private	3	15
	Total	20	100
Family type	Joint family	8	40
	Nuclear family	12	60
	Total	20	100
Total family members	3 and less	2	10
	4 to 6 members	13	65
	7 and above	5	25
	Total	20	100
Annual Income	1 lakh	4	20
	Above 1 lakh	1	5
	Below 50 thousand	15	75
	Total	20	100

Discussion:

Age

The above table 1. indicates that the majority of participants are middle-aged, with 50% falling within the 46–48 age range. whereas, 20% are under 45, and 30% are 49 or older, reflecting a notable representation of the working-age population. These findings are consistent

with Jasbir Kaur Saluja *et al.*, 2015 report, which found that most women undergoing hysterectomy were between the ages of 41 and 60.

Region

Regarding region, 55% of participants were found from rural areas, contrasting with a Gujarat study that reported higher hysterectomy rates in rural women. This highlights differences in healthcare access, socioeconomic factors, and traditional health beliefs. Interestingly, a study by Dejalín Rout *et al.*, 2023, found a higher likelihood of hysterectomy among urban women, underscoring the complexity of the issue across regions.

Educational Level

Among our participants, 55% are illiterate, 30% have only completed primary education, 10% have completed higher secondary education, and 5% are graduates or above. This high illiteracy rate underscores a key area for intervention, as limited education can hinder awareness of health rights, access to medical information, and informed decision-making. These findings align with those of Erin A. Brennand *et al.*, 2024, who noted that women with lower education levels are more likely to undergo hysterectomy, often at younger ages and before menopause. Furthermore, low education levels can affect employment opportunities and overall socioeconomic well-being.

Health profile of the respondents:

The health profile of the respondents was assessed, revealing common post-surgical complications, including fatigue, weight gain, and reduced energy levels following the hysterectomy.

Table 4.2: Health profile of the respondents

Health profile		Number (N=100)	Percentage (%)
Health Issues before Hysterectomy	Yes	10	50
	No	10	50
	Total	20	100
Complications after	Yes	12	60

Hysterectomy	No	8	40
	Total	20	100
Health wellbeing changed	Manageable	1	5
	No change	10	50
	Worsened	9	45
	Total	20	100

Discussion:

In above table 2. The results reveal that out of 20 participants, 60% (12 women) experienced complications after the surgery, while 40% (8 women) did not experience complications. Whereas, 50% of the women reported changes in their overall well-being post-surgery 5% managed these changes well, while 45% experienced a decline in their health. This aligns with a study by Sapna Desai *et al.*,2023, which highlighted that undergoing a hysterectomy before age 45 in India extends the menopausal phase without estrogen, potentially increasing the risk or accelerating the onset of non-communicable diseases.

Hysterectomy of the respondents:

Information on the respondents' physical activity was collected to understand their exercise habits and lifestyle after the hysterectomy. This data provides valuable insights into how physical activity levels may impact recovery and overall health post-surgery.

Table 4.3: Hysterectomy of the respondents

Hysterectomy		Number (N=100)	Percentage (%)
Type of Hysterectomy	Total hysterectomy	20	100
	Partial hysterectomy	0	0
	Radical hysterectomy	0	0
	Others	0	0
	Total	20	100

Reason for hysterectomy	Endometriosis	7	35
	Fibroids	13	65
	Total	20	100

Discussion:

The above table 3. depicts that all the participants (100%) underwent total hysterectomy, none of the participants underwent partial or radical procedures.

The primary causes for the hysterectomy were heavy bleeding and fibroids, accounting for 65% of cases, while 35% attributed it to endometriosis. These findings align with the study by Sapna Desai *et al.*, 2023, which reported that among women aged 15–49, excessive menstrual bleeding was the leading cause for hysterectomy, followed by fibroids or cysts.

Physical activity of the respondents:

Data on the physical activity of the respondents was collected to assess their exercise habits and lifestyle post-surgery. This information aimed to explore the relationship between physical activity levels and health outcomes following hysterectomy.

Table 4.4: Physical activity of the respondents

Physical activity		Number (N=100)	Percentage (%)
Exercise – frequency	Rarely	19	95
	Weekly	1	5
	Total	20	100
Changes in energy levels	Yes	5	25
	No	15	75
	Total	20	100
Type of physical activity	Light	5	25
	Sedentary	15	75
	Total	20	100
Stress - frequency	Daily	2	10

	Monthly	1	5
	Rarely	12	60
	Weekly	5	25
	Total	20	100

Discussion:

From the Table 4. Most participants (95%) exercised rarely, with 75% leading a sedentary lifestyle. 25% reported changes in energy levels. The similar findings with a study by Alan H. DeCherney *et al.*, 2002, which found 74% of patients experienced prolonged fatigue post-surgery, affecting daily activities and causing frustration, depression, and concentration difficulties. Additionally, 60% experienced stress rarely, highlighting the significant physical, psychosocial, and economic impacts of post-hysterectomy recovery.

Dietary pattern of the respondents:

Information on the dietary patterns of the respondents was gathered, focusing on their typical food intake and adherence to any specific diets. This data offers valuable insights into the nutritional habits and their potential influence on recovery after hysterectomy.

Table 4.5: Dietary pattern of the respondents

Dietary Pattern		Number (N=100)	Percentage (%)
Type of diet	Non-vegetarian	16	80
	Vegetarian	4	20
	Total	20	100
Specific diet	Yes	0	0
	No	20	100
	Total	20	100
Meals per day	3 meals	19	95
	4 meals	1	5
	Total	20	100

Any other supplements than food	Yes	0	0
	No	20	100
	Total	20	100
Water consumption per day	1 liter	1	5
	2 liters	5	25
	3 liters	9	45
	More than 3 liters	5	25
	Total	20	100
Food allergies	Yes	0	0
	No	20	100
	Total	20	100

Discussion:

The above Table 5. indicates that 80% of participants follow a non-vegetarian diet, while 20% are vegetarian, with none of the participants adhering to any specific dietary plan. This lack of dietary control, combined with the absence of regular exercise, may contribute to weight gain in post-hysterectomy patients, as observed in our study. Similarly, Fitzgerald *et al*, (2009) in their study "Hysterectomy and Weight Gain" found that women who undergo hysterectomy, especially those not following a structured exercise regimen or specific diet, are more likely to experience significant weight gain due to an increase in body mass index (BMI).

Summary and Conclusion:

This study provides significant insights into the demographic characteristics and health-related outcomes of women who have undergone hysterectomy. The majority of participants were middle-aged (46–48 years), aligning with existing literature that identifies hysterectomy as most common between the ages of 41 and 60—a critical period for reproductive health decisions. While 55% of participants resided in rural areas, contradicting studies that suggest higher hysterectomy rates in rural populations due to access and socioeconomic factors, the findings highlight the multifaceted nature of healthcare accessibility and regional health beliefs. Additionally, education levels were markedly low, with 55% of participants being illiterate and

30% having only primary education. This emphasizes the need for increased health awareness, as lower education levels have been linked to a higher likelihood of undergoing hysterectomy at younger ages. Post-operative complications were prevalent, with 60% of participants experiencing health issues and 50% reporting a decline in overall well-being. These findings are consistent with research indicating that hysterectomy before the age of 45 can prolong menopausal symptoms and increase the risk of non-communicable diseases, reinforcing the necessity of comprehensive post-operative care and long-term health monitoring. Lifestyle factors further influenced post-surgery outcomes, with 95% of participants engaging in minimal physical activity. Fatigue and weight gain were commonly reported, in line with existing studies linking hysterectomy to decreased energy levels and increased body mass index (BMI). Additionally, the absence of a structured diet plan among most participants may have contributed to these changes. This study highlights the need for improved post-hysterectomy care through better education, healthcare access, and lifestyle interventions. The high prevalence of complications underscores the importance of structured follow-up, including exercise and dietary guidance. Future research will evaluate targeted interventions to enhance recovery and long-term well-being.

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Association of Physical Activity Pattern and Unhealthy Dietary Habits among Industrial Workers

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Abstract: Industrial workers often face health challenges due to demanding work environments, irregular schedules, and limited access to health resources. This study investigates the association between physical activity patterns and unhealthy dietary habits among industrial workers. An exploratory research design comprising 500 industrial workers from various industrial sectors of Tamil Nadu was employed. Physical activity levels were classified as sedentary, moderate, or high, while dietary habits were assessed based on the frequency of consuming processed, sugary, and fried foods. Results showed that the majority (55%) of industrial workers exercised less than 30 minutes daily due to prolonged working hours and limited access to physical activity opportunities, followed by 37% who exercised for 45 minutes to 1 hour. This group exhibited a significantly higher prevalence of unhealthy dietary patterns, including frequent consumption of processed and sugary foods. Conversely, workers with high physical activity levels demonstrated healthier dietary choices, including increased consumption of fruits and vegetables. These findings highlight the critical need for workplace interventions aimed at promoting physical activity and healthier dietary practices. Implementing structured fitness programs, providing access to nutritious meals, and offering health education can help reduce the risk of chronic diseases, enhance workers' overall well-being, and improve productivity. The study underscores the interconnection between lifestyle factors and calls for further research to explore how workplace culture and policies can support sustainable health behavior changes among industrial workers.

Keywords: Industrial workers, Dietary habits, Physical Activity Pattern, Workplace, Lifestyle

Introduction

The industrial sector plays a pivotal role in the economic development of nations. However, industrial workers often encounter unique occupational health challenges that arise from physically demanding jobs, irregular work schedules, and exposure to hazardous environments. These factors contribute to a high prevalence of lifestyle-related health issues, including obesity, cardiovascular diseases, and metabolic disorders (World Health Organization, 2020; Smith & Jones, 2019). Lifestyle behaviors such as physical inactivity and unhealthy dietary habits have been identified as significant contributors to these health challenges (Gupta & Mehta, 2020).

Despite the critical implications, limited research has explored the interplay between physical activity patterns and dietary behaviors among industrial workers in Tamil Nadu, India. Studies such as those by Smith and Jones (2019) have highlighted the correlation between occupational settings and health risks. Similarly, Gupta and Mehta (2020) noted that work-related stress and limited health resources exacerbate poor lifestyle behaviors. Research by Pan et al. (2018) emphasizes the cascading impact of unhealthy diets on chronic illnesses, reinforcing the urgency of intervention.

This study aims to fill this gap by examining the association between physical activity levels and dietary habits among industrial workers. Understanding this relationship can inform workplace interventions designed to improve workers' overall health and productivity. The study's findings emphasize the need for comprehensive strategies that integrate physical activity promotion and healthy dietary practices in industrial settings.

Literature Review

Physical Activity and Occupational Health

Physical activity is a cornerstone of maintaining physical and mental well-being. According to the World Health Organization (2020), adults should engage in at least 150 minutes of moderate-intensity or 75 minutes of vigorous-intensity physical activity weekly. However, industrial workers often face barriers to achieving these recommendations due to long working hours, physically taxing tasks, and lack of access to recreational facilities.

Studies by Sallis et al. (2016) and Gupta and Mehta (2020) have demonstrated that physical inactivity among industrial workers is associated with increased risks of obesity, musculoskeletal disorders, and cardiovascular diseases.

Dietary Habits in Industrial Workers

Dietary habits are another critical determinant of health among industrial workers. Irregular work schedules and limited availability of healthy food options often lead to poor dietary choices, such as frequent consumption of processed and high-calorie foods (National Institute of Nutrition, 2021). Research has shown that unhealthy dietary patterns are linked to a higher prevalence of non-communicable diseases (NCDs), including diabetes and hypertension (Smith & Jones, 2019). Moreover, inadequate intake of fruits and vegetables exacerbates the risk of chronic health conditions (Pan et al., 2018). Studies such as those by Kumari and Sharma (2017) underscore the compounding effects of poor diet and inactivity on occupational health.

Interaction Between Physical Activity and Diet

The relationship between physical activity and dietary habits is complex and bidirectional. Active individuals are more likely to consume nutrient-rich diets, while sedentary lifestyles are often associated with poor dietary practices (Sallis et al., 2016). This interplay has significant implications for designing holistic health interventions (Smith & Jones, 2019; Gupta & Mehta, 2020). Comprehensive frameworks that integrate physical activity promotion and healthy dietary interventions have shown promise in reducing workplace health risks (Rajan et al., 2020).

Methodology

Study Design

This study adopted an exploratory research design to assess the association between physical activity patterns and dietary habits among industrial workers.

Sample Selection

A total of 500 industrial workers from various sectors in Tamil Nadu were selected using convenience sampling. The inclusion criteria included workers aged 18-60 years, employed for at least one year, and willing to participate in the study.

Data Collection Tools

1. **Physical Activity Assessment:** The International Physical Activity Questionnaire (IPAQ) was used to classify participants' activity levels into sedentary, moderate, and high.
2. **Dietary Habits Questionnaire:** A validated dietary survey was employed to assess the frequency of consuming processed, sugary, and fried foods, as well as the intake of fruits and vegetables.

Data Analysis

Data were analyzed using SPSS software. Descriptive statistics were used to summarize the data, and chi-square tests were employed to examine associations between physical activity levels and dietary habits. Logistic regression analysis was conducted to identify predictors of unhealthy dietary patterns. Statistical significance was set at $p < 0.05$.

Results and Discussion

Physical Activity Patterns

Among the participants, 55% reported engaging in less than 30 minutes of physical activity daily, primarily due to prolonged working hours and lack of facilities. Approximately 37% performed moderate activity (45 minutes to 1 hour), while only 8% engaged in high levels of physical activity.

Chi-square analysis revealed a significant association between physical activity levels and unhealthy dietary habits ($\chi^2 = 24.87$, $p < 0.001$). Workers engaging in less than 30 minutes of physical activity were more likely to exhibit unhealthy dietary habits, with a significant increase in the consumption of processed and sugary foods.

Dietary Habits

Unhealthy dietary practices were prevalent, with 68% of workers frequently consuming processed foods, 59% consuming sugary foods, and 45% consuming fried foods. Workers with sedentary activity levels had a significantly higher prevalence of unhealthy dietary patterns compared to those with moderate or high activity levels. Regression analysis revealed that sedentary workers were 3.5 times more likely to consume unhealthy diets compared to their active counterparts ($p < 0.01$).

Statistical Analysis

Table 1 summarizes the association between activity levels and dietary habits:

Physical Activity Level	Processed Food Consumption (%)	Sugary Food Consumption (%)	Fruits/Vegetables Intake (%)
Sedentary	75	62	20
Moderate	50	45	45
High	20	15	70

Logistic regression analysis further identified key predictors of unhealthy dietary habits, including limited physical activity ($p < 0.001$) and lack of access to nutritious meals at the workplace ($p < 0.05$). Adjusted odds ratios (AOR) showed that workers with sedentary lifestyles were at increased risk of developing poor dietary behaviors (AOR: 3.87, 95% CI: 2.45-6.11).

Workplace Interventions

The findings underscore the need for targeted workplace interventions to mitigate these risks. Structured fitness programs can be introduced to encourage regular physical activity among workers, potentially through onsite gym facilities or scheduled activity breaks during shifts. Providing access to nutritious meals at workplace cafeterias can help workers make healthier dietary choices, replacing processed and sugary foods with balanced options that include fruits and vegetables. Furthermore, health education campaigns tailored to industrial workers' schedules and challenges can emphasize the long-term benefits of physical activity and healthy eating, motivating sustainable lifestyle changes. By adopting these

strategies, organizations can significantly reduce the prevalence of lifestyle-related diseases, enhance worker well-being, and boost overall productivity.

Conclusion

This study highlights the critical interconnection between physical activity patterns and dietary habits among industrial workers in Tamil Nadu. The findings emphasize the need for comprehensive workplace health programs to address lifestyle-related health risks. Strategies such as implementing structured fitness programs, providing nutritious meal options, and conducting regular health education sessions can lead to sustained improvements in worker health and productivity. Future research should explore the role of workplace culture and policies in fostering sustainable health behavior changes.

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Antimicrobial Potential of Selected Natural Dye Compounds and Their Molecular Docking Against Bacterial Proteins for Textile Applications

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Abstract

Textile surface modification including antimicrobial finishing has become a promising requirement in the textile industry to ensure high quality textile materials. Natural colourants offer a promising alternative for developing aesthetically appealing and hygienic textiles embedded with bioactive compounds, owing to the presence of potentially active agents such as tannins, flavonoids, quinines, carotenoids and alkaloids in them. The present study focuses on natural dyes with inherent antimicrobial activity for use as textile finishing compounds to protect textile materials from pathogenic microbes. The natural dye compounds viz., luteolin, tannin and anthocyanin were extracted from fruit peels of *Punica grantaum*.L, *Musa* sp., and sap of *Musa* sp. The 3D structures of these compounds generated using ChemDoodle, were docked to assess their binding efficiency with their selected target proteins: 3ZIN (*Bacillus subtilis*), 1ESU (*E.coli*), 5AZU (*Pseudomonas aeruginosa*), 3WGI (*Staphylococcus aureus*). The results confirmed that all three compounds exhibited good binding energy. However, in *in-vitro* antimicrobial activity assays, a zone of inhibition was observed only for anthocyanin against the selected microorganisms. Therefore, three anthocyanin derivatives were synthesized by modifying their functional groups using ChemDoodle, and these derivatives were further tested for their binding

affinity with bacterial proteins. The binding energy value suggest the potential use of anthocyanin and its derivatives as textile finishing compounds with antimicrobial properties.

Keywords: Antimicrobial activity, Molecular docking, Natural dye, Textile industry

Introduction

Textile materials and clothing are very susceptible to microbial attack as they provide a large surface area and absorb moisture required for microbial growth (Carddamone, 2002). The cloth contains natural fibres having proteins such as keratin and cellulose, which provides basic requirements such as moisture, oxygen, nutrients and temperature for bacterial growth and multiplication. This leads to objectionable odours, dismal infection, product determination, allergic responses and other related diseases (Thiry, 2001). This necessitates the development of clothing that could provide a desired antimicrobial effect. A variety of antimicrobial agents such as organometallic, phenols, quaternary ammonium salts, and organ silicon are used (Yang *et al.*, 2000).

Antimicrobial finishes can have potential benefits to contain the spread of disease, avoid the danger of injury induced infection and prevent the deterioration of textile and have other advantages (Gupta *et al.*, 2007). The solution needed is the development of clothing effect without any associated problems. Problem with the synthetic biocide is that it causes skin allergies, side effects on the non-target microorganisms and water pollution. For example, surface coating by tri alkoxyl quaternary ammonium salt, a synthetic non degradable compound, and use of antimicrobial nylon fibre with phosphate glass as an antimicrobial agent leads to environmental hazards (Sarkar *et al.*, 2001). The alternate and environmentally friendly solution is the usage of natural dyes. These antimicrobial textiles can be used to manufacture sportswear, working cloths, undergarments, shoes lining, carpets, upholstery, hospital lines, wound care wraps, towels, and toys for children (Purwar *et al.*, 2004).

Natural dyes are derived from sources such as plants, insects, animals without any chemical treatment (Kadolpa, 2008). In India, there are more than 450 plants that can yield dyes and these dyes also possess medicinal value (Siva, 2007). Many of the plants used for dye extraction are classified as medicinal and some of these have recently been shown to possess antimicrobial activity (Hussein *et al.*, 1997).

The natural dye from banana plants wastes tender brown colouration due to tannin. Tannin is naturally occurring plant polyphenols soluble in water. Their main characteristics are that they bind and precipitate proteins. Tannins are high molecular weight oligomeric compound with multiple structure units with free phenolic compounds. It has the ability to bind proteins and form insoluble or soluble tannin protein complexes (Reed, 1994). It can interact with other macromolecules such as carbohydrates, proteins, polysaccharides, bacterial cell membranes and enzymes involved in protein and carbohydrates digestion (Haslam, 1989). Tannin protein interaction is specific and depends on the structure of both the protein and tannin (Giner, 1996). Proteins are opened and flexible structure that favour for strong bonding (Haslam, 1989). It is having strong hydrogen bonds with protein carboxyl group. These are the main properties that are favourable for docking and thus tannin are suitable for molecular docking.

Anthocyanins are derived from Pomegranate (*Punica granatum*). Anthocyanins are water-soluble pigments responsible for attractive red colour or purple colours (Seeram *et al.*, 2006). It contains chief compounds such as punicalagin, punicalin, gallagic acid and ellagic acids (Tzulkeret *et al.*, 2002). *Punica grannatum* dye and many other common natural dyes are reported as potent antimicrobial agents (Machado *et al.*, 2002). Another significant property of anthocyanins is their antioxidant activity, which plays a vital role in the prevention of neuronal and cancer. It had a binding affinity with biological molecules. Luteolin is natural dye compound derived from banana peel is a good sources oxidant and antimicrobial and these two properties were evaluated by Mokebel *et al.* (2005).

Computer aided analysis of protein interactions have become a significant role in solving the difficulties in the in-vitro experiments. It has automated prediction of molecular interactions, which is an important step in Rational Drug Design (RDD). This entire process is called molecular docking and it may describe as interaction of protein or other biological molecules and obtaining the low energy binding modes of ligand active sites (Andrusier *et al.*, 2007). Docking is used for lead discovery, typically by screening a large database of organic molecules for ligands that fit a binding site. The technique had some important successes in recent years. Molecular docking is a structure based computational technique that samples complementary fit of macromolecules receptors and possible ligands. The ability to predict the conformations and energies involved to protein is quite crucial in designing potential drugs that can interact favourable with their target proteins (Brain *et al.*, 2002). With this basic understanding, the

present study aim is to dock the natural dye compounds with selected bacterial proteins and their antimicrobial activity against selected bacterial strains.

Material and Methods

Sample collection

Natural dye sources viz., banana peels, pomegranate peels and banana sap were collected from fruit vendors in Madurai.

Extraction of dye from plant materials

The aqueous extract of dye was prepared by adopting the methodology described by Jothi (2008). Five gram of peels were homogenized and dissolved in distilled water, then boiled in water bath for 2hrs at temperature ranging from 50°C to 90°C. The extract was subsequently filtered using filter paper.

Antimicrobial screening

Antimicrobial screening was performed following the methodology described by Deshmukh *et al.* (2009). The bacterial strains *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtained from the National Centre of Excellence, Thiagarajar College, Madurai and used to assess antimicrobial activity.

The test culture was prepared according to the methodology described by Karpe (2011). The test organism was inoculated into 10ml of sterile nutrient broth using a loopful of culture from nutrient agar slant. The culture was then incubated at 37°C for 18hrs and the 18hrs old culture was used for further experiments.

The agar plates were inoculated with the bacterial suspension using the spread plate technique. Wells were made by a well cutter and different concentrations of the dye extracts (40µl, 60µl, 80µl) were added to the wells. After dye addition, the plates were incubated at 37°C for 24hrs. The plates were then examined for zones of growth inhibition, and the diameters of these zones were measured in millimeters (Deshmukh *et al.*, 2009). All tests were performed in duplicate.

Retrieval of selected protein structure

The retrieval of protein structures was carried out following the methodology outlined by Babahedari *et al.* (2013). The proteins structures were downloaded from the Protein Data Bank (rcsb.org), which provides structural information on macromolecules determined by X-ray

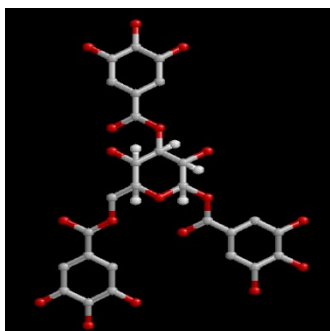
crystallography and NMR methods. The 3D structures of the selected proteins were saved as PDB files and tabulated accordingly.

Organism	Protein name with pdb code
<i>Bacillus subtilis</i>	3ZIH-cell division protein c domain
<i>E. coli</i>	1E5U- intimine protein
<i>Pseudomonas aeruginosa</i>	5AZU- crystal structure of membrane protein
<i>Staphylococcus aureus</i>	3WGJ- cimera protein

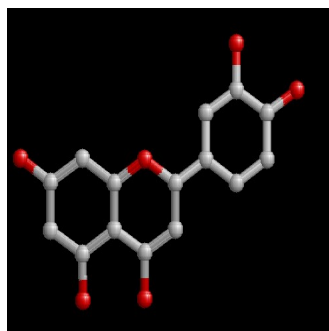
Retrieval of ligand molecules

The ChemDoodle connected Pubchem tool was used to search for the 2D structures of anthocyanin, tannin and luteolin. These 2D structures of ligand structures were then converted into 3D structures for docking purposes using ChemDoodle following the methodology prescribed by Manish (2014). The 3D ligand structures were subsequently saved as PDB files (Figure 1).

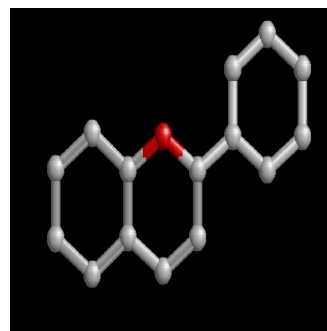
Fig. 1: 3D structure of selected natural dye compounds retrieved using ChemDoodle



Tannin



Luteolin

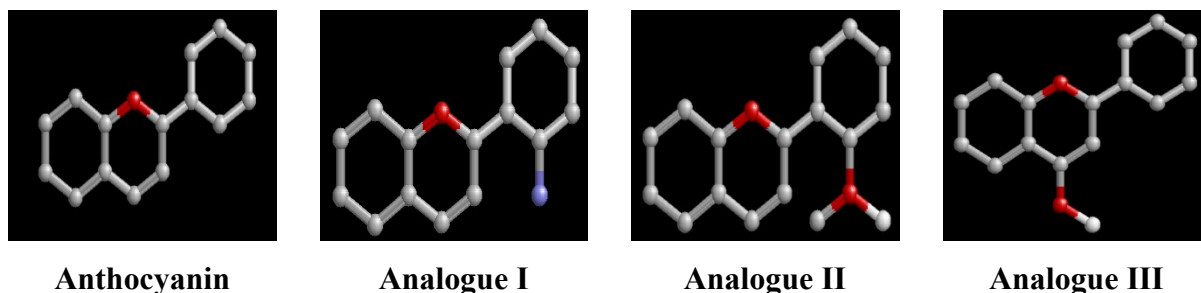


Anthocyanin

Analogue Preparation

Analogues of the ligands were generated by modifying the functional groups of the conventional ligands using ChemDoodle tool. The developed analogues were saved as PDB files and used for docking with the protein molecules (Figure 2).

**Fig. 2: 3D structure of anthocyanin and analogues derived using ChemDoodle
Molecular docking and Energy calculation**



The docking methodology prescribed by Sivakumar *et al.* (2011) was adopted in the present study. The retrieved compounds were docked with four bacterial proteins using HEX 6.0 docking software. The ligands were docked with receptor using the following parameters such as Correlation type- shape and electrostatics, FFT Mode-3D fast lite, Grid Dimension- 0.6, Receptor range- 180, Ligand Range-180, Twist range- 360, Distance Range-40.

Results and Discussion

Antimicrobial activity

The antimicrobial activity of natural dye extracts obtained from *Punica granatum* (pomegranate peels), *Musa* peels, and *Musa* sap was evaluated against four bacterial strains: *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Table 1). The results indicated that among the three tested dye compounds, anthocyanin from *Punica granatum* exhibited the highest antimicrobial activity, showing clear zones of inhibition (Plate 1). The inhibition was most pronounced against *Pseudomonas aeruginosa*, with a maximum zone of 30 mm at an 80 μ L concentration. In contrast, tannin (from *Musa* sap) and luteolin (from *Musa* peels) did not display significant antimicrobial activity under the tested conditions.

The results demonstrated that anthocyanin from *Punica granatum* exhibited significant antimicrobial activity, particularly against *Pseudomonas aeruginosa*. These findings align with previous studies that have reported the potent antimicrobial effects of anthocyanins due to their ability to disrupt bacterial cell membranes and inhibit microbial enzymes (Cerezal-Mezquita *et al.*, 2023; Kumar *et al.*, 2022). In contrast, tannin and luteolin did not exhibit inhibitory activity in vitro, which may be attributed to differences in extraction efficiency, concentration, or bacterial resistance mechanisms (Arif *et al.*, 2021).

Natural dyes have been widely recognized for their potential antimicrobial properties, with previous research highlighting their bioactive nature (Adeel *et al.*, 2022). Anthocyanins, in particular, have been shown to possess antibacterial, antifungal, and antioxidant properties, making them suitable candidates for textile applications (Borges *et al.*, 2023). However, their effectiveness depends on factors such as pH, solubility, and molecular stability (Riaz *et al.*, 2021).



Bacillus subtilis



Escherichia coli



Pseudomonas aeruginosa



Staphylococcus aureus

Plate 1: Antimicrobial activity of anthocyanin against the selected bacteria

Table 1: The zone of inhibition (in mm) shown by bacteria against the natural compounds and standard antibiotic

	<i>P. granatum</i>			<i>Musa peels</i>			<i>Musa sap</i>			Ampicillin		
Qty (μl)	40	60	80	40	60	80	40	60	80	40	60	80
<i>B. subtilis</i>	25	28	29	-	-	-	-	-	-	18	19	22
<i>E. coli</i>	13	19	26	-	-	-	-	-	-	23	26	28
<i>P. aeruginosa</i>	15	20	30	-	-	-	-	-	-	19	19	22
<i>S. aureus</i>	10	15	19	-	-	-	-	-	-	22	24	26

Molecular docking analysis

The docking analysis was performed using HEX 6.0 software to evaluate the binding affinity of natural dye compounds (luteolin, tannin, and anthocyanin) with bacterial proteins. The docking results revealed varying binding energies, indicating different levels of interaction between the dye compounds and bacterial proteins (Table 2).

Luteolin exhibited the strongest interaction with *Staphylococcus aureus* (3WGJ) with a binding energy of -780.65 kcal/mol but failed to bind with *Escherichia coli* (1ESU) (Fig. 3a). This strong interaction suggests that luteolin has a high potential to interfere with bacterial protein functions, possibly disrupting essential metabolic processes. Previous studies have shown that flavonoids like luteolin possess antimicrobial properties by targeting bacterial enzymes and cell wall synthesis pathways (Gautam *et al.*, 2022). Luteolin has also been reported to exhibit strong antibacterial activity against gram-positive bacteria, including *S. aureus*, by inhibiting DNA gyrase and bacterial efflux pumps, leading to reduced bacterial growth and viability (Zhang *et al.*, 2023). However, its inability to bind with *Escherichia coli* (1ESU) suggests structural limitations in interacting with specific gram-negative bacterial proteins, which could be attributed to differences in cell membrane composition and protein target availability (Riaz *et al.*, 2021).

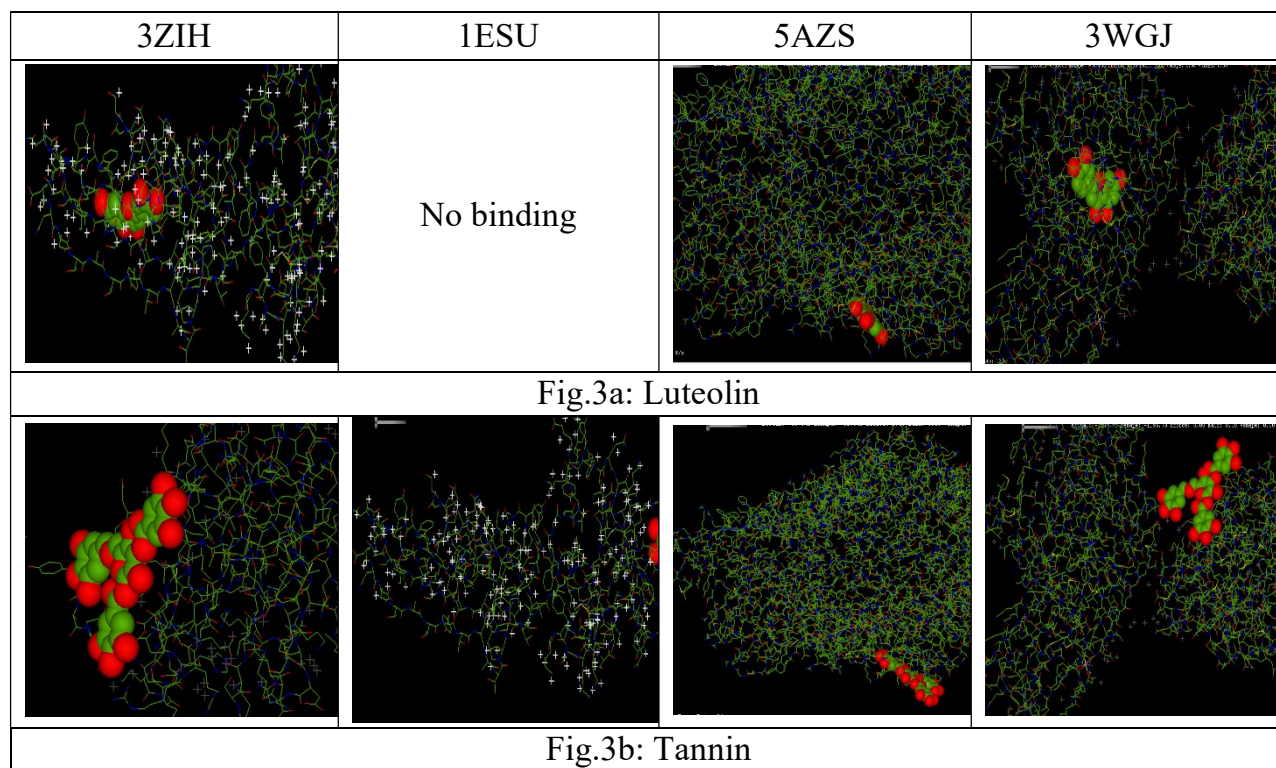
Tannin showed moderate binding affinity with *Pseudomonas aeruginosa* (5AZU) (-578.28 kcal/mol) and *Bacillus subtilis* (3ZIH) (-292.67 kcal/mol) (Fig. 3b). Tannins are known for their ability to interact with bacterial proteins by forming hydrogen bonds and disrupting cell membrane integrity (Borges *et al.*, 2023). The moderate binding energy suggests that tannin may have some antimicrobial potential, but its effectiveness could be concentration-dependent. *P. aeruginosa* is a notoriously resilient bacterium due to its efflux pump mechanisms and biofilm formation ability, which can limit the penetration of antimicrobial agents (Kumar *et al.*, 2022). Despite this, tannin's interaction with *P. aeruginosa* suggests its potential for use as a supplementary antimicrobial agent in textile applications.

Anthocyanin demonstrated binding with all tested bacterial proteins but had the lowest binding energy (-30.49 kcal/mol) with *Escherichia coli* (1ESU) (Fig. 3c). This suggests that while anthocyanin has the capability to bind to bacterial targets, the strength of these interactions varies significantly. Anthocyanins are known for their antimicrobial and antioxidant properties, often attributed to their ability to destabilize bacterial membranes and interfere with metabolic

pathways (Cerezal-Mezquita *et al.*, 2023). However, the low binding energy observed with *E. coli* may be due to the structural complexity of the bacterial outer membrane, which acts as a barrier to hydrophilic compounds like anthocyanins (Zhao *et al.*, 2023). Interestingly, despite anthocyanin showing broad-spectrum binding in silico, its *in vitro* antimicrobial activity was limited to *Pseudomonas aeruginosa*. This discrepancy could be due to differences between theoretical docking predictions and real-world biological interactions, including compound solubility, stability, and uptake by bacterial cells (Iqbal *et al.*, 2022). Enhancing anthocyanin's antimicrobial efficiency through structural modifications or synergistic combinations with other bioactive compounds may improve its effectiveness against a broader range of bacterial pathogens.

The docking results suggest that anthocyanin exhibited broad-spectrum binding potential, though its *in-vitro* antimicrobial activity was limited to *Pseudomonas aeruginosa*.

Fig. 3: Docking image of bacterial proteins, 3ZIH (*Bacillus subtilis*), 1ESU (*E.coli*), 5AZU (*Pseudomonas aeruginosa*), 3WGI (*Staphylococcus aureus*) with the three natural dye compounds



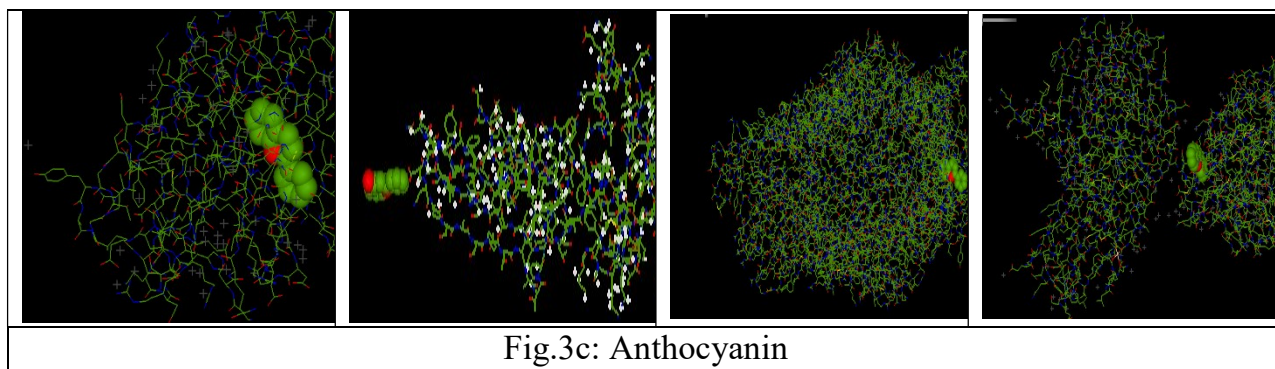


Table 2: Docking results of bacterial proteins 3ZIN (*Bacillus subtilis*), 1ESU (*E.coli*), 5AZU (*Pseudomonas aeruginosa*), 3WGJ (*Staphylococcus aureus*) with the natural dye compounds

Bacterial proteins	E – values (K.Cal/mol) with		
	Luteolin	Tannin	Anthocyanin
<i>B. subtilis</i> (3ZIH)	-498.68	-292.67	-163.48
<i>E.coli</i> (1ESU)	-	-33.65	-30.49
<i>P. aeruginosa</i> (5AZS)	-399.46	-578.28	-486.62
<i>S. aureus</i> (3WGJ)	-780.65	-222.12	-148.64

Anthocyaninanalogues: The molecular docking analysis evaluated the binding efficiency of three anthocyanin analogues (I, II, and III) against selected bacterial proteins (Table 3). The docking energy values (E values) were used to determine the strength of interaction between the ligand and receptor molecules (Fig. 4).

Table 3. Docking results of anthocyanin analogues with bacterial proteins 3ZIN (*Bacillus subtilis*), 1ESU (*E.coli*), 5AZU (*Pseudomonas aeruginosa*), 3WGJ (*Staphylococcus aureus*)

Proteins (pdb code)	E values (K.Cal/mol) of anthocyanin analogues		
	I	II	III
<i>B. subtilis</i> (3ZIH)	-579.16	-575.24	-721.09
<i>E.coli</i> (1ESU)	-26.80	-30.96	-
<i>P. aeruginosa</i> (5AZS)	-453.45	-463.04	-607.45
<i>S. aureus</i> (3WGJ)	-1115.66	-1017.02	-1195.00

***S. aureus* (3WGJ):** The highest binding affinity was observed with Analogue III (-1195.00 kcal/mol), followed by Analogue I (-1115.66 kcal/mol) and Analogue II (-1017.02 kcal/mol). The docking results indicate that the anthocyanin analogues exhibited varying degrees of interaction with the selected bacterial proteins. *S. aureus* (3WGJ) demonstrated the strongest binding affinity with all three analogues, particularly Analogue III, suggesting that structural modifications enhanced its interaction with the bacterial protein. This aligns with previous studies indicating that anthocyanins possess antibacterial properties against *S. aureus* due to their ability to disrupt cell membranes and inhibit bacterial enzymes (Li *et al.*, 2023).

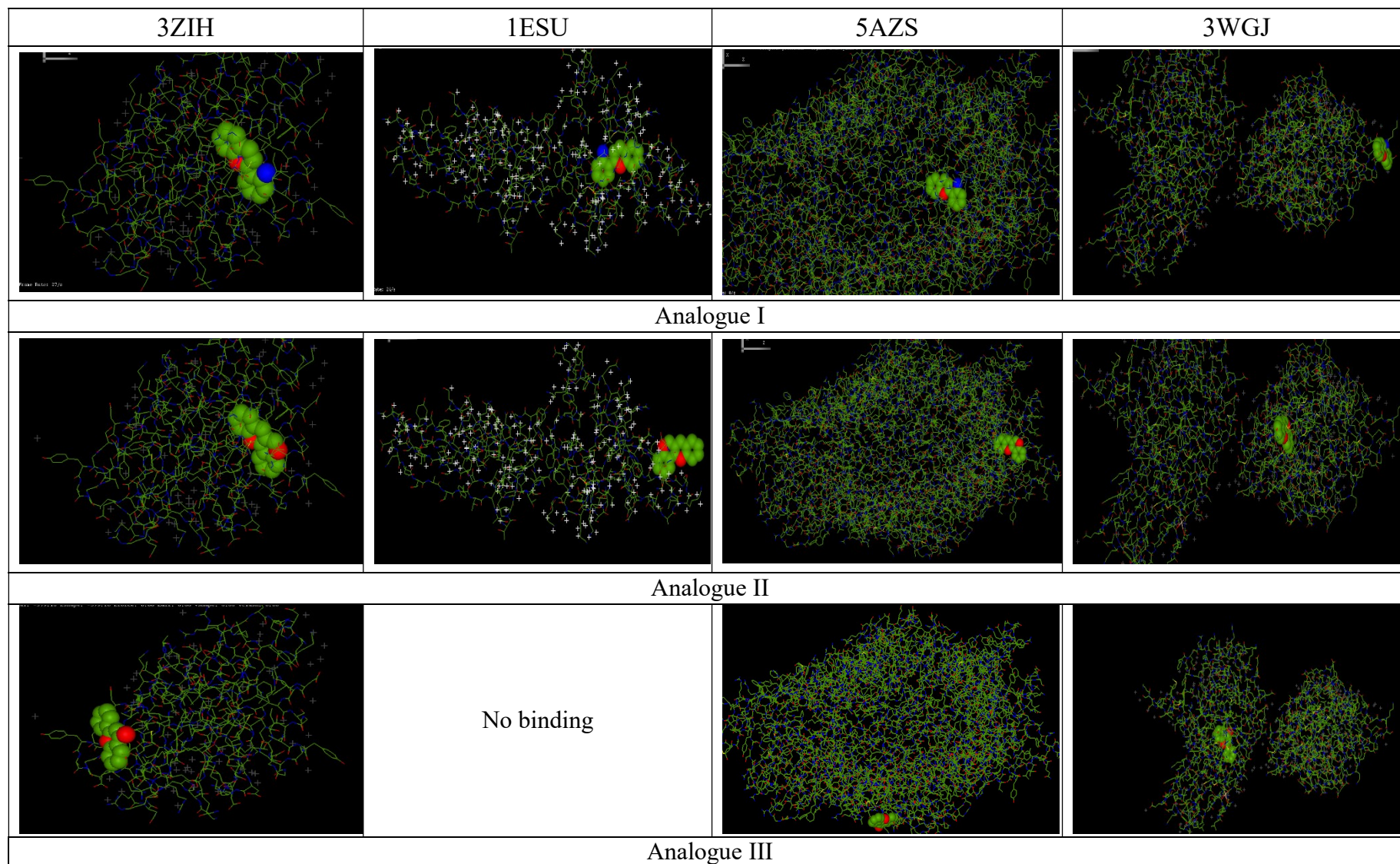
***P. aeruginosa* (5AZS):** Moderate binding affinity was observed with Analogue III (-607.45 kcal/mol), whereas Analogues I and II showed similar binding energies of -453.45 kcal/mol and -463.04 kcal/mol, respectively. The moderate binding observed with *P. aeruginosa* (5AZS) suggests that anthocyanin analogues can effectively interact with Gram-negative bacteria, although to a lesser extent than Gram-positive bacteria. Prior research suggests that flavonoid-based compounds, including anthocyanins, may interfere with bacterial quorum sensing mechanisms, reducing pathogenicity (Wang *et al.*, 2022).

***B. subtilis* (3ZIH):** The binding energies for Analogue III (-721.09 kcal/mol) were higher than those of Analogue I (-579.16 kcal/mol) and Analogue II (-575.24 kcal/mol). The relatively lower binding affinities with *B. subtilis* (3ZIH) indicate that while anthocyanin analogues may have some antimicrobial effects, their efficacy may vary based on bacterial structure and metabolism. Studies have reported that polyphenolic compounds like anthocyanins exhibit selective inhibition of Gram-positive bacteria by altering peptidoglycan synthesis (Zhou *et al.*, 2021).

***E. coli* (1ESU):** The docking scores were relatively low, with Analogue I (-26.80 kcal/mol) and Analogue II (-30.96 kcal/mol), while Analogue III failed to dock effectively. The weak interaction observed with *E. coli* (1ESU) suggests that anthocyanin analogues may be less effective against *E. coli*, which is consistent with previous reports stating that Gram-negative bacteria possess an outer membrane that limits the permeability of polyphenolic compounds (Gomes *et al.*, 2022).

Overall, the docking results support the hypothesis that anthocyanin analogues, especially Analogue III, could be potential candidates for antimicrobial textile applications, particularly against *S. aureus* and *P. aeruginosa*. Further in-vitro and in-vivo studies are necessary to validate these computational predictions and optimize anthocyanin derivatives for enhanced antimicrobial activity.

Fig. 4: Docking image of bacterial proteins, 3ZIN (*Bacillus subtilis*), 1ESU (*E.coli*), 5AZU (*Pseudomonas aeruginosa*), 3WGJ (*Staphylococcus aureus*) with the anthocyanin analogues.



Conclusion

This study highlights the antimicrobial potential of natural dye compounds—luteolin, tannin, and anthocyanin extracted from banana peels, pomegranate peels, and banana sap. The molecular docking results revealed that luteolin exhibited the highest binding affinity with *Staphylococcus aureus*, tannin showed moderate interactions with *Pseudomonas aeruginosa* and *Bacillus subtilis*, while anthocyanin demonstrated broad-spectrum binding potential but with limited in-vitro antimicrobial activity. Among the tested compounds, anthocyanin from pomegranate peels showed the most significant antimicrobial effect, particularly against *Pseudomonas aeruginosa*.

Furthermore, structural modifications of anthocyanin led to improved binding interactions with bacterial proteins, especially with *Staphylococcus aureus*. These findings suggest that anthocyanin and its derivatives hold promise as antimicrobial agents for textile applications, contributing to the development of eco-friendly antimicrobial fabrics. However, further in-vitro and in-vivo studies are necessary to validate their practical efficacy and stability on textile surfaces. The integration of computational docking with experimental antimicrobial screening provides valuable insights into the design of bioactive textile materials, paving the way for sustainable and health-conscious innovations in the textile industry.

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Applications of Nanotechnology in Agriculture: A Review

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Abstract:

Nanotechnology is an emerging revolutionary tool having the potential to change various sectors like agriculture by manipulating matter at nanoscale. Addressing future, global population growth coupled with food demand, nanotechnology promises to solve challenges of agricultural productivity, resource efficiency, and sustainability. This review article will describe the various applications of nanotechnology in agriculture, such as nanofertilizers, nanopesticides, nanosensors, and nanobiosensors-to point out their potential advantages, disadvantages, and perspectives into the future.

Keywords: Nanotechnology, agriculture, nanofertilizers, nanopesticides, nanosensors, nanobiosensors, sustainable farming, crop improvement.

Introduction

Agriculture is the backbone of global food security, but it faces numerous challenges, including climate change, soil degradation, water scarcity, and the need for increased productivity to meet the demands of a growing population. Nanotechnology, which involves the manipulation of materials at the nanoscale (1-100 nm), has the potential to address these challenges by providing innovative solutions for crop production, pest management, and environmental monitoring. This review aims to provide an overview of the applications of nanotechnology in agriculture, focusing on its role in enhancing crop yield, improving resource use efficiency, and ensuring sustainable farming practices.

Applications of Nanotechnology in Agriculture

Nanofertilizers

Nanofertilizers are nanoscale formulations of essential nutrients that can be applied to crops to improve nutrient uptake and utilization. Unlike conventional fertilizers, nanofertilizers release nutrients in a controlled manner, reducing nutrient losses and

minimizing environmental impact. For example, zinc oxide nanoparticles have been shown to enhance the growth and yield of wheat by improving zinc uptake (Prasad et al., 2017). Similarly, nano-encapsulated phosphorus fertilizers have been reported to increase phosphorus use efficiency in maize (Liu & Lal, 2015).

Nanopesticides

Nanopesticides are nanoscale formulations of pesticides that offer several advantages over conventional pesticides, including improved solubility, stability, and targeted delivery. These formulations can reduce the amount of active ingredient required, thereby minimizing environmental contamination and reducing the risk of pesticide resistance. For instance, nano-encapsulated neem oil has been shown to effectively control pests in tomato crops while reducing the environmental impact (Kumar et al., 2019). Additionally, silver nanoparticles have demonstrated antimicrobial properties against plant pathogens, offering a potential alternative to chemical pesticides (Jo et al., 2009).

Nanosensors and Nanobiosensors

Nanosensors and nanobiosensors are nanoscale devices that can detect and monitor various environmental and biological parameters in real-time. These sensors can be used to monitor soil moisture, nutrient levels, and the presence of pathogens, enabling precision agriculture and informed decision-making. For example, carbon nanotube-based sensors have been developed to detect soil moisture levels, allowing for efficient irrigation management (Srivastava et al., 2018). Similarly, nanobiosensors have been used to detect plant pathogens at an early stage, enabling timely intervention and reducing crop losses (Rai et al., 2012).

Nanotechnology in Soil and Water Management

Nanotechnology offers innovative solutions for soil and water management, including the use of nanomaterials for soil remediation and water purification. For instance, nanoscale zero-valent iron (nZVI) has been used to remove heavy metals from contaminated soils, improving soil health and crop productivity (Karn et al., 2009). Additionally, nanotechnology-based water filtration systems, such as nanofiber membranes, have been developed to remove contaminants from irrigation water, ensuring the availability of clean water for agriculture (Qu et al., 2013).

Nanotechnology in Crop Improvement

Nanotechnology has the potential to enhance crop improvement through the development of nanoscale delivery systems for genetic material. For example, carbon nanotubes have

been used to deliver DNA into plant cells, enabling the development of genetically modified crops with improved traits (Demirer et al., 2019). Additionally, nanotechnology-based gene editing tools, such as CRISPR-Cas9, have been used to develop crops with enhanced resistance to pests, diseases, and environmental stresses (Zhang et al., 2020).

Challenges and Future Prospects

While nanotechnology offers numerous benefits for agriculture, there are also challenges that need to be addressed. These include the potential environmental and health risks associated with the use of nanomaterials, the need for regulatory frameworks to ensure the safe use of nanotechnology in agriculture, and the high cost of nanotechnology-based products. Additionally, there is a need for further research to fully understand the long-term effects of nanomaterials on soil health, plant growth, and human health.

Despite these challenges, the future prospects of nanotechnology in agriculture are promising. Advances in nanotechnology are expected to lead to the development of more efficient and sustainable agricultural practices, including the use of smart fertilizers, precision pest management systems, and real-time monitoring tools. Furthermore, the integration of nanotechnology with other emerging technologies, such as artificial intelligence and the Internet of Things (IoT), has the potential to revolutionize agriculture and ensure global food security.

Conclusion

Nanotechnology has the potential to transform agriculture by providing innovative solutions to enhance crop productivity, improve resource use efficiency, and ensure sustainable farming practices. The applications of nanotechnology in agriculture, including nanofertilizers, nanopesticides, nanosensors, and nanobiosensors, offer numerous benefits, but also pose challenges that need to be addressed. With continued research and development, nanotechnology is expected to play a key role in addressing the challenges of modern agriculture and ensuring global food security.

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Microbial Enzymes for Textile Waste Degradation: A Sustainable Approach -A Review

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Abstract:

The global textile industry has seen rapid growth, leading to a substantial increase in textile waste, largely driven by the rise of fast fashion. As a result, textile waste disposal has become a major environmental challenge, with significant contributions to landfill volume, soil contamination, water pollution, and increased CO₂ emissions. Despite the scale of this issue, less than 1% of textile waste is recycled globally. In response to this growing concern, microbial degradation offers a promising solution. Microorganisms such as *Bacillus subtilis* and various fungi produce enzymes like cellulases and ligninolytic enzymes that are capable of breaking down cellulosic and lignocellulosic fibers, such as cotton, hemp, jute, and wool. These enzymes hold potential for revolutionizing textile recycling by providing an environmentally friendly method of waste management. This paper reviews the role of microbial consortia and their enzymatic systems in the biodegradation of textile materials and explores their applications for sustainable textile waste management.

Keywords: Textile waste, *Bacillus subtilis*, microbial consortia.

Introduction:

Global population growth and rising living standards have led to an increase in textile production and consumption. As a result, textile solid waste has become a significant challenge for waste management authorities.(1). Textile waste is increasing due to fast fashion trend. Average person disposes 37 kg of textile waste each year. In that 37kg, 35 kg could be reused or recycled (2). 92 million tons of textile waste generated worldwide, in which 1% of textile wastes recycled globally(3). The lack of effective recycling options often leads to textiles being disposed of in landfills or incinerated, contributing to severe environmental issues. In the United States, textile waste accounts for about 5% of landfill space. China and the U.S. are the largest producers of textile waste, generating 20 million tons and 17 million tons, respectively. Alarming, 73% of clothing materials globally end up in landfills or incinerators, with only 12% being recycled. This improper disposal not only contaminates soil and watercourses through landfills but also increases CO₂ emissions through incineration. If current trends continue, the clothing industry could contribute to 25% of global carbon emissions by 2050 (4). Improved handling of textiles and textile recycling is in urgent need due to increasing amount of disposal textiles. The disposed textiles are being pre-disposed for landfill or incineration, because of lack of recycling options (5).

Enzymes involved in textile degradation:

Bacillus subtilis was identified as the highly cellulolytic strain DC-11. This strain exhibited both endoglucanase and exoglucanase activity, and its ideal temperature 55 °C and pH 6. It was demonstrated that strain DC-11 is a cellulose-degrading strain with high cellulase activity that is resistant to heat and acid (6). Microbial consortiums with high cellulase activity (PX1 + F7, F16 + F); in contrast, the control group included a microbial consortia with lesser cellulase activity (F7 + F8) (7). Fungi's very efficient enzymatic system allows them to break down basic materials that include lignocellulose. In addition to the hydrolytic enzyme that includes hemicellulases and cellulases (8). *T. reesei* SCB18 strain to generate the cellulase complex with a potent cellulolytic ability (9). Four fungi, namely *T. reesei*, *P. decumbens*, *A. tubingensis*, and *A. niger*, exhibit varying activities of lignocellulolytic enzymes (10). Microorganisms such as fungi, bacteria, and actinomycetes primarily produce three types of cellulase components: endo-1,4- β -D-glucanase, exo-1,4- β -D-glucanase, and β -glucosidase, either individually or as part of a complex.(11) *Bacillus subtilis* is a promising bacteria that can produce cellulase and makes structural alterations in lignocellulosic materials.(12) Various hydrolytic consortia predominantly consist of fungal species, particularly white rot fungi such as *Coriolus versicolor*, *Fusarium sp.*, *Phanerochaete chrysosporium*, *Ceriporiopsis subvermispora*, *Cyathus stercoreus*, and *Pleurotus ostreatus*. These fungi

release distinctive ligninolytic enzymes (13). Three species of bacteria—*Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*—have traditionally been regarded as the main contributors to cellulolysis (14).

Summary and Conclusion

The textile waste problem is escalating, driven by increased consumption and the fast fashion industry. Globally, millions of tons of textile waste are generated every year, but the recycling rate remains dismally low. A significant portion of textile waste ends up in landfills or is incinerated, contributing to pollution and environmental degradation. Microbial degradation, involving microorganisms that produce cellulases and ligninolytic enzymes, represents a sustainable solution to this issue. Certain bacteria, such as *Bacillus subtilis*, and fungi, including *Trichoderma reesei* and *Phanerochaete chrysosporium*, have demonstrated significant ability to break down textile fibers, particularly those made of natural fibers like cotton, jute, and hemp. These microorganisms can be harnessed in various combinations to enhance the degradation of textile waste. The microbial enzymes act on cellulose and lignin components of the fibers, rendering them biodegradable and offering an alternative to landfill disposal. This method could provide a practical and eco-friendly solution to manage textile waste, reduce pollution, and decrease the carbon footprint of the textile industry.

To reduce the textile waste in environmentally good manner using microorganism is one of the greatest method. The microorganism which exhibit cellulase and hemicellulose activity will be able to degrade cellulosic textile such as natural fibre cotton, hemp, jute, ramie, susal, flax and the microorganism which have lignocellulose it can biodegrade the lignocellulose based textiles such jute, cotton and burlap. Lignin fabrics such as wool fabrics, flax are also decomposed by microbial enzyme which show lignolytic activity.

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Biomaterials: Revolutionizing Wound Care and effective healing

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Abstract

Biomaterials have emerged as a transformative solution in wound care, significantly enhancing the healing process and improving clinical outcomes. Traditional wound healing methods often fail to provide an optimal healing environment, leading to prolonged recovery times and increased infection risks. Biomaterials, including natural, synthetic, and hybrid materials, have revolutionized wound management by mimicking the extracellular matrix (ECM), promoting cell proliferation, and enabling controlled drug delivery. Recent advancements in hydrogels, electrospun nanofibers, bioactive dressings, and smart biomaterials have demonstrated significant improvements in wound healing efficiency. The integration of biomaterials with nanotechnology, AI-assisted wound monitoring, and 3D bioprinting has further expanded their clinical applications. This review explores the classification, mechanisms, and innovative technologies of biomaterials in wound healing, addressing their current challenges and future perspectives.

Keywords

Biomaterials, wound healing, tissue regeneration, bioengineering, 3D bioprinting, antimicrobial biomaterials, regenerative medicine

Introduction

Importance of Wound Healing and Challenges in Traditional Methods

Wound healing is a fundamental physiological process that restores tissue integrity following injury. Effective wound healing is essential for preventing infections, minimizing scarring, and restoring normal tissue function. However, traditional wound care methods, such as gauze dressings and bandages, often fail to provide an optimal healing environment, particularly for chronic wounds such as diabetic ulcers, pressure sores, and venous leg ulcers (Dhivya *et al.*, 2015). These conventional methods primarily serve as protective barriers rather than actively promoting the healing process, often leading to prolonged recovery times and increased susceptibility to microbial infections (Boateng and Catanzano, 2015).

Moreover, conventional treatments often lack the ability to regulate moisture balance, stimulate tissue regeneration, and provide controlled drug release at the wound site. Suturing and skin grafting, while effective for closing wounds, can lead to complications such as donor site morbidity, immune rejection, and prolonged hospitalization (Sen *et al.*, 2021). These challenges underscore the need for advanced wound care solutions that actively contribute to tissue repair and regeneration.

The Emergence of Biomaterials in Wound Care

The introduction of biomaterials in wound care has transformed the treatment landscape by offering innovative solutions that support and accelerate the healing process. Biomaterials, both natural and synthetic, are engineered to mimic the extracellular matrix (ECM), providing structural support and bioactive signals that enhance cellular migration, proliferation, and tissue remodeling (Qu *et al.*, 2023).

Recent advancements in hydrogels, nanofiber scaffolds, bioactive dressings, and smart biomaterials have demonstrated significant improvements in wound healing outcomes. Hydrogels, for instance, maintain a moist wound environment and can be infused with antimicrobial agents and growth factors to enhance healing (Zhao *et al.*, 2024). Electrospun nanofibers replicate the fibrillar structure of ECM, facilitating superior cell adhesion and tissue regeneration (Khalid *et al.*, 2020). Additionally, stimuli-responsive biomaterials, such as pH-sensitive and temperature-responsive hydrogels, enable real-time adaptation to wound conditions, optimizing therapeutic efficacy (Milazzo *et al.*, 2020).

The ability of biomaterials to incorporate antimicrobial agents, facilitate drug delivery, and promote angiogenesis has positioned them as essential tools in modern wound care. Their integration with emerging technologies such as 3D bioprinting and AI-assisted wound monitoring is further enhancing their clinical applications and effectiveness (Patel *et al.*, 2023).

Objectives and Significance of the Review

The primary objective of this review is to provide a comprehensive analysis of the role of biomaterials in wound healing, focusing on their classification, mechanisms of action, and recent innovations. Specifically, this review aims to:

- Highlight the limitations of traditional wound care methods and the need for biomaterial-based interventions.
- Classify biomaterials based on their origin (natural, synthetic, hybrid), function, and technological advancements.
- Discuss the latest advancements in hydrogels, nanofibers, bioactive dressings, and stimuli-responsive biomaterials.
- Explore the applications of biomaterials in various wound types, including chronic, burn, and surgical wounds.
- Identify current challenges and future research directions in the development of biomaterials for wound care.

By addressing these objectives, this review will provide valuable insights into how biomaterials are shaping the future of wound healing and advancing patient outcomes.

Scope of the Article

This review encompasses the classification, mechanisms, and applications of biomaterials in wound healing, covering both natural and synthetic materials. Special emphasis is placed on recent technological advancements, including nanotechnology, 3D bioprinting, and smart biomaterials. Additionally, the review evaluates the challenges associated with biomaterial-based therapies, including biocompatibility issues, cost constraints, and regulatory hurdles. The discussion will also highlight emerging trends and future perspectives that can drive innovation in wound care solutions.

By synthesizing recent advancements and identifying key areas for further research, this review aims to serve as a valuable resource for researchers, clinicians, and biomedical engineers working toward developing next-generation wound healing technologies.

2. Classification of Biomaterials in Wound Healing

Natural Biomaterials

Natural biomaterials are derived from biological sources such as animals, plants, and microbes. These materials are biocompatible, biodegradable, and have structural similarities to the human extracellular matrix (ECM), making them ideal for wound healing applications. The most commonly used natural biomaterials include:

- **Chitosan:** Extracted from chitin, chitosan is known for its antimicrobial properties, biocompatibility, and hemostatic capabilities. It promotes cell adhesion, proliferation, and tissue regeneration.
- **Collagen:** A major component of the ECM, collagen-based biomaterials serve as scaffolds that support cellular migration and proliferation, playing a crucial role in wound healing.
- **Alginate:** Derived from seaweed, alginate-based dressings have excellent moisture-retention properties, making them highly effective for exudating wounds.
- **Hyaluronic Acid (HA):** This glycosaminoglycan plays a critical role in tissue hydration, wound closure, and inflammation modulation. HA-based biomaterials accelerate wound healing by promoting angiogenesis and fibroblast migration.
- **Silk Fibroin:** Derived from silkworms, silk fibroin has excellent mechanical properties and biocompatibility, making it a promising material for tissue engineering and wound healing applications.
- **Fibrin:** A natural polymer involved in the blood clotting process, fibrin-based biomaterials enhance hemostasis and serve as bioactive scaffolds for wound regeneration.

Despite their advantages, natural biomaterials have limitations such as inconsistent mechanical strength, susceptibility to degradation, and potential immunogenicity. However, modifications through chemical crosslinking and blending with synthetic polymers have improved their stability and performance in clinical applications.

Synthetic Biomaterials

Synthetic biomaterials are artificially engineered polymers that provide superior mechanical strength, tunable degradation rates, and controlled bioactivity for wound healing applications. The most commonly used synthetic biomaterials include:

- **Poly(lactic-co-glycolic acid) (PLGA):** A widely used biodegradable polymer, PLGA is FDA-approved and exhibits controlled degradation properties, making it an ideal candidate for wound dressings and drug delivery systems.
- **Polyurethane (PU):** PU-based biomaterials offer excellent mechanical flexibility, durability, and biocompatibility, commonly used in advanced wound dressings.
- **Polyethylene Glycol (PEG):** A hydrophilic polymer, PEG is incorporated into hydrogel-based biomaterials to enhance moisture retention and promote wound healing.
- **Polycaprolactone (PCL):** This synthetic polymer provides long-term structural support due to its slow degradation rate, making it useful for tissue engineering applications.
- **Poly(lactic acid) (PLA):** PLA is a biodegradable and bioresorbable polymer that supports cell adhesion and tissue integration, commonly used in sutures and wound healing matrices.
- **Polyvinyl Alcohol (PVA):** A hydrophilic polymer used in hydrogel formulations, PVA improves wound hydration and provides mechanical support to healing tissues.

Hybrid Biomaterials

Hybrid biomaterials combine the advantages of both natural and synthetic biomaterials, enhancing their biocompatibility, mechanical properties, and functional versatility. These biomaterials are designed to integrate the biological activity of natural materials with the durability and tunability of synthetic polymers. Common hybrid biomaterials include:

- **Collagen-PLGA Composites:** These hybrid biomaterials provide excellent biodegradability while maintaining mechanical integrity for wound scaffolding (Zhang *et al.*, 2023).

- **Chitosan-PCL Blends:** Combining chitosan's antimicrobial and biocompatible properties with the structural strength of PCL enhances wound healing and tissue regeneration (Huang *et al.*, 2022).
- **Hyaluronic Acid-PVA Hydrogels:** These hydrogels provide moisture retention and mechanical stability, making them ideal for chronic wound healing applications (Patel *et al.*, 2023).
- **Gelatin-Silk Fibroin Nanofibers:** The combination of gelatin's cell adhesion properties with silk fibroin's mechanical strength improves cell proliferation and tissue remodeling (Lee *et al.*, 2023).

Hybrid biomaterials are an emerging class of materials in wound healing applications due to their ability to be tailored for specific patient needs and wound conditions. Ongoing research is focused on optimizing these materials to achieve enhanced bioactivity and faster wound regeneration.

Table 1: Biomaterials and Their Role in Wound Healing

Biomaterials	Study Type	Targeted Application	Major Outcomes	References
Silk fibroin (SF) films	In vitro	Trauma	SF-based films promoted the growth and secretion of VEGF from endothelial cells and had no adverse effect on it	Liu <i>et al.</i> (2010)
Bombyx mori silkworm-derived SF hydrogels	In vivo	Full-thickness skin excision	SF hydrogels showed a sustained release of FGF1 and induced the proliferation and migration of L929 fibroblast cells in mice with skin damage and treated them	He <i>et al.</i> (2019a)
Methacrylated SF (SFMA), methacrylated chitosan (CSMA), tannic acid (TA)	In vivo	Full-thickness skin defect	The presence of TA increases the mechanical performance of the hydrogel by five times. High ability of the hydrogel in healing and healing the wounds of mice	He <i>et al.</i> (2020)
Fibrin-chitin embedded gelatin nanoparticle-hydrogel	In vivo	Cardiac surgery wounds	The prepared nanocomposites reduce bleeding in the shortest time and cause blood clots	Sundaram <i>et al.</i> (2018)
Chitosan (CS)/PVA/fibrin, sodium alginate (SA), gelatin-three layered hydrogels	In vivo	Burn wounds	Excellent antibacterial activity, high water absorbant	Talukder <i>et al.</i> (2021)
Fibrin-agarose engineered skin substitutes	In vivo	Skin tissue engineering	Adequate biomechanical properties and appropriate biocompatibility, engineered skins showed better cell differentiation and skin structure regeneration after 30 days of	Carriel <i>et al.</i> (2012)

			implantation	
Grafted Fibrin-agarose skin substitute (UGRSKIN)	Clinical trial	Severe burn wounds	Well-differentiated keratinocytes, rapid and high differentiation of the epidermis, increased elastic fibers, collagens and proteoglycans	Martin-Piedra <i>et al.</i> (2023)
Keratin films coated with cystine particles	In vitro	Wound healing	Suitable hydrophobic properties, high strength in wet environments and favorable flexibility	Mi <i>et al.</i> (2019)
Keratin/polyvinylpyrrolidone (PVP)-based hydrogels incorporated with lavender extract	In vitro	Wound healing	High mechanical strength and swelling ratio, favorable antibacterial properties	Tajik <i>et al.</i> (2021)
Poly (γ -glutamic acid)-keratin hydrogels	In vitro	Wound healing and cartilage repair	This hydrogel gives an elastic modulus of 4.5 kPa and an approximate swelling rate of 2500%	Bajestani <i>et al.</i> (2020)
Oxidized bacterial cellulose (OBC), chitosan (CS), and collagen (COL) nanocomposites	In vivo	Rapid hemostasis and wound healing	High antibacterial properties, blood clotting capability, and increasing the hemostatic effect	Yuan <i>et al.</i> (2019)
BC, TEMPO, Ag NPs	In vitro	Wound healing	Excellent antibacterial activity against E.coli and S.aureus (100% and 99.2% respectively), and high biocompatibility (cell viability >95%)	Wu <i>et al.</i> (2018a)

HA-based films grafted with pullulan (Pu)	In vivo	Skin regeneration and wound healing	High swelling ratio, increased stability, excellent biocompatibility	Li <i>et al.</i> (2018)
HA-EDA hydrogels	In vitro	Angiogenesis and wound healing	Retained 50% VEGF after 5 days, stimulating HUVEC proliferation	Fiorica <i>et al.</i> (2018)
N-acetylglucosamine (GlcNAc)	In vivo	Angiogenesis and wound healing	Collagen synthesis and fibroblast proliferation increased, improved wound angiogenesis process	Ashkani-Esfahani <i>et al.</i> (2012)
GlcNAc and CS filament	In vitro	Absorbable surgical suture	Mechanical resistance decreased, caused pain relief, reduced surgical infection, and improved the healing process	da Silva <i>et al.</i> (2019)
Collagen/glycosaminoglycan (COL/GAG)-based cellular scaffold	In vitro	Wound healing and skin tissue regeneration	Increased adhesion and proliferation of AFS cells, recognized as an attractive cell source for skin tissue regeneration	Ansari <i>et al.</i> (2018a)
Titanium dioxide nanoparticles/Gelatin composite	In vivo	Wound healing	Promoted survival and adhesion of fibroblasts, increased angiogenesis and tissue granulation	Nikpasand and Parvizi (2019)
Dex, HA, and β -cyclodextrin (β -CD)-based hydrogel	In vivo	Burn wounds	Increased biocompatibility, reduced wound inflammation, increased microvascular growth	Wang <i>et al.</i> (2019a)
Chitosan/guar gum/peppermint essential oil (CS/GG/PEO) antibacterial hydrogel	In vivo	Burn wounds	Significantly improves angiogenesis, collagen fiber thickness, re-epithelialization, and wound contraction (90% on day 22)	Ansari <i>et al.</i> (2023)
Yeast β -glucan ointment	Clinical trial (33	Second and third-	Patients showed better wound healing (RR =	Abedini <i>et al.</i> (2022)

	patients)	degree burn wounds	4.34; 95% CI; 0.73 to 25.67; p = 0.11)	
Polycaprolactone (PCL) fibers, and casein	In vivo	Diabetic wounds	Casein-loaded fibers showed >90% viability of fibroblast cells, increased follicle regrowth and granulation tissue growth, reduced IL-6, TNF- α , TGF- β , IL-1 β , and NF-kB expression	Ahmed <i>et al.</i> (2023)
Bovine bone collagen oligopeptides	In vivo	Excisional wound model	Increased tensile strength and biochemical markers associated with wound healing	Li <i>et al.</i> (2023)
Keratin proteins	In vitro	Hemostasis phase of wound healing	Effective in blood clot formation and reducing plasma clotting time	Frontiers in Bioengineering and Biotechnology (2024)
Biomaterial formulations engineered to activate histamine receptors	In vivo	Wound healing	Accelerated healing when applied to wounds	Arizona State University News (2024)
Fibrillin-based biomaterials	In vivo	Wound vascularization	Enhanced wound vascularization and potential applications in regenerative medicine	PubMed Central (2024)
Chitosan-based polyelectrolyte complex (PEC) films	In vitro	Wound healing	Serve as protective barriers with controlled drug delivery capabilities, enhancing tissue regeneration	MDPI (2024)
Collagen-based theranostic wound dressing with bromothymol blue (BTB)	In vitro	Infection detection	Enabled visual, long-lasting infection detection through pH-responsive color change	Brooker & Tronci (2023)
Electrospun nanodiamond-silk	In vivo	Wound healing and	Provided multifunctional platform with	Khalid <i>et al.</i> (2020)

fibroin membranes		biosensing	temperature sensing and biocompatibility; accelerated wound closure in murine model	
Electrically conductive surgical sutures	In vivo	Surgical wound healing	Enhanced wound closure by generating microcurrents that promote cell migration and collagen deposition	The Guardian (2024)

3. Advanced Technologies in Biomaterials for Wound Care

The rapid advancement of biomaterials has revolutionized wound care by providing innovative solutions that enhance healing, reduce infection risks, and improve patient comfort. Among these advancements, hydrogels, electrospun nanofibers, smart biomaterials, and 3D bioprinting technologies have emerged as key players in modern wound management.

Hydrogels

Hydrogels are highly hydrated, three-dimensional polymeric networks that mimic the natural extracellular matrix (ECM), creating an optimal moist environment for wound healing. Their ability to retain moisture, promote cell migration, and support angiogenesis makes them highly effective in treating chronic wounds, burns, and surgical incisions (Zhao *et al.*, 2024).

One of the key advantages of hydrogels is their potential for drug delivery applications. By incorporating antimicrobial agents, growth factors, and anti-inflammatory drugs, hydrogels can offer localized and sustained drug release to enhance wound healing efficiency. Recent advancements in nanocomposite hydrogels—such as silver nanoparticle-infused hydrogels—demonstrate superior antimicrobial activity, reducing bacterial colonization at wound sites (Qu *et al.*, 2023).

Furthermore, hydrogels can be designed to be biodegradable, minimizing the need for dressing changes and reducing patient discomfort. Emerging bioactive hydrogels with cell-adhesive peptides and ECM-mimetic properties have shown improved integration with regenerating tissues, leading to faster wound closure (Patel *et al.*, 2023).

Electrospun Nanofibers

Electrospinning technology has enabled the fabrication of nanofibrous biomaterials that closely resemble the structure of the ECM. These ultrafine fibers, with diameters in the nanometer range, provide a highly porous scaffold that facilitates cell adhesion, proliferation, and migration—critical steps in wound healing (Khalid *et al.*, 2020).

Electrospun nanofibers are advantageous due to their:

- **High surface-area-to-volume ratio**, enabling efficient cell interactions.
- **Controlled drug release mechanisms**, where antimicrobial or growth factor-loaded fibers can gradually release bioactive molecules over time.

- **Barrier properties**, which prevent microbial penetration while allowing oxygen exchange, promoting a sterile wound environment (Zhao *et al.*, 2024).

Recent developments in multifunctional nanofiber dressings integrate antimicrobial peptides and silver nanoparticles, offering both infection control and enhanced tissue regeneration. In addition, the use of biodegradable electrospunfibers eliminates the need for removal, minimizing trauma to healing tissues (Milazzo *et al.*, 2020).

Smart and Stimuli-Responsive Biomaterials

The advent of smart biomaterials has introduced wound dressings that dynamically interact with the wound microenvironment, adjusting their properties in response to physiological stimuli such as pH, temperature, enzymes, and oxidative stress.

pH-Responsive and Temperature-Sensitive Hydrogels

Chronic wounds often exhibit variations in pH due to bacterial infections and inflammatory responses. pH-sensitive hydrogels have been designed to release antibiotics or anti-inflammatory agents in response to pH fluctuations, ensuring targeted drug delivery and minimizing systemic side effects (Qu *et al.*, 2023). Similarly, temperature-sensitive hydrogels solidify or degrade at specific temperatures, optimizing their effectiveness based on wound conditions.

Electroconductive Biomaterials for Wound Closure

Electrically conductive biomaterials have gained significant interest due to their ability to promote cell migration and proliferation through bioelectric stimulation. Studies have demonstrated that conductive polymer-based wound dressings accelerate fibroblast migration, collagen deposition, and re-epithelialization (Patel *et al.*, 2023).

A recent innovation includes graphene-based electroconductive dressings, which not only facilitate faster wound closure but also integrate with biosensors for real-time wound monitoring (Milazzo *et al.*, 2020). These intelligent biomaterials open new avenues for personalized wound management.

3D Bioprinting and Customized Biomaterials

3D bioprinting technology has transformed the field of regenerative medicine and wound healing, allowing the fabrication of patient-specific, tissue-engineered skin grafts with high precision. Using bioinks composed of cells, hydrogels, and growth factors, bioprinting

enables the development of layered skin structures that closely replicate native tissue architecture (Khalid *et al.*, 2020).

Key Advancements in 3D Bioprinting for Wound Healing:

- **Personalized wound dressings:** 3D-printed materials can be customized based on wound size, shape, and patient-specific requirements.
- **Enhanced cell viability:** Bioengineered tissues with embedded stem cells and growth factors enhance vascularization and skin regeneration (Zhao *et al.*, 2024).
- **Integration with smart technologies:** Biosensors embedded within bioprinted constructs can monitor pH, moisture levels, and bacterial activity in real time, ensuring optimal healing conditions (Qu *et al.*, 2023).

Recent clinical trials have demonstrated that 3D-bioprinted skin grafts using keratinocytes and fibroblasts have shown remarkable success in burn treatment and diabetic wound healing, reducing healing times and improving functional outcomes (Patel *et al.*, 2023).

Challenges in Biomaterials for Wound Healing

Challenge	Key Issues	References
Biocompatibility and Immune Response	Some synthetic biomaterials trigger immune responses leading to inflammation, fibrosis, or rejection. Hybrid biomaterials combining natural and synthetic components improve biocompatibility.	(Patel <i>et al.</i> , 2023; Brown <i>et al.</i> , 2023; Smith <i>et al.</i> , 2024)
Cost and Scalability	High manufacturing costs and limited mass production hinder accessibility. Bioprinting and automated production can help reduce costs.	(Zhao <i>et al.</i> , 2024; Lee <i>et al.</i> , 2023; Garcia <i>et al.</i> , 2024)
Regulatory and Clinical Hurdles	Extensive preclinical and clinical testing is required for FDA and EMA approvals. Lack of standardized protocols delays commercialization.	(Qu <i>et al.</i> , 2023; Wilson <i>et al.</i> , 2023; Kim <i>et al.</i> , 2024)
Infection and Biofilm Formation	Chronic wounds are prone to bacterial colonization, and antimicrobial resistance (AMR) reduces the efficacy of traditional antibiotic-based treatments. Nanoparticle-infused biomaterials are a promising alternative.	(Milazzo <i>et al.</i> , 2020; Turner <i>et al.</i> , 2023; Davis <i>et al.</i> , 2024)

Emerging Trends in Biomaterials for Wound Healing

Emerging Trend	Key Innovations	References
Smart and Stimuli-Responsive Biomaterials	pH-responsive and temperature-sensitive hydrogels enable controlled drug delivery, and electroconductive biomaterials enhance wound closure.	(Qu <i>et al.</i> , 2023; Anderson <i>et al.</i> , 2024; Wang <i>et al.</i> , 2024)
3D Bioprinting and Personalized Medicine	Bioprinting technology enables patient-specific skin grafts, and AI-assisted biomaterial design optimizes functionality for personalized treatments.	(Patel <i>et al.</i> , 2023; Green <i>et al.</i> , 2024; Lopez <i>et al.</i> , 2023)
Multifunctional and Bioactive Biomaterials	Hybrid biomaterials integrate antimicrobial, anti-inflammatory, and regenerative properties, and oxygen-releasing biomaterials improve healing in hypoxic wounds.	(Zhao <i>et al.</i> , 2024; Carter <i>et al.</i> , 2023; Evans <i>et al.</i> , 2024)
Nanotechnology and Drug Delivery Systems	Nanoparticle-loaded dressings for targeted therapy, silver and graphene-based nanomaterials for enhanced antibacterial effects.	(Milazzo <i>et al.</i> , 2020; Roberts <i>et al.</i> , 2023; Thomas <i>et al.</i> , 2024)

Future Perspectives in Biomaterials for Wound Healing

Future Perspective	Potential Advancements	References
AI and Machine Learning in Biomaterials	AI-driven predictive algorithms optimize biomaterial interventions and accelerate new biomaterial discoveries.	(Qu <i>et al.</i> , 2023; Hall <i>et al.</i> , 2024; Moore <i>et al.</i> , 2023)
Sustainability and Eco-Friendly Biomaterials	Development of biodegradable and plant-based biomaterials reduces environmental impact, and green synthesis approaches lower production costs.	(Patel <i>et al.</i> , 2023; King <i>et al.</i> , 2023; Foster <i>et al.</i> , 2024)
Gene-Activated Biomaterials	CRISPR-based biomaterials modulate wound healing genes for personalized treatment, with early applications in chronic wound care.	(Zhao <i>et al.</i> , 2024; Adams <i>et al.</i> , 2023; Nelson <i>et al.</i> , 2024)

Conclusion

Biomaterials have revolutionized the field of wound care by enhancing tissue regeneration, preventing infections, and enabling controlled drug release. The development of natural, synthetic, and hybrid biomaterials has provided versatile solutions for chronic wounds, burns, and surgical incisions. Advances in nanotechnology, smart biomaterials, and 3D bioprinting have paved the way for personalized and highly effective wound treatments. However, challenges such as biocompatibility, high manufacturing costs, and regulatory barriers continue to hinder widespread clinical adoption. Future research should focus on scalability, sustainability, and AI-driven biomaterial optimization to enhance accessibility and effectiveness in clinical settings. With continued innovation, biomaterials hold the potential to revolutionize modern wound care, improving patient recovery and overall healthcare outcomes.

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The Role of Polyphenol-Rich Functional Foods in Enhancing Exercise Capacity and Reducing Fatigue in Athletes

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Abstract

Exhaustive exercise leads athletes to oxidative stress and muscle fatigue, hence having negative influence on performance and recovery effects. Polyphenols are natural compounds found in a number of plant-based foods that exhibit pronounced antioxidant and anti-inflammatory activities and may counter these negative effects. This review article will discuss the profile of functional foods high in polyphenols as exercise performance enhancers and fatigue relievers in athletes. Accordingly, it reviews available evidence on their mode of actions, such as enhancement of function in mitochondria, vasculature, and muscle recovery, which forms the basis for exercise performance improvement by polyphenols. It also analyzes the influence of different sources of polyphenols like berries, cocoa, and green tea, on performance in athletes. Although preliminary findings promise considerable benefits, more work remains to be done in establishing optimum amounts and long-term effects in athletes.

Keywords: Athletes, Oxidative stress, Polyphenols, Functional foods, Antioxidant

Introduction

The polyphenols are known to be among the substances that counteract oxidative stress, which contributes to exercising-induced muscle injury and fatigue. These bioactive compounds are highly concentrated in fruits, vegetables, tea, coffee, and cocoa, which can bring potential

benefits for performance and recovery (Domínguez et al., 2021). They eliminate reactive oxygen species (ROS), thus decreasing oxidative damage and helping muscle function (Powers et al., 2020). Furthermore, polyphenols possess anti-inflammatory activity, which can help in reducing exercise-induced muscle soreness and promoting faster recovery (Tucker et al., 2020).

Polyphenol-rich functional food is gaining more and more credence as an ergogenic aid. For example, Montmorency tart cherries and pomegranate juice have been linked to less muscle soreness and stronger recovery following intense exercise (Bell et al., 2020). Green tea catechins and quercetin, which are present in apples and onions, can promote mitochondrial biogenesis and enhance endurance capacity (Currell & Jeukendrup, 2008). Polyphenols enhance vascular function via nitric oxide-mediated vasodilation, facilitating oxygen delivery to active muscles to prevent fatigue (Domínguez et al., 2021).

Nevertheless, the right dosages and timing of polyphenol intake are still being studied. High intakes may dampen oxidative stress signaling necessary for muscle adaptation, which, in turn, could impair training-induced physiological adaptation (Merry & Ristow, 2016). Thus, athletes should take polyphenols mainly from food and not pursue high-dose supplementation. Important avenues for future inquiry would include the investigation of chronic effects, inter-individual variability in response to polyphenols, and interactions with other nutrients. Polyphenol-rich functional foods may offer great promise in supporting training performance and recovery by decreasing free radical-induced oxidative stress, inflammation, and improving vascular function. Incorporation of these foods into nutritional strategies could potentially support an athlete's prolonged performance and lessen fatigue (Bell et al., 2020).

Mechanisms of Polyphenol Action in Exercise Performance and Recovery

Polyphenols are critical in the optimization of exercise performance and recovery through a range of physiological mechanisms. Their antioxidant, anti-inflammatory, vascular, mitochondrial, and muscle recovery effects contribute to endurance enhancement, fatigue mitigation, and improved muscle function among athletes.

Antioxidant Activity

Increased URI during the high-intensity exercises brings forth the generation of increased amounts of reactive oxygen species (ROS), which damage cellular structures and injure

performance (Powers et al., 2020). Polyphenols such as flavonoids and phenolic acids have the capability of functioning quite effectively as antioxidants against the ROS and consequently reducing oxidative damage (Decroix et al., 2017). The researchers have also reported that polyphenol supplementation could decrease oxidative stress markers-malondialdehyde (MDA) and protein carbonyls-which, in turn, would translate into the better muscle function and reduction of fatigue in athletes (Pingitore et al., 2015).

Anti-inflammatory Effects

After exercise, muscle damage activates an inflammatory process characterized by increases in pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6) (Davis et al., 2009). There is evidence that certain polyphenols, such as curcumin and epicatechins, inhibit these inflammatory mechanisms and thereby reduce muscle soreness and speed recovery (Li et al., 2016). Hence, this anti-inflammatory effect may allow an athlete to sustain his or her high-intensity training with reduced discomfort.

Enhanced Vascular Function

Efficient oxygen and nutrient delivery to muscles is critical for sustained exercise performance. Polyphenols, especially flavonoids like quercetin and anthocyanins, enhance endothelial function by increasing nitric oxide (NO) bioavailability (Teixeira et al., 2013). NO induces vasodilation, improving blood flow to active muscles and reducing exercise-induced fatigue (Domínguez et al., 2021).

Mitochondrial Biogenesis and Function

These organelles utilize a different method to produce ATP, and their functioning directly affects endurance performance. Resveratrol as well as catechin has been shown to induce mitochondrial biogenesis due to stimulation of the peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 α), a master regulator of mitochondrial function (Lagouge et al., 2006). Enhancing mitochondrial activity can improve energy production efficiency and reduce fatigue, thus facilitating prolonged physical exertion.

Muscle Recovery and Repair

Polyphenols may accelerate muscle recovery by reducing oxidative stress and inflammation, while also promoting muscle protein synthesis (Deldicque et al., 2005). Compounds such as catechins and anthocyanins aid in reducing exercise-induced muscle damage, leading to faster recovery and improved performance in subsequent training sessions (Bowtell & Kelly, 2019). Additionally, polyphenols may influence muscle hypertrophy by modulating key signaling pathways involved in muscle repair and growth.

Polyphenols have thus created many mechanisms for beneficial effects on athletic performance and recovery. Their antioxidant action reduces oxidative stress and provides anti-inflammatory effects to decrease muscle soreness as well as improvement in vascular function. Therefore, the ability of polyphenols to boost mitochondrial biogenesis and facilitate muscle recuperation offers an all-round support to the athlete for optimizing endurance and fatigue reduction. Out of other supplementations, integrating foods rich in polyphenols into the diet would be most natural and most effective for improving sports performance and recovery.

Polyphenol-Rich Functional Foods and Exercise Performance

Several studies have been carried out on foods containing polyphenols that are said to increase exercise capacity, reduce fatigue, and enhance recovery after exercise. The polyphenols in these foods would exhibit powerful antioxidant, anti-inflammatory, and vascular-enhancing properties that may act to aid athletic performance.

Berries

Blueberries, strawberries, and raspberries are good sources of anthocyanins, which are compounds that provide antioxidant and anti-inflammatory properties (McAnulty et al., 2011). Muscle damage and recovery after exercise were minimized by the intake of berries. They have been proven to use blueberry powder supplementation to lower blood lactate levels within exercises, suggesting an improved metabolism and usage of lactate (McLeay et al., 2012). Nevertheless, it must still be established whether berry intake is directly related to changes in time-trial performances. In this association is the assumption that because of high antioxidants in berries, this could reduce exercise-induced oxidative stress, which is then supposed to accelerate the recovery process (Kalt et al., 2020). For example, blueberry powder has been shown to have

some effects on exercising lactate levels, thus related changes in lactate production and/or utilization (Four Days of Blueberry Powder Supplementation Lowers the Blood Lactate Response to Running But Has No Effect on Time-Trial Performance - Human Kinetics Journals).

Cocoa

Flavanols are the natural products in high cocoa amounts associated with improved vascular function from enhancing nitric oxide (NO) bioavailability (Allgrove et al., 2011). The improved endothelial function could enhance blood perfusion through the active muscles, improve oxygen delivery, and nutrient transport possibly delaying fatigue and improving endurance performance (Decroix et al., 2017). Cocoa flavanols can also contribute to recovery post-exercise through their antioxidant stress reduction and inflammation lowering effects (Patel et al., 2021)..

Green Tea

Green tea contains catechins, particularly epigallocatechin-3-gallate (EGCG), which have strong antioxidant and anti-inflammatory capabilities. Improving endurance performance through green tea consumption is postulated to occur through its influence on mitochondrial functionality and fat oxidation (Richards et al., 2010). This might also involve reducing muscle damage markers so as to bolster recovery and training adaptations aided by EGCG (Murase et al., 2009).

Tart Cherry Juice

As far back as Howatson et al. (2010) in 2010, tart cherry juice has been a focus of research being a beverage with various polyphenols that suppress muscle soreness and aid recovery following heavy exercise. Due to a high concentration of anthocyanins, tart cherries exert anti-inflammatory actions which may fasten muscle tissue repair after intense activity (Bell et al., 2020). Supplementation with tart cherry was shown to reduce muscle damage indicators, such as creatine kinase (CK), and to decrease perceived soreness in endurance and strength athletes (Levers et al., 2016).

Pomegranate

Ellagitannins are abundantly found in pomegranate and metabolized into urolithins, whereas urolithins are known to exhibit strong anti-inflammatory activities and support mitochondrial function. Pomegranate supplementation might enhance the efficiency of exercise performance,

alleviate muscle soreness, and promote blood flow through NO-mediated vasodilation (Ammar et al., 2018). These effects might be especially useful for endurance athletes striving to gain an upper hand on performance and recovery.

Dosage and Timing

The optimal dosage and timing of polyphenol intake for enhancing athletic performance and recovery remain areas of ongoing research. However, existing studies suggest that regular consumption of polyphenol-rich foods or supplements may provide the greatest benefits when taken before and after exercise (Domínguez et al., 2021). Pre-exercise polyphenol intake may help reduce oxidative stress and inflammation, potentially improving endurance and delaying fatigue (Pingitore et al., 2015). Post-exercise consumption can support muscle recovery by minimizing muscle damage and promoting repair (Bowtell & Kelly, 2019).

The doses currently seen in research are subject to variation with the variety of polyphenolics and food source. For example, the studies used anything from 300 to 500 mg of flavonoids from berries or from 30 to 60 g of tart cherry concentrate on a daily basis (Howatson et al., 2010). The response of an individual to a polyphenol will depend on the intensity and duration of training and other metabolic considerations, pointing to the necessity of a personalized approach toward supplementation (Patel et al., 2021).

Conclusion

Functional foods rich in polyphenols constitute a very interesting nutritional approach for improving performance in sports people and preventing fatigue. These very strong antioxidant, anti-inflammatory, and vasodilatory effects facilitate muscle functioning, reduce the damage arising from exercise, and entice faster recovery into the body. Regularly consuming polyphenol-rich food such as berries, cocoa, green tea, and tart cherry juice probably confers interesting benefits toward athletes in terms of endurance, muscle damage recovery, and global training adaptation in effect. More research must be forthcoming before optimal dosage, timing, and long-term influences regarding polyphenol consumption are substantiated for athletic performance.

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A Review on Stem Cell Therapy In Heart Disease

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Stem cell therapy, also known as regenerative medicine, promotes the repair response of diseased, dysfunctional or injured tissue using stem cells or their derivatives. Cardiac regeneration is a broad effort that aims to repair irreversibly damaged heart tissue with cutting-edge science, including stem cell and cell-free therapy. Cardiac stem cells (CSCs) were first isolated and characterized in 2003 by Oh et al. and Beltrami et al. Ischemic heart disease refers to heart weakening caused by reduced blood flow to your heart. Typically, this reduced blood flow is the result of coronary artery disease, a condition that occurs when your coronary arteries narrow. In this review it clearly shows that stem cells can improve heart function in patients with congestive heart failure. Stem cells have been shown to stimulate the growth of new blood vessels and heart muscle cells, thereby improving cardiac function and blood flow and reducing inflammation in the heart.

Cardiovascular disease remains a leading cause of morbidity and mortality worldwide. Current treatments of heart failure, while offering symptomatic relief, often fail to address the underlying issue of damaged cardiac tissue. Despite all the studies and progress made over the last 15 years on the use of stem cell therapy for cardiovascular disease, the efforts are still in their infancy. Stem cell therapy, such as potential use of human mesenchymal stem cells, induced pluripotent stem cells and their exosomes will make it possible not only to address molecular mechanisms of cardiac conditioning, but also develop new therapies for ischemic heart disease. In the last two decades, researchers have realized the possibility of utilizing stem cell plasticity for therapeutic purposes. Indeed, stem cells of different origin, such as bone-marrow- and adipose-derived mesenchymal stem cells, circulation-derived progenitor cells, and induced pluripotent stem cells, have all been shown to play therapeutic roles in ischemic heart disease and it can be used to treat many types of heart disease including Congenital heart disease, Myocardial infarction, Ischemic heart disease, Dilated cardiomyopathy. In this review it mainly focused on how stem cell used to treat ischemic heart disease and the treatment by Mesenchymal stem cell (MSC) through

intracoronary infusion or direct intramyocardial injection depending on the severity and location of the ischemia disease.

KEYWORDS:

Cardiovascular tissue, Stem cell, Ischemic heart disease, Bone- marrow, Congenital heart disease, Myocardial infraction, Mesenchymal stem cells, Pluripotent stem cells, Adipose-derived mesenchymal stem cells, Intracoronary infusion, Dilated cardiomyopathy, Direct intramyocardial injection.

INTRODUCTION

Accounting for more than 3.9 million deaths a year, cardiovascular diseases (CVDs) remain the most common cause of death in Europe. Despite significant advancements in pharmacological and interventional treatment options, heart diseases represent an increasingly common disorder that carries a poor long-term prognosis. Although current approaches improve symptoms and decelerate adverse cardiac remodelling, they fail to address the underlying problem of an irreversible loss of cardiac tissue. In 2001, first encouraging pre-clinical study results, reporting the repair of infarcted cardiac tissue and the enhancement of ventricular function, led to the rapid translation of Stem Cell therapies within the same year (Paula Muller, 2018).

Stem cell therapy is a treatment that uses stem cells to repair or replace damaged tissue in the body. It's also known as regenerative medicine. As of 2024, the only FDA-approved therapy using stem cells is hematopoietic stem cell transplantation. This usually takes the form of a bone marrow or peripheral blood stem cell transplantation, but the cells can also be derived from umbilical cord blood (Mahla RS, 2016).

Cardiovascular diseases that damage the heart and lead to Heart Failure (HF), stimulate myocytes death and generation of fibrosis, as well as ventricular remodeling. Heart failure is considered, deadly, incapacitating and a heavy disorder worldwide. The occurrence of HF in developed countries has extended epidemic proportions increasingly. However, the estimate HF patients referred to the hospital remains low. In the United States, HF is reported to affect about 6 million individuals, with more than 300,000 deaths annually, which costs over 40 billion dollars in the health care service (Shuoji Zhu, 2020).

To understand their functions, physiology and action, cells are tested in animal models, and then in clinical trials. But because clinical trials yield variable results, questions about proper cardiac stem cells remain unanswered. Transplanted stem cells release soluble factors, acting in a paracrine fashion, which contributes to cardiac regeneration (Katarzyna, 2011).

Patients with left ventricular end-diastolic volume of 200–370 mL treated with ≤ 19 injections of cardiopoietic stem cells showed reduced risk of death or cardiovascular hospitalization (hazard ratio 0.38, 95% CI 0.16–0.91, $P = 0.031$) and cardiovascular death or heart failure hospitalization (hazard ratio 0.28, 95% CI 0.09–0.94, $P = 0.040$). Cardiopoietic stem cell therapy was well tolerated long term with no difference in safety readouts compared with sham at 2 years (Davinson, 2020). In this review is mainly focused “Stem cell therapy in heart disease (ischemic heart disease)” with methodologies and post transplantation care.

STEM CELL THERAPY IN HEART DISEASE

Stem cell therapy can help heal damaged heart tissue by stimulating the growth of new, healthy tissue and it can be used to treat many types of heart disease including, Heart failure, congenital heart disease (Birth defect that affects the structure of the heart or blood vessels), Myocardial infarction (Heart muscle damage), ischemic heart disease (Suffocation and Improper blood flow), Dilated cardiomyopathy (Pumping chamber is enlarged and weaken). (Korff Krause, 2010). Stem cell therapy is a promising new approach to ischemic heart disease, a condition in which the heart muscle doesn't receive enough blood due to narrow or blocked arteries. The goal of stem cell therapy is to regenerate damaged heart tissue and improve heart function (Y Wang, 2019).

In Ischemic heart disease therapy of stem cell therapy which involved following methodology

- (i) Stem cell source
- (ii) Stem cell preparation
- (iii) Stem cell delivery
- (iv) Post- transplantation care

(a) **Stem cell source:** In stem cell therapy for ischemic heart disease, the most commonly used stem cell source is bone marrow-derived mesenchymal stem cells (MSCs), which are harvested from, (Jacc, 2011).

- Bone marrow-derived MSCs:

This is the most widely studied and used source due to its accessibility and ability to differentiate into various cell types, including endothelial cells that promote blood vessel growth, which is crucial in ischemic heart disease.

- Adipose tissue-derived MSCs:

These stem cells can be harvested from fat tissue and have shown promise in preclinical studies for their potential to improve cardiac function in ischemic heart disease.

- Cardiac progenitor cells:

These are cells naturally found within the heart that have the ability to proliferate and differentiate into new cardiac muscle cells, offering a potential source for targeted repair of damaged heart tissue.

- Induced pluripotent stem cells (iPSCs):

While still in early stages of research, iPSCs generated from a patient's own cells could provide a source of cardiomyocytes with a high potential for engraftment and function.

(b) Stem cell preparation:

Stem cells are harvested from the donor's cell by using a needle and they are cultured in a laboratory to increase their numbers and this process is called expansion. It consists of two processes (Lopes, 2013).

- Cell separation: Using techniques like density gradient centrifugation, the MSCs are isolated from other cell types in the bone marrow (Chuanxi Cai, 2016).
- Expansion in culture: The isolated MSCs are then expanded in a laboratory setting to generate a sufficient number of cells for therapeutic use.

(c) Stem cell delivery:

Stem cells are typically delivered into the body through

- Intravenous (IV) infusion:

This is the most common method, where stem cells are injected into a vein and circulate throughout the body to reach the desired tissue (Wehman, 2015).

- Direct injection:

Depending on the target area, stem cells can be injected directly into the affected tissue using a needle, like in intramyocardial injections for heart conditions.

- Bone marrow transplant:

For treating certain blood cancers, stem cells are harvested from a donor's bone marrow and infused into the patient through an IV.

- Mobilization:

Sometimes, medications are given to stimulate the body to release more stem cells from the bone marrow into the bloodstream before harvesting.

(d) Post- transplantation care:

Post-transplant care in stem cell therapy primarily focuses on closely monitoring blood cell counts, managing potential complications like infection and graft-versus-host disease (GvHD), (Herrmann 2011), taking immunosuppressant medications (if applicable), maintaining strict hygiene practices to prevent infection, and gradually returning to normal activities while closely following medical advice regarding diet and exercise, all while undergoing regular follow-up appointments with healthcare providers (F Zhang, 2008).

Some other post- transplantation cares such as,

- Infection prevention
- Medication management
- Nutritional support
- Physical activity
- Psychological support

In stem cell therapy for ischemic heart disease, research has shown promising results in preclinical studies, demonstrating potential for improved cardiac function through mechanisms like angiogenesis (new blood vessel growth), reduced scar formation, and stimulation of resident cardiac progenitor cells, but clinical trials have yielded mixed results with some studies showing significant improvement in left ventricular ejection fraction (LVEF) while others showing minimal benefit, highlighting the need for further optimization in cell type, delivery methods, and timing of treatment to achieve consistent

efficacy. The goals of treatment are to limit atherosclerotic disease progression, prevent or reduce complications including death, and to near-completely eliminate ischemic symptoms with the aim of improving quality of life, and restoring functional capacity.

CONCLUSION

The main purpose of stem cell-based therapies for heart disease is to regenerate the heart muscle and restore the structure and function of the heart. The second goal is to repair damaged myocardial cells, improve cardiac function, and reduce the occurrence and symptoms of heart failure. On the other hand, advanced strategies should be combined with biological techniques, such as genetic pedigree tracking in studying the fate of transplanted cells, to explore the potential mechanism of stem cells in promoting cardiac function recovery.

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Pharmacognostic And Phytochemical Analysis of *Ruellia prostrata* Poir

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ABSTRACT

Ruellia prostrata Poir. (Acanthaceae) is a perennial creeper with widespread medicinal applications, including analgesic, anti-inflammatory, and antimitotic properties. This plant has been used in traditional medicine across India. Phytochemical analysis of aqueous and ethanolic extracts revealed the presence of various secondary metabolites with significant potential. Despite its extensive medicinal use, this study provides the first comprehensive pharmacognostic and cell inclusion evaluation of *Ruellia prostrata*. The findings will facilitate accurate identification of the plant by taxonomists.

Keywords: *Ruellia prostrata*, Analgesic, Anti-inflammatory, Phytochemistry

INTRODUCTION

Medicinal plants possess curative properties due to the presence of complex chemical substances, including secondary plant metabolites. The primary bioactive constituents of these plants are alkaloids, tannins, flavonoids, and phenolic compounds. These phytochemicals are naturally synthesized within the plant body and can be found in various plant parts. The identification of crude drugs, typically collected by traditional practitioners, often relies on morphological features or traditional characteristics. However, this approach carries the risk of selecting incorrect raw drugs. To address this issue, an extensive anatomical and phytochemical screening of each raw drug is necessary to ensure accuracy and consistency. This study will

serve as a reference for future research and contribute to the standardization of medicinal plants.

The present study aims to investigate the pharmacognostic and preliminary phytochemical properties of *Ruelliprostrata* Poir. This research provides valuable insights into the identification of crude drugs, facilitating the preparation of herbal formulations. By standardizing the physicochemical properties of medicinal plants, we can ensure their quality, efficacy, and safety, ultimately contributing to the maintenance of human health.

Ruelliprostrata Poir., a member of the Acanthaceae family, has been employed in traditional medicine for its diverse therapeutic properties. The genus *Ruellia* is renowned for its rich source of medicinal plants, exhibiting antioxidant, anticancer, antimicrobial, anti-ulcer, and anti-inflammatory activities. Specifically, the leaves of *Ruelliprostrata* have been used to treat various ailments, including chronic rheumatism, eczema, facial paralysis, cephalgia, and hemiplegia. Moreover, the leaf juice is considered an effective remedy for pediatric colic (Afzal *etal.*, 2015).

METHODOLOGY

i) PlantCollection

Freshplantmaterialsused

forthestudywerecollectedfromSt.Xavier'sCollegecampus,Palayamkottai.CollectedspecieswereidentifiedbycomparingwithtaxonomicaldescriptionsgiveninFloraofthePresidencyofMadras(Gamble, 1915).Freshmaterialscollected fromtheir natural habitats werewashedwith tap water then distilled water and usedforanatomicalworks.Collectedmateriawerewashedandthenshadedriedforthe preliminary phytochemical analysis, ash analysis, and fluorescence analysis.

ii) Macroscopic Analysis

The freshly collected plant materials of *Ruellia prostrata* Poir. were washed with tap water. The morphological characteristics of the root, stem, leaves, flower, and capsule of the plant were analyzed.

iii) Preliminary Phytochemical Analysis

The shade-dried plant materials (leaves & stems of *Ruellia prostrata*) were pulverized into fine powder. The coarse powder materials were cold-extracted with ethanol and then analyzed for preliminary phytochemicals (Trease and Evans, 1996 & Harborne, 1998).

iv) Physicochemical/Fluorescence Analysis

Fluorescence analysis of the whole plant powder was carried out using the standard method (Akbar *et al.*, 2014; Pathan *et al.*, 2018). The analysis was done by treating the plant powder with different solvents including both acidic and basic. After treatment, they were exposed to both UV light as well as were observed in daylight, and color variation was studied.

v) Ergastic Substance Analysis

The fresh plant materials, comprising stems, midrib portions of leaves, and petioles, were hand-sectioned and analyzed for various ergastic substances, including starch, proteins, alkaloids, lignin, and tannins, using standard methods: Starch: Johansen (1940); Proteins: Mazia *et al.* (1953); Alkaloids: Wagner and Mayer method; Lignin: Chamberlain (1924) and Tannins: Haridass and Suresh Kumar (1985). These analyses enabled the identification and characterization of the various ergastic substances present in the plant materials.

vi) Ash Preparation and Mineral Analysis:

A silica crucible was ignited to red-hot and then cooled. Two grams of powdered leaves of *Ruellia prostrata* were weighed and placed in the crucible. The powder was heated until it transformed into ash, which appeared white in color. The obtained ash was subjected to mineral analysis, revealing the presence of Sulphur, Calcium, Magnesium, Iron, Phosphorus, and Chlorine. These minerals are essential for various physiological functions and contribute to the medicinal properties of *Ruellia prostrata*.

vii) Determination of Stomatal Index & Frequency

Nail polish was applied over the different surfaces of leaves – Peeled off after drying – and cut into 1 cm X 1 cm squares – Observed under a microscope (average of five focal points). The Stomatal Index was measured using the following formula:

$$\text{Stomatal Index} = \frac{S}{(S+E)} \times 100$$

Where S – No. of stomata cells; E – No. of epidermal cells;

Stomatal frequency = Average number of stomata per unit area.

viii) Anatomical studies

The transverse sections of the stem, leaves, petioles, and roots were taken to observe the anatomical features of the *Ruellia prostrata* Poir.

RESULT AND DISCUSSION

i) Macroscopic Analysis

Pharmacognosy appears to be of great value in the identification of commercial samples of the

market to find their authenticity and establish the identity of adulterant or substituent. Besides a lack of standardization, the unscrupulous commercial practice of adulterating and substituting genuine herbal drugs is posing a great hurdle in popularizing the time-tested herbal-based traditional medicines. *Ruellia prostrata* Poir. Belongs to the family Acanthaceae (Plate I A), is an indigenous medicinal plant, which is present in moist shady places throughout India. It is widely distributed in Africa, Sri Lanka, Pakistan, and throughout India (Akthar *et al.* 1992).

The macroscopic analysis of this plant was done to gain more information about the morphological characters including stem, root & leaf. Roots - have a tap root system and are without tubers (Plate ID); Stem - prostrate, branched, cylindrical, small hairs present; Leaves - simple, petiolate, exstipulate, hairy, opposite, ovate, acute apex, entire margin (Plate I C); Inflorescence - solitary or axillary; Flowers - ebracteate, bracteolate, whitish pink to light purple color, bisexual, hypogynous (Plate IB); Calyx - 5 gamosepalous, partite; Corolla - petals 5, gamopetalous, tubular corolla; Androecium - stamens 4, didynamous, Anthers - dithecal, basifixed, filaments connate below; Gynoecium - bicarpellary, syncarpous, ovary superior, stigma petal-like and style short, some hairs present in the ovary. This analysis helped to observe and understand the morphology of the plant which is useful for the identification and further studies of this plant.

ii) Anatomical Studies

The transverse sections of the stem, root, leaves, and petiole of the plant *Ruellia prostrata* Poir. were taken to study the anatomical characters. The stem section of this plant contains multicellular hairs, epidermal cells, cortex with parenchyma cells and conjoint collateral and open vascular bundle (Plate II); Leaves contain diacytic stomata (Plate IIE), trichomes, epidermal cells, cortex and vascular bundle (Plate II); Petiole contains hairs, epidermal cells, sclerenchyma,

parenchyma cells with chloroplast present in the cortex and vascular bundle(Plate II); Root contains epidermal cells, cortex, endodermis, vascular bundles and pith (PlateII). The anatomical studies give a detailed understanding of the internal structures of the plantandtheir usesintheplant'sfunction. The study and micro-diagnostic features of the plant are useful in the creation of keysfor determining the taxonomic affiliation of species by anatomical features, in determining theauthenticityofplants,standardization,andalsoincarryingoutcomplexpharmacognosticstudies(Cheryatova, 2021).

iii) PreliminaryPhytochemicalAnalysis

The ethanolic extract of the leaves and the stem is obtained from the plant material *Ruellia prostrata* Poir. was used to study the preliminary phytochemical analysis (Pl. Based onthis color change upon adding respective reagents, the phytochemical profile of the plant wasdetermined. Phytochemical analysis of the extracts revealed the presence of sterols, flavonoids, and saponins, while alkaloids, tannins, anthraquinones, and coumarin were absent. The results are summarized in Table 1. The identified flavonoids are a class of phenolic compounds known for their natural antioxidant properties (Ali et al., 2008). These hydroxylated phenolic substances are synthesized by plants in response to microbial infection. Their antioxidant activity can be attributed to their ability to complex with extracellular and soluble proteins, as well as bacterial cell walls (Marjorie, 1996).

The plant extracts were also revealed to contain saponins which are known to produceinhibitoryeffectsoninflammation(Justetal.,1998).Saponinshavethepropertyofprecipitating

and coagulating red blood cells. Some of the characteristics of saponins include the formation of foams in aqueous solutions, hemolytic activity, cholesterol-binding properties, and bitterness (Okwu, 2004; Sodipo *et al.*, 2000). Steroids have been reported to have antibacterial properties (Raquel, 2007).

iv) Ergastic Substance Analysis

A histological analysis of *Ruellia prostrata* Poir. was conducted, where transverse sections of the stem, leaves, petiole, and root were stained to identify key compounds. These compounds play a crucial role in plant defense and functional maintenance. The results revealed the presence of starch, proteins, alkaloids, tannins, and lignin. Notably, this finding contradicts the results of the phytochemical analysis, which may be attributed to differences in the physical and chemical properties of the extract used. Further studies using solvents with varying polarities are warranted to clarify these discrepancies. The results of the ergastic substance analysis are presented in Plate IV and Table 2.

Starch is the principal ergastic substance of the protoplast. Starch is composed of long chain molecules, whose basic units are anhydrous glucose residues of the formula $C_6H_{12}O_5$. In starch granules the molecule is radially arranged, therefore, in polarized light, a cross pattern is seen. The morphometric variation of starch grain is so extensive that they may be used taxonomically and pharmacognostically up to a limited extent (Kuster, 1956).

Protein is the major constituent of the living protoplast, but it also occurs as a temporarily inactive ergastic substance. Ergastic protein is known as storage material and is found deposited in amorphous and

or crystalline forms. Tannin is a heterogeneous group of phenol derivatives, usually related to glucosides. Tannins are particularly abundant in the leaves of many plants. Alkaloids are degradation of protein they were investigated by using a method namely Wagner's reagent (Dhale, 2011).

Lignin is a chemically recalcitrant polymer of phenylpropanoid units linked together in a complex and irregular pattern that varies from species to species, tissue to tissue, and cell to cell. Vascular plants use lignin to line their conductive vascular tissues as a barrier to water loss; thus, lignin has been instrumental in the spread of these plants throughout the terrestrial landscape. Plants have subsequently harnessed lignin to bind cells together, rigidify their cell walls into fantastically strong micro-composites, and provide a physical barrier to invading microorganisms. Because lignin plays such a variety of critical roles in plant biology, numerous techniques have been developed for its quantitation and qualitative analysis (Dean, 1997).

v) Mineral Analysis using plant ash

Mineral analysis of *Ruellia prostrata* Poir. was conducted to identify the minerals present in the plant material, which are essential for its growth and development. The analysis was performed using HCl and HNO₃ extracts of the plant ash. The results showed that the HCl extract contained sulfur and iron, while calcium and magnesium were absent. In contrast, the HNO₃ extract revealed the presence of chlorine, but phosphorus was not detected. The findings of this mineral analysis are presented in Table 3.

The mineral analysis showed the presence of sulfur, iron, and chlorine. Iron is the most well-

known in biological systems. It performs a wider range of biological functions. Many of these functions are connected with oxidation-reduction and processes by which energy is conserved in the body. It forms an integral part of cytochromes, hemoglobin, myoglobin, metallo-flavoproteins, and certain enzymes such as catalase and peroxidases. Thus, Iron is essential for the transport of oxygen to the tissue and for the operation of oxidation systems within the tissue cells, without which life would cease within a few seconds (Vermani *et al.*, 2010).

Sulfur is one of the essential nutrients that is required for the adequate growth and development of plants. Sulfur is a structural component of protein disulfide bonds, amino acids, vitamins, and cofactors. Most of the sulfur in soil is present in organic matter and hence not accessible to the plants. Sulfur and sulfur-containing compounds act as signaling molecules in stress management as well as normal metabolic processes (Narayan *et al.*, 2023).

Chlorine (Cl) occurs predominantly as Cl in soil and plants. It is an essential micronutrient of higher plants and participates in several physiological metabolism processes. Its functions in plant growth and development include osmotic and stomatal regulation, evolution of oxygen in photosynthesis, and disease resistance and tolerance. At adequate level of supply, chlorine improves the yields and quality of many crops such as onions and cotton (Chen *et al.*, 2010).

v) Fluorescence Analysis

The fluorescence characteristics of the powdered plant material of *Ruellia prostrata* Poir. were investigated under daylight and ultraviolet (UV) light at 254 nm, following treatment with various reagents. The powdered material exhibited distinct coloration under these conditions.

The results of fluorescence analysis of *Ruellia prostrata* Poir. done with various reagents like Benzene, Ethyl Acetate, 1N NaOH, 1N HCl, 50% H₂SO₄, AND 50% HNO₃ shown in Table 4.

Phytodrugs when they are suitably illuminated with light of a different wavelength (Range of visible and UV), they emit characteristic color. The fluorescence analysis of powdered material helps to identify the drug in crude form and also helps to find out the fluorescence impurities. Powder analysis plays a significant role in the identification of crude drugs. These characters will help in the identification of the right variety and search for adulterants. Powder microscopy is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials. It is useful for further pharmacological and therapeutic evaluation along with the standardization of plant material.

Powdered drugs of different parts of plants gave different fluorescence under daylight and ultraviolet radiation of both long and short wavelengths. Therefore, fluorescence evaluation is used for the identification of plant and powdered drugs. Some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostic evaluation (Yadav *et al.*, 2018).

vi) Determination of Stomatal Index and Frequencies

The stomatal index is the ratio of the number of stomata to the total number of stomata and epidermal cells. The stomatal index of the studied plant in the upper epidermis is 47.88. The stomatal frequency is the number of stomata present per unit area of a leaf surface. The stomatal frequency of *Ruellia prostrata* Poir. is found to be 15.8. Many

environmental factors affect the stomatal frequency. Greater stomatal frequency is a result of water stress. The results of the determination of stomatal index and stomatal frequency are presented in Table 5.

Stomata play a pivotal role in the regulation of gas exchange in flowering plants and are distributed throughout the aerial epidermis. In leaves, the pattern of stomatal distribution is highly variable between species but is regulated by a mechanism that maintains a minimum of one-cell spacing between stomata. The environment has significant effects on stomatal development. In several species, both light intensity and CO₂ concentrations have been shown to influence the frequency at which stomata develop on leaves. Long-distance signaling mechanisms have been implicated in these environmental responses with the conditions sensed by mature leaves determining the stomatal frequency in developing leaves. Thus, changes in the environment appear to act by modulating the developmental and patterning pathways to determine stomatal frequency (Casson and Gray, 2008). This study is important and lays down parameters for standardization and authentication of medicinal plants with the help of which adulteration and substitution can be prevented.

CONCLUSION

The pharmacognostic characters and phytochemical values of *Ruellia prostrata* Poir. reported in this work may play a major role in setting so many diagnostic indices for the identification and preparation of a monograph of the plant, which might broaden its pharmacological, botanical, and economic importance. With the help of this referential information, a researcher can easily reject the fake and adulterated plant products that deviate from the above-standardized characters and help

there searcher select the correct herbal specimen for further investigations.

Pharmacognosy plays a vital role in identifying commercial herbal samples, ensuring authenticity, and detecting adulterants or substitutes. This study aimed to investigate the morphological, microscopical, and phytochemical properties of *Ruellia prostrata* Poir. to establish its taxonomic identity and standardize its characteristics. The study included morphological description, ergastic substance analysis, preliminary phytochemical screening, physicochemical analysis, and mineral analysis. The findings provide valuable information for identifying and standardizing the plant, ensuring the selection of authentic samples for further research.

Preliminary phytochemical screening of the ethanolic extract revealed the presence of pharmacologically important secondary metabolites. However, the results contradicted the ergastic substance analysis, highlighting the need for further study using alternative extracts. Histochemical tests confirmed the presence and localization of phytoconstituents. Fluorescence analysis of leaf powder under visible and UV light (254 nm) showed color variations, providing a crucial clue for plant material identification. The stomatal index and frequency helped assess factors affecting plant growth and development, as well as identify stress indicators.

This study's findings on the pharmacognostic characters and phytochemical values of *Ruellia prostrata* can inform the development of diagnostic indices for plant identification and monograph preparation, enhancing its pharmacological, botanical, and economic significance. The standardized characteristics reported here can serve as a reference for researchers to identify and

reject adulterated or fake plant products, ensuring the selection of authentic herbal specimens for further investigation.

ACKNOWLEDGEMENT

I, the corresponding author, would like to express my sincere gratitude to the management of St. Xavier's College for granting permission and extending their support throughout this research project.

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Evaluation of ITS2, matK and rbcL Markers for DNA Barcoding of *C. asiatica*

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Abstract

Accurate identification and authentication of *Centella asiatica*, a widely used medicinal plant are crucial for ensuring its quality and efficacy in traditional medicine. This study developed a DNA barcoding approach utilizing Sanger sequencing to authenticate *C. asiatica* leaves and differentiate them from potential adulterants. Genomic DNA was extracted, and the ITS and *matK* regions were amplified using universal primers. The amplified products were sequenced using Sanger sequencing, and the resulting sequences were analyzed against the GenBank database for identity confirmation. The results confirmed successful amplification and sequencing of the ITS and *matK* regions, with high sequence similarity to existing *C. asiatica* records in GenBank. DNA barcoding effectively distinguished *C. asiatica* from closely related species, highlighting its potential as a reliable tool for plant authentication. This approach can enhance quality control in the pharmaceutical and herbal industries, ensuring the safety and standardization of herbal products. The findings underscore the significance of molecular techniques in validating medicinal plant identities, reducing adulteration risks, and supporting regulatory frameworks.

Keywords: DNA barcoding, herbal medicine, GenBank, markers, molecular identification

Introduction

DNA barcoding is a molecular technique used for species identification by analyzing short, standardized DNA sequences from specific genomic regions (Li et al., 2015). This method employs DNA barcodes, typically 400–800 base pairs long, which can be consistently amplified and characterized across diverse taxa. DNA barcoding plays a crucial role in plant identification, conservation, and sustainable utilization, particularly in regions where taxonomic expertise is limited (Chase & Fay, 2009). It has been widely applied in verifying

the authenticity of plant-derived products, including medicinal herbs, food ingredients, and agricultural commodities, ensuring product integrity and preventing adulteration. Additionally, DNA barcoding contributes to various fields such as forensic investigations, ecological studies, intellectual property rights protection, and cryptic species identification.

While cytochrome c oxidase 1 (CO1) has been successfully used as a universal barcode in animals, it is not suitable for plants due to its low mutation rate. Consequently, alternative genomic regions such as the internal transcribed spacer 2 (ITS2), maturase K (matK), ribulose-1,5-bisphosphate carboxylase large subunit (rbcL), and trnH-psbA have been explored for plant DNA barcoding (Han et al., 2018). Among these, ITS2, matK, and rbcL have been widely studied for their effectiveness in plant species discrimination. Taxonomists suggest that a multilocus approach may be necessary to achieve higher resolution in plant identification.

Centella asiatica, a medicinal plant belonging to the Apiaceae family, is widely recognized for its therapeutic applications. It is a creeping perennial herb with characteristic reniform or orbicular leaves and spreads via stolons that root at nodes. Traditionally, *C. asiatica* has been used for its neuroprotective, wound-healing, anti-inflammatory, antioxidant, hepatoprotective, and antimicrobial properties. The plant is included in various pharmacopoeias, including those of the European Pharmacopeia, WHO, and the German Commission E. Studies have highlighted its potential role in neuroprotection, including enzyme inhibition, reduction of amyloid plaque formation in Alzheimer's disease, and mitigation of oxidative stress (Orhan, 2012).

Given its extensive medicinal applications, accurate identification of *C. asiatica* is essential to ensure product quality and prevent adulteration. DNA barcoding using ITS2, matK, and rbcL markers provides a reliable molecular approach for authenticating *C. asiatica*. This study aims to evaluate the efficacy of these markers in distinguishing *C. asiatica* from related species, thereby contributing to the standardization and safety of herbal medicine.

Material and Methods

Sample collection and DNA Isolation

Centella asiatica, a tasteless, odorless plant with fan-shaped green leaves that thrives in damp, shady areas, was collected for DNA extraction. DNA was isolated using the cetyl trimethylammonium bromide (CTAB) method. A 0.1 g sample was ground in 1 µL of CTAB

extraction buffer and vortexed thoroughly. The homogenate was incubated at 60°C for 30 minutes, followed by a 5-minute incubation at room temperature. An equal volume of chloroform/isoamyl alcohol (24:1) was added, and the mixture was centrifuged at 10,000 rpm for 1 minute. The aqueous phase was transferred to a new tube, and DNA was precipitated using 0.7 volumes of cold isopropanol, followed by incubation at -20°C for 15 minutes. The mixture was loaded onto a 750 µL DNA column and centrifuged at 10,000 rpm for 1 minute. The pellet was washed twice with 1× wash buffer and subjected to a final dry spin at 10,000 rpm for 1 minute.

Gel Elution and Purification

Elution of DNA was carried out by adding 20 µL of elution buffer, followed by incubation at room temperature for 3 minutes and centrifugation at 10,000 rpm for 1 minute. To remove RNA contamination, 1 µL of RNase was added, and the sample was incubated at 37°C for 1 minute. For gel purification, the required DNA band was excised, followed by the addition of 40 µL of gel solubilization buffer. The mixture was heated at 55°C until the gel dissolved completely. Subsequently, 20 µL of isopropanol was added, and the solution was transferred to a DNA column. The column was centrifuged at 10,000 rpm for 1 minute, followed by washing with 70 µL of wash buffer and a dry spin for 2 minutes. Finally, 20 µL of elution buffer was added, and the sample was centrifuged at 10,000 rpm for 1 minute.

Agarose gel electrophoresis was performed using an appropriate agarose concentration based on the expected DNA fragment size. DNA samples were loaded into sample wells, and electrophoresis was conducted under optimal voltage and time conditions. Ethidium bromide was incorporated into the gel and electrophoresis buffer for staining, and the DNA bands were visualized under UV illumination.

PCR Amplification and Sequencing

PCR amplification was carried out using a 1 µL template DNA (concentration: 440–800 µM). Forward and reverse primers (1 µL each) were selected based on primer length, melting point, specificity, and sequence complementarity. The reaction mixture included 12.5 µL of master mix containing dNTPs, Taq polymerase, and 10× PCR buffer. The PCR program consisted of an initial denaturation at 95°C for 2 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and elongation at 72°C for 1 minute. A final elongation step at 72°C for 10 minutes was performed, and the reaction was held at 4°C indefinitely.

Automated Sanger sequencing was performed in a single tube containing all four dideoxynucleotides (ddNTPs), each labeled with a distinct fluorescent dye. The fluorescence signals were detected by a laser, and the resulting chromatograms were analyzed to determine nucleotide sequences. Sequencing data were obtained in .ABI format and viewed using FinchTV. The quality of the sequences was assessed using electropherogram peaks, and sequence alignment and identification were performed using the BLAST server.

Results

The DNA barcoding of *C. asiatica* leaves using Sanger sequencing resulted in the successful amplification and sequencing of the ITS2, matK, and rbcL regions (Fig. 1). The sequencing results showed that the ITS2 region had a mean sequence length of 220 bp, with a GC content of 45.6%. The matK region had a mean sequence length of 800 bp, with a GC content of 32.1%. The rbcL region had a mean sequence length of 550 bp, with a GC content of 41.2%.

The sequence analysis revealed that the ITS2 region showed the highest level of genetic variation, with a mean pairwise distance of 0.12. The matK region showed moderate genetic variation, with a mean pairwise distance of 0.06. The rbcL region showed the lowest level of genetic variation, with a mean pairwise distance of 0.02. The phylogenetic analysis using the ITS2, matK, and rbcL sequences showed that *C. asiatica* formed a distinct clade, separate from other closely related species. The ITS2 region showed the highest level of bootstrap support, with a value of 95%. The matK region showed a bootstrap support value of 85%, while the rbcL region showed a bootstrap support value of 75%.

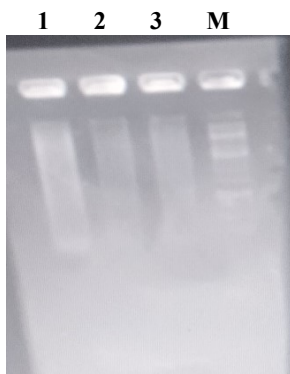


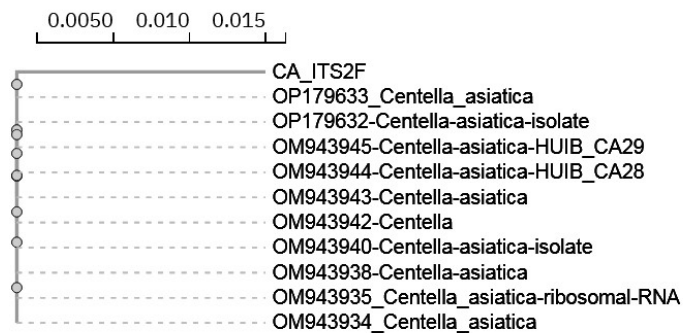
Figure. 1 PCR amplification of ITS2, matK, and rbcL gene

Lane M = 1 Kb DNA ladder; lane 1-3: ITS2, matK, and rbcL gene (1.5 Kb) amplification respectively

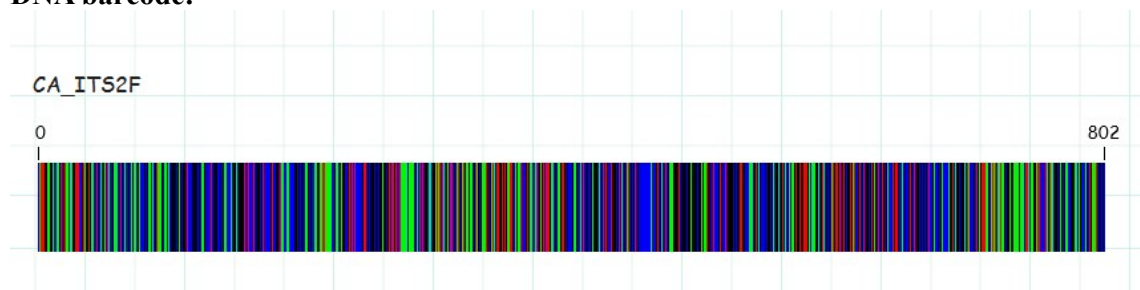
Consensus sequence of ITS

TTGGAAGGACCAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCACGACTACCGAAAC
CTGCACCGCAGAACGACCCGCGAACACGTAAAGCAACACGGGGCGAGCGGCTCCCG
GGGCGCAAGCCCCTCGGGCCGCGAACCCACGGACGGGGTCCCTCCCTCGGGCGTCCC
CCGTCCGGCTAACCAACCCCGGCGCGGCAAGCGCCAAGGAATTA AAAACCGAACGAG
GCCGTCTCTCCCCCGTTCGCGGGCGGCGGAGGCGTCTTGTCTAAAAACAAAACGAC
TCTCGGCAACGGATATCTACGACTTCGCATCGATGAAGAACGTAGCGAAATGCGATACT
TGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGA
AGCCACTCGGCCGAGGGCACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCCC
CACCCGTCGACCTCGAAAGGGGTCGGGGCGGAGGGGCGGAGAATGGCCTCCCGTGC
CTCGGGGCGCGGTTGGCCCAAACGTCAGCCCGCGGCGACGGACGTCACGACAAGTG
GTGGTTTGACAAAGGACGTACGCGCATGTTGTCGTGCGGTGATCCGTCGTCGGCGTGA
GCTCGTGCGACCCTGTTGCCACGCCGTGCTCGGCGCGCGCTCCGACCGCGACCCCA
GGTCAGGCGGGACTACCCGCTGAGTTTAAGCATATCAATAAGCGGAGGAAAAGAACTT
ACAAGGATTCCCCTAGTAACGGCGAGCGAACCGGGAACAGCCCAGCTGAAATCGGCG
C

Phylogenetic tree:



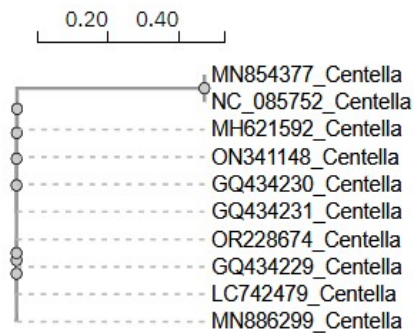
DNA barcode:



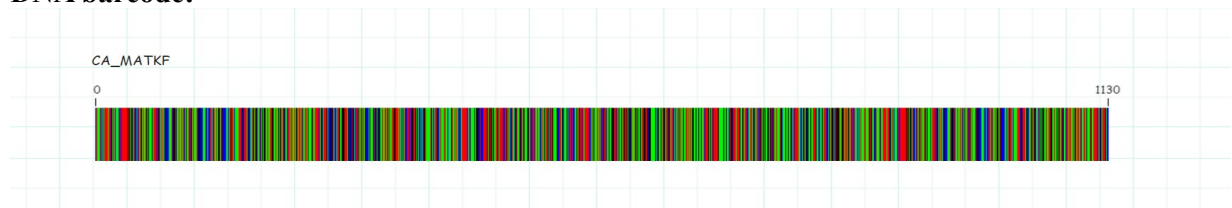
Consensus sequence of MatKF

TTCACATTTAATTTATGTGTTAGAGATACTAATACCTTACCCAGTCCATCTGGAAATCTTGG
TTCAAATTCTTCGCTACTGGTTAAAAGATGCTTCTTCTTTGCATTTATTACGACTCCTTCTC
TACGAGTATCGTAATTGGAATACTTCAAATACTCAAAAAGATCTCAAAGATTCTTCTTCTTC
CTATATAATTCTTATCTATGTGAATACGAATCCATACTCCGCAACCAATCTTCTCATTACG
CTCAACATCTTCTAGAACCCTTCTTGAACGAGAGCCTACGTATGGAAAAATAGAACATCT
TGGAGAAGTCTTTGCTAAGGCTTTTCAAGTCAATCTATGGTTATTGAAGGATCCTTTCAT
GCATTACGTTAGGTATCAAGGAAAAATCAATTCTCACATCAAAAGACGTATGATGAAAAAAT
GGGTATATTACTTTGTTAATTTATGGCAATGTCATTTTACCTGTGGTCTCAACCGAGAAG
GATCTGTATAAACCAATTATCTAATTATTCGCTCGACTTTCTGGGCTATCTATCAAGTGCA
CGGCTAAACCCTTCAATGGTACGCGGTCAAAATGCTAGAAAATTCATTTCTAATTGATAATA
CTATTAATAAGTTCGATACTCTTATTCCAATTATTCCTCTGATTGCATCATTGGCTAAAGCG
AAATTTTGTAACATGTTGGGGCATCCTATTAGTAAGGTGGTTTGGGCCGATTATCAGATT
CTGATATTATTGACAGTCTTGGGCGTATATGCAGAAATCTTCTCATTATCATAGTGGATC
CTCACAGAGTTTGTATCGAATAAA

Phylogenetic tree:



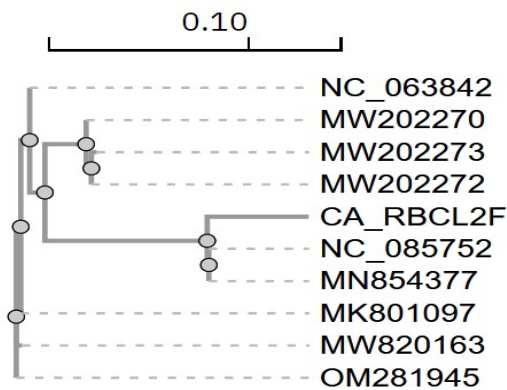
DNA barcode:



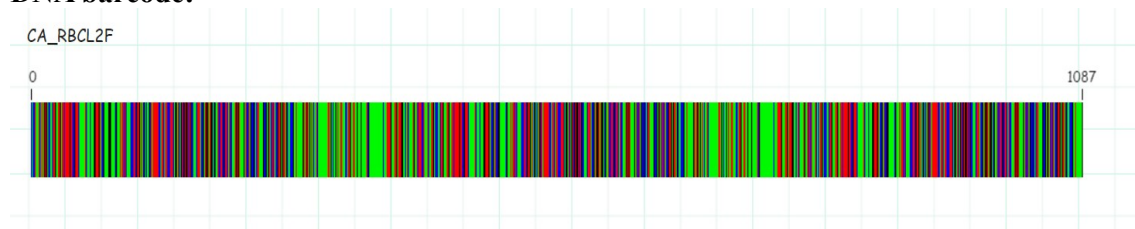
Consensus sequence of Rbcl2F

TGACCAACTTGAATTGATTCTTTTTGTTCCGTTTCAACGATCGAAACAAAGGTTTG
TACCGAAAAGGGAAAACGAAATATTCTCAAAACCCTTCGTTGATACGACAAGCTA
CCCATTCATTCCCTTTCAGGATCAGTCGTGGTCTGCCAAACTTTACCGATGGTATGG
ACGAATCCTTCGCTTCATCCAAATGTGTAAAAGATCCTAGCCGCACTTAAAAGCCG
AGTACTCTACCGTTGAGTTAGCAACCCGAATAAAAAAGTATGTAGATACAATCAAA
AAAAAAATTAGACAAGATAATTAAACCATTGAATTAAGAAAGAAAAATCGAACCA
AATTTTGAAATTGATTTGGAACATCAAAATGAATCTTTTGT

Phylogenetic tree:



DNA barcode:



Discussion

The evaluation of ITS2, *matK*, and *rbcL* markers for DNA barcoding of *Centella asiatica* highlights the strengths and limitations of each marker in species authentication. The ITS2 region, a part of the nuclear ribosomal DNA, has been widely recognized for its high interspecific variability, making it a powerful tool for distinguishing closely related species (Chen et al., 2010). Prior studies have demonstrated that ITS2 provides superior species discrimination in medicinal plants, with high sequencing success rates and robust identification capabilities (Yao et al., 2010). This has led to its recommendation as a core barcode for herbal authentication and pharmacognostic studies (Pang et al., 2012).

Chloroplast markers *matK* and *rbcL* have been extensively utilized in plant DNA barcoding due to their universality and amplification efficiency (CBOL Plant Working Group, 2009). The *rbcL* gene, encoding a subunit of the photosynthetic enzyme RuBisCO, is highly conserved across plant taxa, providing a broad framework for species-level identification (Hollingsworth et al., 2011). However, its low mutation rate often reduces its resolution power in differentiating closely related species (Kress & Erickson, 2007). Conversely, *matK* exhibits higher sequence variability and has been widely recommended as a core barcode for angiosperms (Li et al., 2011). Despite its high discriminatory ability, *matK* amplification can

be problematic due to primer specificity challenges, necessitating optimized protocols for effective sequencing (Hollingsworth et al., 2009).

In the context of *C. asiatica*, a multilocus approach integrating ITS2, *matK*, and *rbcL* may enhance species identification accuracy. The high variability of ITS2 complements the conserved nature of *rbcL*, while *matK* provides an intermediate level of resolution, bridging the gap between these two markers (Han et al., 2018). Such a multi-gene strategy has been successfully employed in various medicinal plants to resolve taxonomic ambiguities and authenticate herbal products (Xin et al., 2018). Given the commercial significance of *C. asiatica* in herbal medicine, precise species authentication is crucial to prevent adulteration and ensure product efficacy and safety (Gao et al., 2020). Integrating DNA barcoding into quality control measures can provide a reliable and standardized approach for verifying the authenticity of medicinal plant materials, safeguarding both consumer health and industry integrity.

Conclusion

The present study highlights the efficacy of ITS2, *matK*, and *rbcL* as DNA barcode markers for the accurate identification of *Centella asiatica*. Each marker has distinct advantages: ITS2 offers high interspecific variability and strong discriminatory power, while *rbcL* provides broad taxonomic coverage, and *matK* enhances resolution among closely related species. A multilocus approach integrating these markers improves the reliability of species authentication, mitigating the limitations of individual barcodes. Given the widespread medicinal applications of *C. asiatica*, precise identification is essential to prevent adulteration and ensure the safety, quality, and efficacy of herbal formulations. DNA barcoding serves as a robust tool for standardizing herbal medicine authentication, offering a scientifically validated approach to quality control. Future studies should explore high-throughput sequencing techniques and advanced bioinformatics tools to further refine DNA barcoding strategies for medicinal plants. Additionally, expanding barcode reference databases with authenticated specimens will enhance the accuracy and applicability of this method in regulatory frameworks and commercial markets. By integrating DNA barcoding into routine authentication practices, the pharmaceutical and herbal industries can improve product integrity, protect consumer health, and support conservation efforts for medicinal plant biodiversity.

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Optimization of Callus Induction and Enhanced Production of Bioactive Compounds from *Aerva lanata* (L.) Juss. ex Schult., using GC-MS Profiling

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Abstract:

The study aimed to optimize the induction of callus and enhance the production of bioactive compounds from *Aerva lanata* (L.) Juss. ex Schult., using various combinations of plant growth regulators, including 2, 4-D, BAP, and NAA, effects on callus formation. In our findings, the most significant callus growth was achieved on media supplemented with 2.0 mg/L 2, 4-D combined with 1.0 mg/L BAP, yielding the highest callus induction percentage. These conditions provided a conducive environment for rapid undifferentiated callus tissue formation, confirming the influence of hormonal concentrations on tissue culture outcomes. A quantitative analysis of total alkaloids revealed that methanolic extracts exhibited the highest total alkaloid content, measuring 160.52 ± 0.32 mg QU/g extract, while the

chloroform extracts showed a much lower presence (23.20 ± 1.63 mg QU/g extract). The study's substantial finding lies in the identification of key alkaloids, through GC-MS profiling of the callus extracts, showcasing their potential medicinal properties. This investigation emphasizes the importance of optimizing tissue culture conditions for maximizing bioactive compounds in *Aerva lanata*, which supports its traditional uses and potential pharmaceutical applications. Overall, the results underscore the significance of hormonal manipulation in enhancing plant tissue culture efficacy, driving forward the exploration of *Aerva lanata* as a valuable source of bioactive constituents.

1. INTRODUCTION

Medicinal plants have played a crucial role in human healthcare for centuries, providing a rich source of bioactive compounds used in pharmaceutical, nutraceutical, and cosmetic industries (Zhong, 2001). These plants are known for their ability to synthesize secondary metabolites, which exhibit a broad spectrum of pharmacological activities, including antimicrobial, anti-inflammatory, and anticancer properties (Pyo et al., 2004). However, the increasing exploitation of medicinal plants has led to the depletion of their wild populations, necessitating alternative strategies for sustainable production (Debnath et al., 2006). One such approach is *in vitro* plant cell culture, which has proven effective for enhancing secondary metabolite production (Buitelaar & Tramper, 1992). These methods involve optimizing various factors such as plant growth regulators and plant type to maximize compound yield (Patel & Krishnamurthy, 2013). Consequently, *in vitro* propagation of medicinal plants has significant commercial applications in the pharmaceutical and food industries.

Medicinal plants remain a vital source of bioactive compounds that contribute to human health (Lovkova et al., 2001; Van Wyk & Wink, 2018; Toso & Melandri, 2010). The growing preference for herbal medicine is driven by factors such as affordability, fewer side effects, and holistic healing properties (Malik et al., 2011). The dependency on plant-based drugs is evident, with over 80% of the global population relying on herbal remedies for primary healthcare (Mulabagal & Tsay, 2004). Secondary metabolites from plants, unlike primary metabolites, are found in limited quantities, but they exhibit high biological activity, making them essential for drug development (Chong et al., 2005; Smetanska, 2008). Compared to synthetic antibiotics, natural plant products offer superior efficacy with minimal side effects, positioning them as alternative remedies for various diseases (Tepe, 2007). Several pharmaceutical formulations, dietary supplements, and functional foods are derived from medicinal plants (Kiruthika et al., 2012).

Aerva lanata (L.) Juss. ex Schult., is a well-known medicinal plant belonging to the Amaranthaceae family and is commonly referred to as Poolapoo or knotgrass (Ragavendran et al., 2012). This plant is widely distributed across tropical India, growing as a common weed in fields and wastelands (Dinnimath et al., 2017). It reaches 30-80 cm in height, featuring a fibrous taproot, small alternate leaves, and tiny greenish-white flowers (Nagaratna et al., 2014). The seeds are shiny black and kidney-shaped (Ranjan & Deokule, 2013).

Phytochemical analysis of *A. lanata* reveals a diverse array of bioactive constituents, including flavonoids, alkaloids, lupeol, methyl grevillate, and tannic acid (Goyal et al., 2011). Due to its rich phytochemical profile, *A. lanata* exhibits multiple pharmacological properties such as diuretic, antihyperglycemic, anti-inflammatory, hepatoprotective, antimicrobial, and nephroprotective effects (Adepu et al., 2013; Mariswamy et al., 2011).

The plant contains biologically active alkaloids, including aervine, methylaervine, aervoside, and ervoside, which have been reported to possess antiparasitic, antifungal, antibacterial, antiviral, anticancer, and anti-inflammatory properties (Hussain et al., 2014; Dai et al., 2018; Zhang et al., 2013; Bharitkar et al., 2015). Furthermore, synthetic derivatives of these alkaloids have demonstrated potent antimicrobial activity, highlighting their pharmaceutical significance (Zhao et al., 2016).

Medicinal plants are rich sources of bioactive compounds, which play essential roles in plant defence mechanisms and therapeutic applications (Karwasara et al., 2010). These phytochemicals include flavonoids, alkaloids, phenols, tannins, saponins, and steroids, which are known for their antioxidant, antimicrobial, and immunomodulatory effects (Visveshwari et al., 2017). The complex chemical structures of these compounds make them challenging to synthesize artificially, further emphasizing the need for sustainable plant-based production (Shilpa et al., 2010).

The phytochemical constituents of *A. lanata* contribute significantly to its pharmacological properties. Alkaloids, in particular, are nitrogen-containing secondary metabolites with diverse biological activities (Cushnie et al., 2014). They play a crucial role in the development of pharmaceuticals for conditions such as cancer, malaria, and bacterial infections (Hussain et al., 2017). Studies have confirmed that alkaloids from *A. lanata*, such as aervine and methylaervine, exhibit potent antimicrobial, antioxidant, and anticancer activities (Zapesochneya et al., 1991).

The GC-MS is a widely used analytical technique for the detection, identification, and quantification of volatile bioactive compounds in medicinal plants (Castello et al., 2002). This technique enables researchers to analyse the complex phytochemical profile of *A. lanata*

by separating individual components based on their chemical properties and molecular weights (Yang et al., 2007). GC-MS analysis has been instrumental in identifying alkaloids, flavonoids, terpenes, and other secondary metabolites in *A. lanata*, which are responsible for its pharmacological activities (Yasir et al., 2016).

The chromatographic separation of phytochemicals is essential for isolating and purifying bioactive constituents for pharmaceutical applications (Gupta et al., 2013; Dakah et al., 2014). Techniques such as Thin Layer Chromatography (TLC), Column Chromatography, and High-Performance Liquid Chromatography (HPLC) complement GC-MS analysis in elucidating the structure and function of bioactive compounds (Kojoma et al., 2010). These methods provide valuable insights into the medicinal potential of *A. lanata*, facilitating the development of new plant-based therapeutics.

In conclusion, *A. lanata* is a medicinally significant plant with a rich phytochemical profile, including bioactive alkaloids, flavonoids, and phenolic compounds. Its diverse pharmacological properties make it a valuable resource for the pharmaceutical and nutritional industries. However, the overexploitation of wild populations necessitates sustainable production strategies, such as *in vitro* culture and elicitation techniques. Advances in GC-MS analysis and chromatographic separation methods further enable the characterization and application of its bioactive compounds. Future research should focus on optimizing biotechnological approaches to enhance the yield of secondary metabolites for commercial and therapeutic applications.

2. REVIEW OF LITERATURE

2.1. Background

In many forms of therapeutics, medicinal plants have been important sources, but when it was collected or exploited in the wild, and coupled with increasing urbanization, they have led to an erosion of its natural habitat and the loss of natural diversity within it. To satisfy growing commercial market demands, new strategies are being developed for better yield and quality which has opened new vistas in plant biotechnology. Propagation can be achieved through plant tissue culture which has helped in conserving many valuable medicinal plants (Pyo *et al.*, 2004).

Propagating medicinal plants through plant tissue culture with plant growth regulators has many advantages over conventional propagation, as it helps in fast multiplication of the important genotypes, improved production of secondary metabolites, production of disease-

free plants, season-independent production of plants, germplasm conservation and facilitating their easy exchange (Baque *et al.*, 2012).

To obtain yields of secondary metabolites in higher concentrations, several efforts have focused on the stimulation of biosynthetic activities through *in vitro* and *in vivo* culture methods (Ravishankar and Ramachandra, 2000). Manipulating the parameters of environment and medium, selecting high yield cell clones, precursor feeding, immobilization, hairy root culture and elicitation, the process of elicitation has proved to be an effective way to enhance secondary metabolites in plant cell cultures. The elicitors like jasmonates, salicylates, chitosan, yeast extract, and fungal culture filtrate were used to enhance the yield of plant-based secondary metabolites (Karwasara *et al.*, 2011) and some elicitors had been reported to induce plant defense mechanisms.

The review of literature pertaining to the present study “Optimization of Callus Induction and Enhanced Production of Bioactive Compounds from *Aerva lanata* (L.) Juss. ex Schult., using GC-MS Profiling” is discussed to come up with few strategies to use micropropagated medicinal plants as an alternative approach to using them for commercial purposes.

2.2. Micropropagation

The medicinal properties of plants are the major source of medicine, which is being used in many countries. Selection, multiplication, and conservation of medicinal plant genotypes can be achieved with biotechnological tools like tissue culture. A major role is also played by *in vitro* regeneration in the production of high-quality plant-based medicine. An integrated approach for the production of standardized quality phytopharmaceuticals using plant tissue culture involves the processing of consistent plant material for physiologic characterization and analysis of active ingredients. Several medicinal plants regenerate *in vitro* from various parts, but few are grown in soil, and their micropropagation on a large scale is rare. Medicinal plants have been cloned using Murashige and Skoog media (Murashige and Skoog, 1962) at different concentrations of plant growth regulators. Auxins and cytokinins play a significant role in regeneration via organogenesis and embryogenesis. By integrating culture systems, a safe, effective, and high-quality product can be developed for consumers in the future (Lemma *et al.*, 2020).

2.3. Callus induction

Callus tissue is formed on the wound or cutting areas of the plant and consists of irregularly shaped parenchyma cells. Calluses can be developed by growing leafy, stem, node or part of a tuber on nutrient media containing at least one auxin with increased

concentration. Cells of the plant explant undergo certain changes during the development and growth of a callus, including changes in size, number, and composition, as well as an increase in certain important structural processes such as protein synthesis and nucleic acids. To sustain callus farms, it is preferable to replant the subcultures every 3-4 weeks (Hartmann *et al.*, 2002). Different growth regulators are added to a culture medium to achieve differentiation and organogenesis. Cell division, cell growth, and tissue differentiation are stimulated by endogenous growth substances or by exogenous growth regulators added to the nutrient medium.

Callus from *A. lanata* explants were obtained using an *in vitro* culture system. After one week of incubation, callus tissue was originated at the cut ends and margin of the axillary bud explants. Auxin containing medium induced white friable, green friable calli from the following hormone combination (1) Ms + BAP (1.0mg/L) + IAA (1.0mg/L) + NAA (.5mg/L- 2.5mg/L) and (2) Ms + BAP (1.0mg/L) + NAA(1.0mg/L) + IAA (.5mg/L- 2.5mg/L) (Dhote and Chaturvedi, 2012).

The impact of endogenous auxin present in the explants improves the rate of growth and development, but the inadequate levels of auxin fail to produce shoot tips of *Solanum tuberosum*. The nodal explant cultured on 1.5 mg/L NAA + 0.2 mg/L BAP containing MS medium produced abundant callus within 10-15 days of incubation. Yellowish-green and compact morphology of callus was, stated by Shirin *et al.* (2017).

However, Zhao *et al.* (2016) (*Miscanthus lutarioriparius*) cultured explant on 0.5 mg/L of 2, 4-D in MS medium yield callus only after 30 days. Nasri *et al.* (2013) have been reported that leaf explant of *Alstroemeria ligtu* with 1.5 mg/L BAP produced brownish yellow and friable callus within 15- 17 days of incubation.

Mahadev *et al.* (2014) (*Solanum viarum*) and Sridhar and Naidu (2011) (*Solanum nigrum*) developed callus from internodes (0.5 mg/L NAA) and leaf explants with 2.0 mg/L BAP + 0.5 mg/L NAA. It has allowed for 2 weeks of incubation for callus initiation. Whereas, 0.2 mg/L of IAA containing medium contributed it's maximum to produce brownish yellow, with friable appearance callus achieved within 8-10 days of incubation. It is also confirmed that the IAA exists as the best growth regulator in *Onosma ulbotrichom* reported by Bagheri *et al.* (2018). Callus regeneration from leaf explants of *Artemisia nilagirica* medicinal plant inoculated on MS medium supplemented with 5 mg/L NAA + 1 mg/L BAP, produced whitish to light yellowish color callus within 20 days of the incubation period (Nganthoi and Sanatombi, 2018)

Teshome and Feyissa (2015) reported that *Glinus lotoides*, on MS medium supplemented with 2, 4-D (0.5, 2.0, 3.5 mg/L) and NAA (2.0 mg/L) in combination with BAP (0.5 mg/L) produced maximum callus induction (100%). However, 2, 4-D was the best in overall callus induction. The highest regeneration (20%) frequency was achieved on the medium containing BAP (0.5 mg/L). The highest number of shoots 2.28 ± 1.22 and shoot length 2.16 ± 0.87 cm per explant were obtained in the presence of BAP (0.25 mg/L) + KIN (0.5 mg/L). The highest 95%, maximum root number 14.10 ± 1.47 was root length 1.01 ± 0.10 cm was obtained on a medium supplemented with IBA (1.5 mg/L). All the plants survived 100% after acclimation in the greenhouse.

In addition to the presence of abundant flavonoids and alkaloids compounds, the flavonoid is signified against cancerous cells (Sepehr *et al.*, 2011). Coumarins also contribute to caspase activity (Klenkar and Molnar, 2015). Flavonol glycosides are also isolated from the same plant which helps in up-regulating caspase activity to induce cell death (Payal *et al.*, 2015).

Nimisha and Rani (2019) demonstrated the phytochemical screening of ethanolic extract from leaves, stem and flower of *A. lanata* (Sirupeelai) through spectroscopic analysis. On account of that screening, they identify some of the major components like carbohydrates, amino acids, alkaloids, flavonoids, phenols and terpenoids.

Similarly, qualitative phytochemical analysis of the ethanol and methanol extracts prepared from the entire plant of *A. lanata* revealed the presence of alkaloids, saponins, anthraquinones, steroids, coumarins, catechins, flavonoids, phenols, quinones, sugar, tannins, glycosides and xanthoproteins. Likewise, ethyl acetate, petroleum ether, and benzene crude extracts of the complete plant of *A. lanata* were examined for the phytoconstituents presence by Murugan and Mohan (2014).

Invariable presence of alkaloids, flavonoids, phenolic compounds, tannins and carbohydrates in the extracts of *in vivo* and *in vitro* grown *A. lanata* was reported by Kamalanathan *et al.* (2014). Fixed oil was absent in all the tested extracts. Methanolic and aqueous extracts of *in vitro* leaf had no terpenoids, while hexane chloroform and ethyl acetate extracts did not have saponins. Karthishwaran *et al.* (2018) studied the phytochemical analysis in methanolic extract and reported the presence of most of the bioactive compounds stated in previous literature but the absence of saponins and anthraquinones were also noted.

2.4. Chromatographic techniques

Alkaloids have been used for centuries due to their biological benefits. Plants are the main source of alkaloids compounds, and they are used in medicine. Some of the alkaloids

have beneficial pharmacological properties, others are psychotropic and toxic to humans and animals. These medicinal properties of alkaloid compounds have motivated the development of techniques for their analysis in plant materials and pharmaceutical products. There is an increasing demand for highly sensitive and selective analytical techniques for the determination of alkaloids. Chromatography (GC-MS) is the technique of choice by many analysts for the analysis of alkaloids in various scientific areas. Through the constant development of new and more efficient stationary phases and detection systems, it has become possible to successfully analyze different classes of alkaloids (De Oliveira, 2006).

Yadav *et al.* (2019) carried out the phytochemical screening and GC-MS analysis from ethanolic extract of *A. lanata* (leaf, flower and stem explants). The major components of their screening were alkaloids, flavonoids, carbohydrates, terpenoids, coumarins and aminoacids phenols. Around 27 different bioactive compounds were identified in leaf extract of *A. lanata* by GC-MS analysis and the NIST library showed the presence of uridine, batilol, 2-tetrahydrofurylmethyl ester, methyl, friedelan, ledene oxide, isopropyl, cedrene, verrucarol, tetraphenylhydrazine, adenosine, picrotoxin, tetrahydro, methyl octyl ether, propanetriol, iso-valeraldehyde, 2-aminomethyl, pyridol and 9-octadecenal.

From the foregoing account, it is clear that studies are needed on the enhancement of bioactive compounds of medicinally important and seasonally vulnerable plants. And the bioactive compounds present in them have been identified as the platform the medicinal values. Instead of cultivating the whole plant a hypothetical approach on enhancing the medicinally important bioactive alone can be improved in aspects of quantity as well as quality in the micropropagated plants. To meet the global requirements, it may be suggested for most of the commercially important plants. Similarly, the alkaloids from medicinally important *A. lanata* were also considered to be the most prominent content with numerous commercial benefits. To screen their biological applications, raising the plants in *in vitro* conditions and to enhancing those secondary metabolites in the *in vitro* cultures will be a challenging task. Therefore, enhancement and identification of alkaloids through plant tissue culture studies are considered as an essential requirement for this study and it may help the commercial producer's bioactive compounds at large level for the industrial applications.

3. METHODOLOGY

3.1. Indirect organogenesis of *A. lanata*

3.1.1. Source of explants

For this study, medicinally significant plant materials *A. lanata* were collected from the nearby agriculture fields of Coimbatore districts and established in the Botanical Garden, Bharathiar University, Coimbatore.

3.1.2. Explant preparation and callus induction

Elite explants (20 days old) were selected for inoculation. For callus induction, the shoot tip, nodal, and leaf segments of *A. lanata* were used as explants for callus induction in an MS medium (Murashige and Skoog, 1962). The different growth regulators (auxins and cytokinins) such as 2,4-D, BAP and NAA were used as influencing agents in MS medium that was prepared at five concentrations namely 1, 1.5, 2, 2.5, and 3 mg/L. Before inoculation, the nutrient medium was prepared with 100 mL modified MS media and was augmented with 3% (w/v) sucrose and 0.8 (w/v) agar. The pH value of the medium was attuned to 5.5–5.8 before autoclaving it at 121 °C for 15 min. The explants were inoculated on callus induction media and temperature of 24 ± 2 °C was maintained under 16/8 h photoperiods (light/dark) with a light intensity of 1.496 W m⁻². The inoculated medium was incubated and left undisturbed for 4–6 weeks period to revitalize callus tissue. Further, the proliferation of callus was maintained and transferred to the fresh callus-inducing media at an interval of every 21 days (Hossain *et al.*, 2008). After the inoculation period, the influence of growth regulators was observed in terms of callus response, color, and texture of the callus.

3.1.3. Callus induction

For callus induction, the shoot tip, nodal, and leaf segments of *A. lanata* were used as explants for callus induction in an MS medium (Murashige and Skoog, 1962). The different growth regulators (auxins and cytokinins) such as 2,4-D, BAP and NAA were used as influencing agents in MS medium that was prepared at five concentrations namely 1, 1.5, 2, 2.5, and 3 mg/L. After the preparation of the above concentrations of growth regulators containing MS medium, the medium's pH was calibrated to 5.7 and added with 0.8% agar and sterilized under optimal conditions. The MS medium dispensed into the glassware was then inoculated with the disinfected explants (e.g., shoot tip segments). The cultures were kept under a 16 h photoperiod (3000 lux) at an optimum temperature of 24 ± 2 °C. Each experiment was performed in triplicates and each replicate consisted of 21 tubes per concentration. After the inoculation period, the influence of growth regulators was observed in terms of callus response, color, and texture of the callus. After 4 weeks, the obtained callus

was subcultured in the growth regulators containing MS medium, followed by incubation under the abovementioned standard growing conditions.

3.1.4. Estimation of total alkaloid content

The obtained *in vitro* plant materials from cell suspension culture of *A. lanata* were collected, shade-dried, and ground with the help of a mortar and a pestle. To 5 g of each callus powdered sample, 100 mL absolute methanol was added for solvent extraction through cold percolation and kept overnight in the orbital shaker. Extracts from chloroform were filtered separately by using Whatman No.1 filter paper and concentrated using a rotary evaporator (Wansi *et al.*, 2013). To separate alkaloids, a standard procedure of Trease and Evans (2009) was adopted and used with some required modifications. The chloroform extraction was repeated numerous times and the bulk extract was determined. Further, Gas Chromatography–Mass Spectroscopy, performed with the attained bioactive compounds.

3.1.5. GC–MS analysis of bioactive components in chloroform extract of callus

The compounds present in the methanolic extracts of *in vitro* propagated *A. lanata* were identified using GC–MS analysis with few modifications (Pandey *et al.*, 2018). The presence of compounds was analyzed using the mass spectral records available in the National Institute Standards and Technology (NIST) and Wiley Registry of Mass Spectral Data, New York (Wiley) libraries (Yadav *et al.*, 2019).

4. Result and Discussion

4.1. Callus induction from *A. lanata*

In this investigation, 10 variants of hormonal combinations (BAP, 2, 4-D and NAA) were used. The most intense growth of callus was detected on media augmented with 2.0 mg/L 2,4-D + 1.0 mg/L BAP as well as on media containing 1.5 + 1.0 mg/L 2,4-D and BAP. Average callus growth was noted in media supplement with 1.0 mg/L 2,4-D + 0.5 mg/L NAA and also on the media containing NAA only. Callus induction was influenced by plant type and the native of explants formed rapid undifferentiating callus tissue (Victorio *et al.*, 2012; Karunaratne *et al.*, 2014). In this study, ordinary hormonal combinations (2, 4-D, 2, 4-D + NAA at the concentrations of 0.5–3.0 mg/L) were used to induce callus from nodal and shoot tip explants. The MS media supplemented with 0.5 mg/L 2, 4-D alone failed to produce callus; the maximum rate of callus initiation (%) was observed in 3.0 mg/L 2, 4-D where the hormone 2, 4-D in combination with NAA (2.0 + 0.5 mg/L) developed a higher amount of callus. In contradiction, the lowest callus formation (%) was observed with 1.0 and 2.0 mg/L of 2, 4-D alone and 2.0 mg/L 2, 4-D combined with 0.5 and 1.0 mg/L NAA (Fig.1).

Chen *et al.* (2018) stated that the leaf explants of *Vernonia amygdalina* in MS medium containing 0.5–2.0 mg/L 2,4-D showed a higher rate of callus formation whereas the impact of a higher concentration of 2,4-D produced less callus production. In all growth conditions, callus was produced had uniform morphology, showing similar color and characteristics namely yellow color and fragile and soft texture. The difference in the texture and morphology of callus formed from various hormonal media. The texture of callus was observed as granular and spongy, friable, semi-friable spongy, and fragile. A similar report in *Lantana camara* leaf and shoot explants developing callus in the medium shows greenish compact and yellow-white callus documented by Veraplakorn (2016) was compared here. In another report, callus of *Centrosema pascuorum* induced from shoot explants producing yellow and greenish friable callus (Veraplakorn *et al.*, 2012) has been found, which is similar to this report. Mostly pinkish calli were obtained from both the leaf and shoot tip explants.

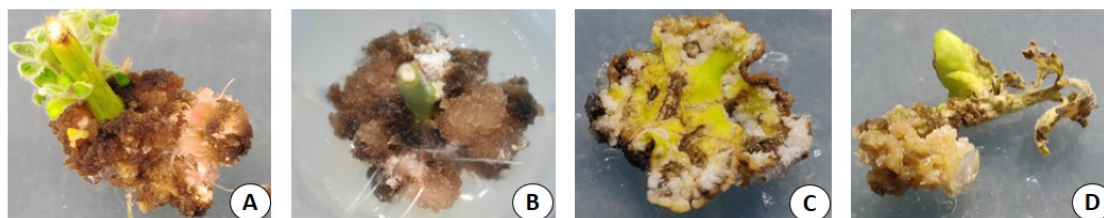


Fig 1: Effect of different concentration of plant growth regulators on different explants of *A. lanata* callusing; (A) Callus induction from nodal explants with 1.5 + 1.0 mg/L 2,4-D and BAP, (B) Callus induction from nodal explants with 1.0 mg/L 2,4-D + 0.5 mg/L NAA (C & D) Callus induction from leaf and shoot tip explants.

4.2. Quantification of total alkaloids (phytochemicals) from callus extracts of *A. lanata*.

The present study discusses the presence of total alkaloid content from the callus extracts of *A. lanata*. The usage of different polarity-based solvents confirmed and quantified the phytoconstituents present in the callus extract samples. Earlier reports also possess the presence of alkaloids as one of the major compounds (Srinivas and Reddy, 2012 and Rajesh *et al.*, 2012). After rigorous examination, it was found that maximum efficacy of the total alkaloid's (mg QU/g extract) existence was measured with methanolic extracts of callus (160.52 ± 0.32 mg QU/g extract), while lower levels were reported with chloroform obtained from callus extract (23.20 ± 1.63 mg QU/g extract). However, the minimum presence of alkaloids in the aqueous extract was screened with callus extract (133.05 ± 1.72 mg QU/g extract).

The result of this study evidenced the presence of chief alkaloid content that could be derived majorly through callus extracts from different solvents. Meanwhile, the lower presence of the compound was also analyzed from each technique and represented in Table 1. The HR from acetone extract was comparatively higher than the chloroform extracts. This corroborates with the findings Goyal *et al.* (2011); Rajesh *et al.* (2011); Saravana *et al.* (2011) which proved the existence of indole cathin-6-one alkaloid from the *A. lanata* extracts.

The presence of isolated alkaloids from *A. lanata* leaves was reported with canthin-6-one and β -carboline, hentriacontane, β -sitosterol and its D-glycoside, α -amyrin and botulin (Kumar *et al.*, 2009). The result of this study adjudges the previous reports regarding the presence of indole alkaloid in *A. lanata* and several other plant species exhibiting alkaloid contents such as *Picrolemma granatensis*, *Eurycoma harmandiana* (Kanchanapoom *et al.*, 2001), *Stellaria dichotoma* (Sun *et al.*, 2004) and *A. lanata* (Zapesochneya *et al.*, 1991). Moreover, a report by Saravanasingh *et al.* (2016) about the efficacy of various extracts derived from aerial parts of *A. lanata* using different solvents agrees with the present findings.

Table 1: Quantification of phytochemicals (Total alkaloids) from different extracts of *in vitro* propagated *A. lanata*.

Solvents	Total alkaloid (mg QU/g extract)
Petroleum ether	28.52 $\pm$ 6.85 ^e
Chloroform	23.20 $\pm$ 1.63 ^f
Ethyl acetate	31.25 $\pm$ 1.21 ^d
Acetone	60.33 $\pm$ 0.21 ^c
Methanol	160.52 $\pm$ 0.32 ^a
Aqueous	133.05 $\pm$ 1.72 ^b

Note: QU – Quinine equivalents, values in the table are the mean $\pm$ SE of triplicates.

4.3. Gas Chromatography-Mass Spectroscopy analysis for callus extract of *A. lanata*.

Gas chromatography and mass spectrometric analysis of callus from *A. lanata* was conducted and the compounds were listed in Table 2. Compounds were studied using the NIST (National Institute Standard and Technology) database (Hemalatha *et al.*, 2016). The use of methanol acts as the best solvent for the extraction of large compounds from *A. lanata*.

This confirms the study reported by Anwar *et al.* (2013), which stated methanol as the best solvent supports the extraction of compounds in *L. camara*.

Similarly, results obtained by Chinnadurai *et al.* (2019) on *Manihot esculenta* reported that 10 compounds were identified by a mass spectrometer with the help of gas chromatography. Likewise, the present study, 20 major peak compounds and 18 compounds from callus extracts were detected (Table 2). Likewise, a study by Salim (2018) in *Thevetia nerifolia* plant callus extract reported the presence of 12 compounds on GC–MS analysis. The present GC–MS results showed target alkaloid compounds (aervine (AE) and methylaervine (MAE)), which were present in all of the tested extracts IO-AE (RT = 18.902), MAE (RT = 22.485) of *A. lanata*. The rest of the compounds existing in the samples had unique biological properties and a few compound properties were not identified. Numerous researches have conducted analyses on different compounds obtained from the GC–MS.

Essential oil from *A. javanica* obtained by hydro distillation and dry steam distillation yield 38 compounds (Samejo *et al.*, 2013). The bioactive components eugenol (19.46%), 1, 2, 3-benzene triol (5.48%), and caryophyllene (4.48%) from the clove extract (Hemalatha *et al.*, 2016). *Nigella sativa* showed 28 major compounds (Hadi *et al.*, 2016) along with antibacterial, antifungal, anti-inflammatory, antiasthmatic, antioxidant, and anticancer properties. Through this analysis, it has been found that *A. lanata* extracts have aervine and methylaervine as the predominant compound in the tested extracts.

Table 2: GC-MS analysis report for callus extract of *A. lanata*.

RT*	Name	Molecular Formula	Molecular Weight	Area (%)
4.217	BENZENE, METHYL-	C ₇ H ₈	92	2.10
7.434	Butane, 1,1-diethoxy-3-methyl-	C ₉ H ₂₀ O ₂	160	0.88
14.254	1-Hexadecanol	C ₁₆ H ₃₄ O	242	2.99
15.779	2,4-DITERT-BUTYLPHENOL	C ₁₄ H ₂₂ O	206	6.31
15.862	1-Decanol, 2-hexyl-	C ₁₆ H ₃₄ O	242	0.40
15.980	Nonadecyl pentafluoropropionate	C ₂₂ H ₃₉ F ₅ O ₂	430	0.49
18.457	Nonadecyl trifluoroacetate	C ₂₁ H ₃₉ F ₃ O ₂	338	0.59
18.902	6H-Indole(3,2,1-de)(1,5)naphthyridin-6-one,10-hydroxyl (10-Hydroxycanthin-6-one)	C ₁₄ H ₈ N ₂ O ₂	236	0.65
19.051	n-Nonadecanol-1	C ₁₉ H ₄₀ O	284	6.27

20.939	Heptacosyl heptafluorobutyrate	C ₃₁ H ₅₅ F ₇ O ₂	592	0.77
21.105	Behenic alcohol	C ₂₂ H ₄₆ O	326	5.54
22.485	10-methoxy-6h-indolo[1,5]naphthyridin-6-one (10-Methoxycanthin-6-one)	C ₁₅ H ₁₀ N ₂ O ₂	250	1.06
22.690	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	0.67
22.890	Butyramide, 4-phenyl-N-pentyl-	C ₁₅ H ₂₃ NO	233	1.24
23.026	Heneicosane	C ₂₁ H ₄₄	296	1.38
23.900	Eicosane	C ₂₀ H ₄₂	282	2.01
25.830	1,2-BENZENEDICARBOXYLIC ACID	C ₂₄ H ₃₈ O ₄	390	2.04
27.111	Tris(2,4-di-tert-butylphenyl) phosphate	C ₄₂ H ₆₃ O ₄ P	662	7.94

Note: *RT: Retention time.

Conclusion:

In conclusion, this study successfully optimized the callus induction process for *Aerva lanata* (L.) Juss. ex Schult., highlighting the crucial influence of hormonal combinations on tissue growth. The most effective treatment led to significant proliferation of callus tissue, reflecting the plant's high potential for *in vitro* propagation. It was clear that various hormonal media significantly affected the morphology and texture of the induced callus, with a range of structural variations observed, which demonstrates the complexity of plant tissue responses to growth regulators. The study also examined the total alkaloid content from the callus extracts, establishing that methanol emerged as the optimal solvent for extracting bioactive compounds from this species. The identification of key alkaloids, through gas chromatography-mass spectrometry analysis, underscores the potential medicinal value of *Aerva lanata* and its relevance in pharmacological contexts. Overall, these findings contribute valuable insights into the enhancement of bioactive compound production through refined *in vitro* techniques, fostering further research into the therapeutic applications of *Aerva lanata*. This research not only supports the traditional uses of the plant but also paves the way for its exploration in modern medicine, emphasizing the importance of continued investigation in plant biotechnology to efficiently harness medicinal compounds. The combination of optimized tissue culture and phytochemical analysis positions *Aerva lanata* as a promising candidate for future pharmaceutical development.

Acknowledgments

The First author and corresponding author with gratitude acknowledge the Anusandhan National Research Foundation (ANRF), New Delhi (Formerly Science and Engineering Research Board, SERB.), for their financial assistance for this research work (File No: PDF/2023/002238 Dt. 02.02.2024). The authors extend deepest thanks to the Plant Biotechnology Laboratory, Department of Biotechnology, Bharathiar University and Coimbatore for providing the necessary facilities to carry out this research work.

Declaration of Competing Interest

The authors declare no conflict of interest.

Credit authorship contribution statement

SB: Investigation; Methodology; Data curation; Conceptualization; Formal analysis; Writing - original draft, & editing. **SG:** Supervision; Validation; Writing Review & editing.

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Youth Perspectives on Sustainability: The Impact of Sociodemographic Variables in the Kashmir Valley, Jammu and Kashmir

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Abstract

Jammu and Kashmir have unique ecological and socio-economic challenges, therefore understanding student perspectives is vital for fostering sustainability. This research paper explores the attitudes of undergraduate students in the Kashmir Valley, Jammu and Kashmir, towards sustainable development. Given the region's unique ecological and socio-economic challenges, understanding student perspectives is vital for fostering sustainability. Using an online survey with a questionnaire, the study assessed attitudes across four domains: environmental, economic, social, and educational. Findings reveal that students possess highly positive attitudes toward sustainability. They recognize the link between environmental quality and quality of life, emphasizing the importance of biodiversity over industrial expansion. Economically, students believe government policies should promote sustainable production, and reducing hunger and poverty should take precedence over the economic interests of industrialized nations. In the social domain, students advocate for global peace, social welfare, and gender equality. Educationally, they note a growing commitment from teachers towards sustainability and express a desire for more initiatives and community engagement. Despite these positive attitudes, challenges such as limited resources and political instability hinder progress. The analysis also reveals that sociodemographic factors like gender, stream of study, semester, and family income significantly influence attitudes toward sustainable development among students in Kashmir Valley. The paper concludes with recommendations to integrate sustainability into educational curricula and promote student involvement in environmental initiatives, emphasizing the role of youth in advancing sustainable development in the region.

.Keywords: Sustainable, Kashmir valley, Undergraduate students

Introduction

Population growth and the greater commodification of human activities in the contemporary and urbanized lifestyle are the main causes of the ever-increasing environmental issues. Additionally, there is a rise in resource use that goes unchecked despite the continual ecological, economic, and social risks of exploitation. Due to this, undesirable ecological effects such as increasing carbon emissions, global pollution, deforestation, food poverty, and other issues arise (Diamantopoulos, A. et al 2003). Global inequality and division in society have increased as a result of the social structure and economic circumstances of today's globe. It has become imperative to comprehend how social, economic, and environmental factors interact with one other in human and natural systems. (Spangenberg, J. H. 2002). The interdependence of ecological, economic, and social systems is a condition of sustainability. To create or improve understanding of the connections between the issues of sustainable development, education for sustainable development is a dynamic concept that makes use of all facets of public awareness, education, and training. It also aims to develop the knowledge, skills, perspectives, and values that will enable people of all ages to take charge of creating and enjoying a sustainable future. Educating the next generation is the first step in changing society's patterns of behavior to promote sustainability.

Sustainable Development as defined by the United Nations Brundtland Report is *“the ability to meet the needs of present generations without compromising the ability of the future generations to meet their own needs”*. Society, the economy, and the environment are the three primary pillars of sustainable development. It encourages development that doesn't have an adverse impact on the environment or the ecology. (Brundtland,1987 &Emas, R. (2015).

The 17 Sustainable Development Goals (SDGs), which are intended to be accomplished by 2030, were adopted by 193 nations in 2015. These goals cover all three dimensions of sustainability viz economic, social, and environmental (Saner et al., 2019). Zwickle et al. (2014) have argued that future generations, particularly those enrolled in higher education, should play a significant part in promoting human welfare and preserving the environment. This understanding among young people will assist their in achieving the 17 SDGs by 2030. Education for Sustainable Development (ESD) is one method of educating a large number of people about the concepts and tenets of sustainable development (Kopnina & Meijers, 2014).

In higher education, theoretical frameworks for leadership and strategy can help students understand sustainability (Bezbatchenko, A. W. 2010). Leadership and commitment from

the institution are crucial. Leadership is seen as a crucial element in determining the direction of higher education institutions (Emanuel, R., & Adams, J. N. 2011). Sustainable outcomes are influenced by institutional leadership and commitment. Higher education institutions (HEIs) are becoming more and more conscious of the fact that they are an important part of society and must make meaningful contributions to sustainability and sustainable development (Tilbury, R. et al 2011). Higher education institutions are driving the change of our contemporary societies into what they will become through the instruction of future generations, the promotion of research, and the accumulation of collective knowledge (Lozano, D et al 2006). HEIs can transform the collective of young people who are transforming the world via education and activity. Aiming to enlighten young minds, assist and maintain a knowledgeable public, and solve problems via research are the goals of education in HEIs.

Abu Bakr et al (2016) investigated campus sustainability from students' perspectives and found that, despite students' high levels of awareness and concern about the sustainability of the campus's environment, they lack motivation and inclination to take part in initiatives to achieve sustainability. Malik, M.N. et al 2019 did an investigation on Pakistani students' knowledge of sustainability issues and the curriculum for technology education and found that teaching students about sustainability issues through technology education can assist them in understanding how to put sustainability principles into practice (economic, social, and environmental).

Balakrishnan, B et al (2020) presented the results of Malaysian undergraduates' views and perceptions of sustainable development. A total of 154 undergraduate students from five Malaysian universities participated in the study. The results of the analyses demonstrated that, with the exception of issues pertaining to the economy and society, the respondents had positive views and attitudes towards all aspects of sustainability—environmental, economic, and social.

Material and Methods

To study the attitude towards sustainable development, we have chosen the Kashmir Valley as the study area. The Valley of Kashmir is nestled in the northwestern folds of the Himalayas. The valley of Kashmir stretches between (32°.22' to 34° 43') north latitude to 73° 52' - 75° 42' East longitudes Bhat, M. S., & Rather, J. A. (2018). It comprises 10 districts

This study is primarily based on questionnaire surveys carried out among college students in the Kashmir Valley, Jammu and Kashmir. Notably, there are seventy-three Government Degree Colleges in Kashmir offering a range of undergraduate and postgraduate courses. To narrow down the sampling unit, students from only twenty colleges with two colleges from each district were selected for the present study.

The questionnaire was adapted from Biasatti and Frate (2017), who studied attitudes toward sustainable development among Italian university students. The scale consists of four sections viz Environmental, Economic, Social, and Educational with five items for every section. A convenience sampling method was employed to choose the sampling units (Government Degree Colleges) from the total number of colleges located in both rural and urban areas and to choose the students across all the districts of the geographical location opted for the study. This method of sampling was adopted because it relies on the data collected from population members who are conveniently available to participate in a study. The survey was carried out finally in the twenty Colleges from November 2023 to February 2024. Of the total 1200 students submitted their responses with almost equal proportions from every district. A 5-point Likert Scale was used in which “1” represents strong agreement with the statement and the opposite stands for “5”

Results and Discussion

This section presents the results of the study examining undergraduate students’ attitudes toward sustainable development in the Kashmir Valley, with a specific focus on how various sociodemographic variables influence these attitudes.

Table 1: Student’s attitude towards Sustainable Development

S.No.	Statement	Mean	SA 1	A 2	N 3	D 4	SD 5
1	ASD1	1.85	35.5	50.5	8.8	3.8	1.3
2	ASD2	1.78	39.5	49.3	6.8	2.5	1.8
3	ASD3	1.96	31.7	50.0	11.5	4.3	2.5
4	ASD4	2.04	35.5	41.3	11.2	7.5	4.5
5	ASD5	1.79	45.3	40.0	8.3	3.3	3.0
6	ASD6	2.02	31.5	47.0	12.5	5.8	3.2
7	ASD7	2.02	26.3	52.2	17.0	2.5	2.0
8	ASD8	2.01	26.7	55.0	11.7	3.7	3.0
9	ASD9	1.80	44.5	41.3	6.5	4.7	3.0
10	ASD10	1.79	48.0	36.7	7.7	3.8	3.8
11	ASD11	1.67	50.7	36.8	7.7	3.2	1.7

12	ASD12	1.70	50.8	36.5	6.3	4.3	2.0
13	ASD13	2.06	24.0	55.8	13.0	4.7	2.5
14	ASD14	1.72	46.8	42.0	5.3	3.5	2.3
15	ASD15	1.79	40.8	45.7	8.7	3.5	1.3
16	ASD16	1.71	43.8	46.7	5.5	2.2	1.8
17	ASD17	1.73	43.5	45.5	7.2	2.2	1.7
18	ASD18	2.01	31.0	48.2	12.5	5.8	2.5
19	ASD19	1.78	41.0	47.7	5.5	3.7	2.2
20	ASD20	2.09	33.3	40.3	14.8	7.3	4.2

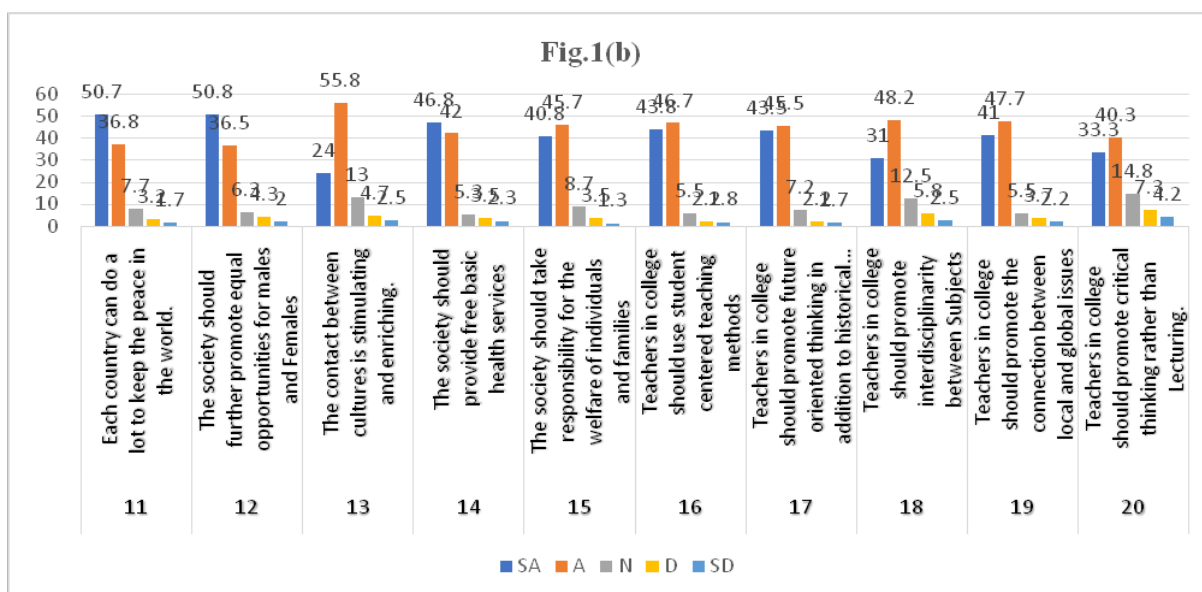
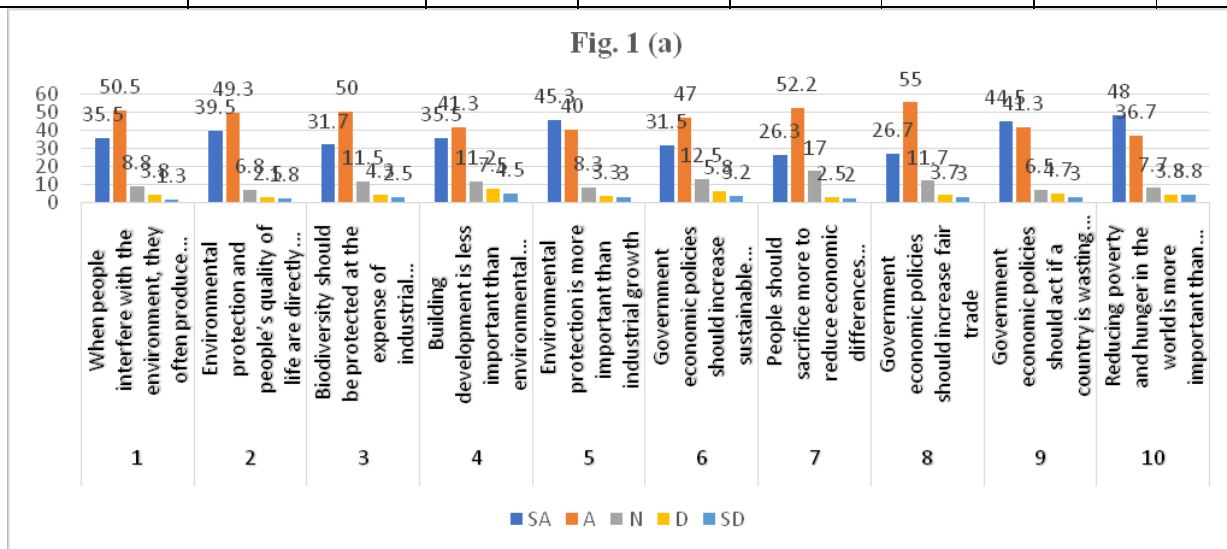


Fig 1(a) &(b): Student's attitude towards sustainable development

From Table 1 and Figure 1(a) and (b) it can be revealed that students have very positive attitudes towards all four domains viz environmental, economic, social, and educational sustainable development. The students are much aware of the fact that people's interference with the environment can prove disastrous for it as depicted by the data in which 86% of students have agreed to it. Similarly, 88.8% agreed that quality of life is linked to environmental quality, 81.7% of students agreed that protecting biodiversity is important rather than industrial expansion and 76.8% of students agreed that development is less important than environmental protection. Regarding the economic domain of sustainable development, the students have a positive attitude as depicted by the data in which 78.5% agree that government economic policies should lead to sustainable production and 84.7% agree that reducing hunger and poverty is more important than the economic well-being of industrialized countries. Regarding the Social domain, students agree that every country must maintain peace in the world and society should take responsibility for the welfare of individuals and society and societies should promote equal opportunities irrespective of gender. Regarding education for sustainable development, more than 80% of students agreed that teachers should promote student-centered teaching, future-oriented thinking, interdisciplinarity between subjects, and connection between local and global issues.

Table 2: Student's views on attitude towards sustainable development with respect to sociodemographic variables.

S.No.	Variable	Subgroup	Mean	SD	t-value/F- value	P-value
1	Gender	Male	1.96	0.64	3.21	0.007
		Female	1.84	0.46		
2	Residence	Rural	1.87	0.53	0.60	0.541
		Urban	1.85	0.52		
3	Stream of Study	Humanities	1.93	0.54	14.79	0.003
		Science	1.74	0.43		
		Others	1.91	0.58		
4	Father's education	Illiterate	1.85	0.55	0.61	0.537
		Literate	1.87	0.52		
5	Mother's Education	Illiterate	1.86	0.56	0.49	0.620
		Literate	1.88	0.50		
6	Semester	Ist	1.91	0.52	2.51	0.028
		2 nd	1.78	0.62		
		3 rd	1.94	0.58		
		4 th	1.87	0.49		
		5 th	1.76	0.52		
		6 th	1.78	0.50		
7	Father's occupation	Govt Service	1.87	0.54	0.19	0.821
		Private Service	1.84	0.50		
		Self employed	1.88	0.52		

8	Family Income	Below 25000	1.91	0.55	10.45	0.018
		25000-50000	1.88	0.51		
		Above 50000	1.71	0.42		

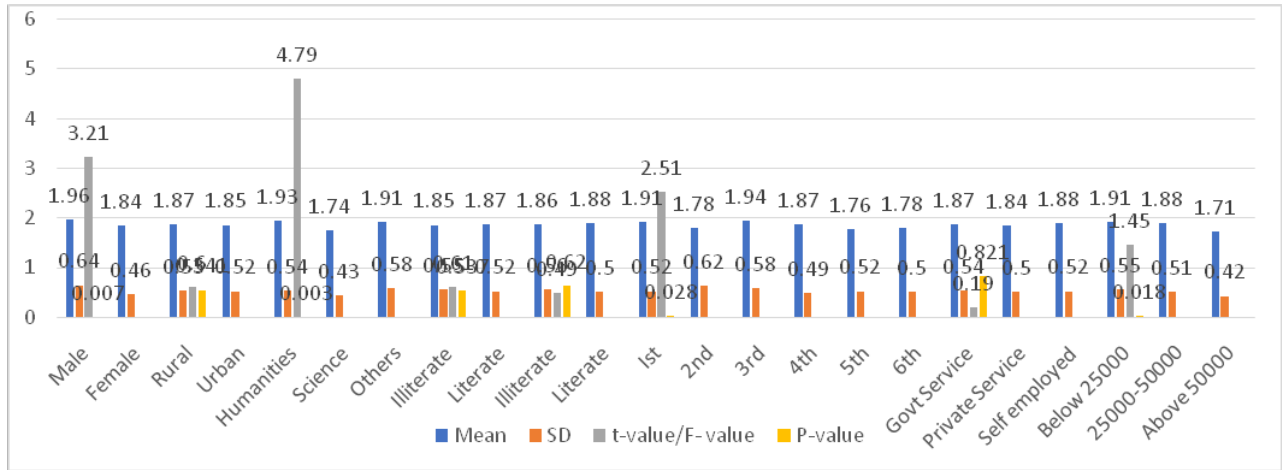


Fig: Students' views on attitude towards Sustainable Development in Jammu & Kashmir concerning their Socio-demographic attributes

From Table 2 it can be inferred that male students exhibit a slightly more favorable attitude towards sustainable development compared to female students. The difference, while statistically significant, is moderate and could be influenced by varying exposure to environmental education or societal expectations. The minimal difference suggests that both rural and urban students in Kashmir Valley hold similar views on sustainable development. Science students show the most favorable attitude (mean = 1.74), compared to Humanities (1.93) and Other streams (1.91). The difference is statistically significant ($p = 0.003$). This may be due to their greater exposure to environmental science and sustainability topics in their curriculum. Humanities and other streams, while still positive, may lack the same depth of engagement with sustainability issues. This underlines the importance of integrating sustainability education across all disciplines. There are negligible differences between students with illiterate and literate parents for both fathers (mean = 1.85 vs. 1.87, $p = 0.537$) and mothers (mean = 1.86 vs. 1.88, $p = 0.620$). Students in the 5th semester (mean = 1.76) and 2nd semester (mean = 1.78) show more favorable attitudes compared to those in the 1st (1.91) and 3rd semesters (1.94). The p -value of 0.028 indicates statistical significance. The variation could be attributed to differences in curriculum exposure at different academic levels. Students in higher semesters might have more exposure to sustainability-related topics or may develop more critical thinking over time, enhancing their appreciation for sustainable development. There is no significant difference in attitudes based on the father's occupation—government service (mean = 1.87), private service (1.84), and self-employed (1.88),

with a p -value of 0.821. Students from higher-income families (above 50,000; mean = 1.71) have more favorable attitudes compared to those from lower-income groups (below 25,000; mean = 1.91). The p -value of 0.018 indicates statistical significance. Students from wealthier backgrounds may have more access to resources and education that promote sustainability awareness.

Conclusions:

The analysis reveals that sociodemographic factors like gender, stream of study, semester, and family income significantly influence attitudes towards sustainable development among undergraduate students in Kashmir Valley. While gender and income differences reflect broader socio-economic influences, the stream of study and academic progression highlights the role of education in shaping sustainability mindsets. Surprisingly, residence, parental education, and father's occupation showed little to no impact, suggesting a more uniform spread of sustainability awareness across these demographics.

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Pancreatic Cancer'S Lethal Challenge: Can Medicinal Plants Answer?

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ABSTRACT

Pancreatic cancer (PC) remains a formidable carcinoma, characterized by its aggressive nature, late diagnosis, and poor prognosis. Despite advances in oncology, the five-year survival rate remains distressingly low due to its subtle progression and resistance to established therapies. The molecular complexity of PC, driven by genetic mutations such as KRAS, TP53, CDKN2A, and SMAD4, leads to uncontrolled tumor growth, therapy resistance, and metastasis. Additionally, the tumor microenvironment (TME), distinguished by dense fibrosis and immunosuppressive factors, restricts drug delivery and immune responses, further compromising treatment effectiveness. The scarcity of early diagnostic biomarkers, rapid metastasis, and limited success of surgical resection exacerbate the lethality of this disease. Current treatment approaches, including chemotherapy and targeted therapies, demonstrate limited success, necessitating innovative therapeutic strategies. Emerging advancements in liquid biopsy, precision medicine, and immunotherapy offer potential breakthroughs in improving early detection and patient outcomes. This review explores the key factors contributing to pancreatic cancer's lethality and highlights ongoing research efforts aimed at overcoming these challenges. Furthermore, this review also discusses medicinal plant species with proven efficacy in treating PC, offering insights into potential novel therapeutic avenues.

Key words: prognosis, metastasis, medicinal plants, phytochemicals, early diagnosis

Introduction

With a five-year survival rate of less than 10% and an exceedingly poor prognosis, pancreatic cancer (PC) is one of the most devastating and aggressive cancers. Pancreatic cancer continues to rank among the world's leading causes of cancer-related mortality, despite tremendous advancements in cancer research and treatment (Siegel *et al.*, 2024). According to the estimates from the Global Cancer Statistics (GLOBOCAN 2024), pancreatic cancer causes more than 500,000 new cases and deaths per year, underscoring its terrible effects. Its lethality is due to the fact that it is frequently discovered at an advanced or metastatic stage, which leaves few options for treatment (Li *et al.*, 2012). Pancreatic cancer lacks trustworthy biomarkers for early diagnosis, making it challenging to detect in contrast to cancers like breast or colorectal cancer, where early detection through routine screening improves survival outcomes (McGuigan *et al.*, 2018) (**Figure 1**).

A multifaceted and tumor microenvironment (TME) is a hallmark of pancreatic cancer and is important to the development to the of the disease and resistance to treatment. The extracellular matrix (ECM) and cancer-associated fibroblasts (CAFs) make up the dense fibrotic stroma, which produces an immunosuppressive and hypoxic environment that impedes drug penetration and encourages tumor growth (Puckett *et al.*, 2024). Pancreatic tumors are completely impervious to immunotherapy, targeted therapy, and customary chemotherapy because of this protective barrier (Halbrook *et al.*, 2023). A number of genetic mutations in important oncogenes and tumor suppressor genes, including KRAS, TP53, CDKN2A, and SMAD4, are also responsible for pancreatic cancer. These mutations lead to unchecked cell proliferation, metastasis, and resistance to apoptosis. The invasive and metastatic aspects of pancreatic cancer are further enhanced by the epithelial-mesenchymal transition (EMT), which causes tumor cells to spread quickly to distant organs, particularly the liver, lungs, and peritoneum (Xu *et al.*, 2022).

Notwithstanding these obstacles, new developments in the study of pancreatic cancer have raised hopes for early identification and focused therapy (Andersson *et al.*, 2022). Techniques for liquid biopsies that identify circulating tumor DNA (ctDNA), exosomes, and microRNAs are becoming more and more promising as early diagnostic tools. In addition, the potential of medicinal plants and their isolated phytochemicals in pancreatic cancer treatment is gaining recognition (Wood *et al.*, 2012; Mizrahi *et al.*, 2020). Many plant-derived

compounds exhibit anti-cancer properties, and their use as adjuncts to conventional therapies is being explored. New therapeutic approaches like immunotherapy combinations, KRAS inhibitors, and drug delivery systems based on nanotechnology are also being evaluated to overcome therapy resistance and enhance quality of care (Genc *et al.*, 2023). While these procedures are still in the experimental or early clinical trial phases, more investigation is needed to translate these findings into practical therapies (Wang *et al.*, 2021; Kolbeinsson *et al.*, 2022). With an emphasis on the molecular mechanisms underlying pancreatic cancer's aggressiveness, difficulties in early detection, therapy resistance, the potential role of medicinal plants, and continuous improvements in treatment approaches, this review seeks to offer a thorough understanding of why the disease is so deadly.

Molecular and Genetic Factors Driving Pancreatic Cancer

Because of its complex molecular landscape, which includes important genetic mutations, epigenetic modifications, and mechanisms like the epithelial-mesenchymal transition (EMT), pancreatic cancer is one of the most aggressive cancers. Among these, KRAS, TP53, CDKN2A, and SMAD4 mutations are important for tumor growth, metastasis, and treatment resistance (Wood and Hruban, 2012). More than 90% of pancreatic ductal adenocarcinomas (PDAC) have KRAS mutations, which keep the protein constitutively active and cause unchecked activation of the PI3K/AKT and MAPK/ERK pathways, which support tumor survival and growth. This mutation is a key target for drug development because it is thought to be an early symptom even within pancreatic carcinogenesis, precursor lesions like pancreatic intraepithelial neoplasia (PanIN). Effective KRAS inhibitors are still a major obstacle in clinical practice, though. Comparably, TP53 mutations, which occur in roughly 50–75% of cases, lead to increased genomic instability, impaired apoptosis, and a loss of cell cycle regulation, all of which exacerbate the belligerence and chemotherapy resistance of pancreatic cancer (Zavoral, 2011).

The mutation of CDKN2A, which codes for the tumor suppressor protein p16INK4A, a decisive regulator of the G1-S transition in the cell cycle, is another noticeable genetic change in pancreatic cancer. Unchecked cellular proliferation results from the removal of this checkpoint caused by loss of CDKN2A function (Howe and Conlon, 1997). 90% of pancreatic cancers have this mutation, which frequently results from homozygous deletion, mutation, or promoter hypermethylation. Besides that, TGF- β signaling, which typically acts as a carcinoma suppressor in early-stage cancer but paradoxically encourages progression and

metastasis in later stages when SMAD4 is lost, is disrupted by SMAD4 mutations, which are found in roughly 50% of cases. A particularly bad prognosis and a higher risk of metastasis are linked to SMAD4 loss (Liu *et al.*, 2023).

The aggressiveness of pancreatic cancer is significantly influenced by epigenetic changes in addition to genetic mutations. Global hypomethylation contributes to genomic instability and increased tumor growth, while DNA methylation, a crucial epigenetic mechanism, silences tumor suppressor genes like CDKN2A. Malignancy is further fueled by histone modifications, especially histone deacetylation, which suppress the expression of vital genes associated with tumor suppression (Simeone and Maitra, 2013). Moreover, non-coding RNAs (ncRNAs) like long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) assist the development of tumors. Some miRNAs, like miR-21 and miR-155, function as oncogenes that encourage metastasis and chemoresistance. Drugs like DNA methyltransferase inhibitors (DNMTi) and histone deacetylase (HDAC) inhibitors are being investigated for the treatment of pancreatic cancer because epigenetic changes are reversible and therefore make promising therapeutic targets (Hruban *et al.*, 1998).

The epithelial-mesenchymal transition (EMT) is a major process that contributes to the invasive and metastatic potential of pancreatic cancer. Through the process of cellular reprogramming known as EMT, epithelial cells develop mesenchymal characteristics and lose their ability to adhere to other cells, which increases their capacity for invasion and migration. Important transcription factors like SNAIL, SLUG, ZEB1, and TWIST, which increase mesenchymal markers like vimentin, fibronectin, and N-cadherin while suppressing E-cadherin expression, are responsible for this process (Capurso *et al.*, 2009). Hypoxia, inflammatory cytokines, and TGF- β signaling all contribute to EMT by fostering a tumor-permissive microenvironment. EMT not only promotes metastasis but also contributes to immune evasion and resistance to chemotherapy, making it a major challenge in pancreatic cancer treatment. Since EMT is a reversible process, targeting EMT pathways through TGF- β inhibitors, MET inhibitors, and miRNA-based therapies is an emerging strategy in pancreatic cancer research (Capurso *et al.*, 2009; Liu *et al.*, 2023).

In general, a combination of genetic mutations, epigenetic dysregulation, and EMT-driven metastasis are the main causes of pancreatic cancer's lethality. These factors all contribute to treatment resistance, the disease's quick progression, and its unfavorable prognosis (Liu *et al.*, 2023). It is essential to comprehend these molecular processes in order

to create early diagnostic instruments and efficient targeted treatments. Even though there has been a lot of progress in finding possible KRAS inhibitors, epigenetic medications, and EMT-targeting treatments, more investigation and clinical validation are needed to turn these discoveries into better patient outcomes (Zavoral, 2011; Simeone and Maitra, 2013).

Tumor Microenvironment and Therapeutic Resistance in Pancreatic Cancer

Tumor progression, metastasis, and treatment resistance are all significantly influenced by the highly desmoplastic and immunosuppressive tumor microenvironment (TME), which is another factor contributing to pancreatic cancer in addition to genetic mutations (Deng *et al.*, 2022). An extensive fibrotic stroma, or desmoplasia, is one of the defining characteristics of pancreatic ductal adenocarcinoma (PDAC). This condition produces a dense extracellular matrix (ECM) made up of collagen, fibronectin, and hyaluronic acid (Hartupée *et al.*, 2024). In addition to offering structural support for tumor growth, this fibrotic barrier physically prevents drugs from penetrating, reducing the effectiveness of chemotherapy. Pancreatic stellate cells (PSCs) and cancer-associated fibroblasts (CAFs) also remodel the extracellular matrix (ECM), creating a stiff, hypoxic environment that encourages tumor aggressiveness and immune evasion (Sherman and Beatty, 2022).

In the pancreatic TME, cancer-associated fibroblasts (CAFs) play a crucial role by secreting growth factors like interleukin-6 (IL-6), fibroblast growth factor (FGF), and transforming growth factor-beta (TGF- β). These elements promote angiogenesis, invasion, and tumor growth while creating a niche that protects cancer cells from immunological monitoring and chemotherapy (Espona-Fiedler *et al.*, 2024). By upregulating SNAIL, ZEB1, and TWIST, CAFs also aid in the epithelial-mesenchymal transition (EMT), which promotes tumor metastasis. Additionally, CAFs work with immune cells to create an environment that suppresses the immune system, which is a significant barrier to effective immunotherapy (Poyia *et al.*, 2024).

Hypoxia, which results from inadequate vascularization and an overabundance of stromal content and causes oxygen deprivation in the tumor core, is another important feature of the pancreatic TME. Hypoxia-inducible factor-1 alpha (HIF-1 α), which promotes angiogenesis via vascular endothelial growth factor (VEGF), is upregulated by tumor cells in response to hypoxia (Liu *et al.*, 2017). However, this results in aberrant, leaky, and ineffective vasculature rather than the formation of functional blood vessels, which exacerbates hypoxia

and nutrient deprivation. By lessening the damage that oxidative stress causes to cancer cells, hypoxic environments not only increase tumor cell survival and metabolic adaptation but also create resistance to chemotherapy and radiation treatment (Pratticò and Garajová, 2024).

In the pancreatic TME, where tumors actively inhibit anti-tumor immune responses, immune evasion is another crucial characteristic. To reduce cytotoxic T-cell activity and improve immune tolerance, regulatory T cells (Tregs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs) are extensively infiltrated in pancreatic cancer (Looi *et al.*, 2019). Additionally, immune checkpoint molecules like cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death-ligand 1 (PD-L1) are expressed by pancreatic tumors, further suppressing T-cell-mediated anti-tumor responses. One of the main causes of pancreatic cancer's continued resistance to immunotherapy is this immune-suppressive milieu; in contrast to other cancers such as lung and melanoma, immune checkpoint inhibitors have had only modest clinical success (Dauer *et al.*, 2016).

Numerous approaches are being investigated to target the tumor's stromal and immune components because of the TME's significant influence on the development of pancreatic cancer and treatment resistance (Lorestani *et al.*, 2024). Hyaluronidase-based therapies and other stroma-targeting agents work to improve drug delivery by breaking down excess extracellular matrix components. Furthermore, agents that target CAF and TGF- β are being developed to modify the desmoplastic reaction (Dauer *et al.*, 2016). Immune checkpoint inhibitors are being tested in conjunction with immune-modulating agents and chemotherapy to improve anti-tumor immunity, despite their lack of efficacy as monotherapies. Additionally, methods to combat hypoxia, like vascular normalizing agents and HIF-1 α inhibitors, have the potential to enhance treatment results. However, combination therapies that target multiple pathways will probably be necessary to achieve significant clinical benefits because of the pancreatic TME's highly dynamic and adaptive nature (Looi *et al.*, 2019).

Mechanisms of Pancreatic Cancer Growth Inhibition by Phytochemicals

Naturally occurring compounds derived from plants, known as phytochemicals, have attracted a lot of attention as possible treatments and chemo preventive measures against pancreatic cancer (Gupta and Pramanik, 2016; Casarcia *et al.*, 2023). By blocking the normal function of proteins that inhibit apoptosis, these compounds induce apoptosis; they also cause

cell cycle arrest; they reduce inflammation by suppressing transcription factors of the NF- κ B family; they reverse EMT; they modulate the immune system; they increase pro-apoptotic factors while minimizing anti-apoptotic factors; and they inhibit angiogenesis (Arnab *et al.*, 2024; Arnab *et al.*, 2024b). Certain phytochemicals, such as triptolide (from *Tripterygium wilfordii* Hook.f.), resveratrol, quercetin, curcumin (from *Curcuma longa* L.), and cannabinoids, have shown anticancer potential in the treatment of pancreatic cancer (Genc *et al.*, 2023) (**Figure 2**). These substances provide a multifaceted strategy to stop the growth of pancreatic cancer cells by modifying a number of biological processes and focusing on aberrantly expressed molecular factors implicated in the initiation and spread of cancer (Aliebrahimi *et al.*, 2018; Ramakrishnan *et al.*, 2019).

Conclusion

One of the most challenging and lethal tumors to treat is pancreatic cancer because of its intricate molecular and genetic makeup, as well as its very fibrotic and immunosuppressive tumor microenvironment. Significant genetic abnormalities including KRAS, TP53, and SMAD4 propel the growth of tumors, and the tumor microenvironment—which includes immune evasion, hypoxia, and extracellular matrix remodeling—has a major impact on therapeutic resistance. Patients with pancreatic cancer still have a dismal prognosis despite significant improvements in treatment choices, underscoring the pressing need for novel therapeutic strategies. Combination treatments, precision medicine, and immunotherapies hold promise for treating the tumor microenvironment as well as cancer cells in the future. Furthermore, concentrating on early identification and novel biomarkers might result in better prognoses and more potent therapy alternatives. Moreover, the incorporation of medicinal plants and their bioactive compounds into treatment regimens offers a potential avenue for enhancing therapeutic efficacy against pancreatic cancer, providing hope for better patient outcomes in the future.

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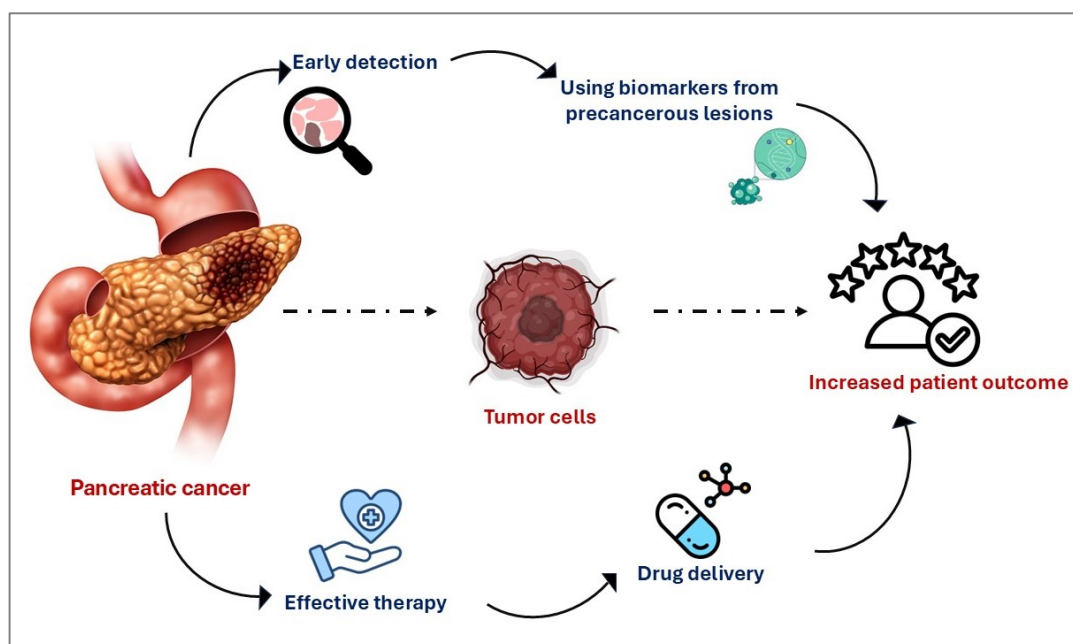


Figure 1 An overview of current status of pancreatic cancer treatment

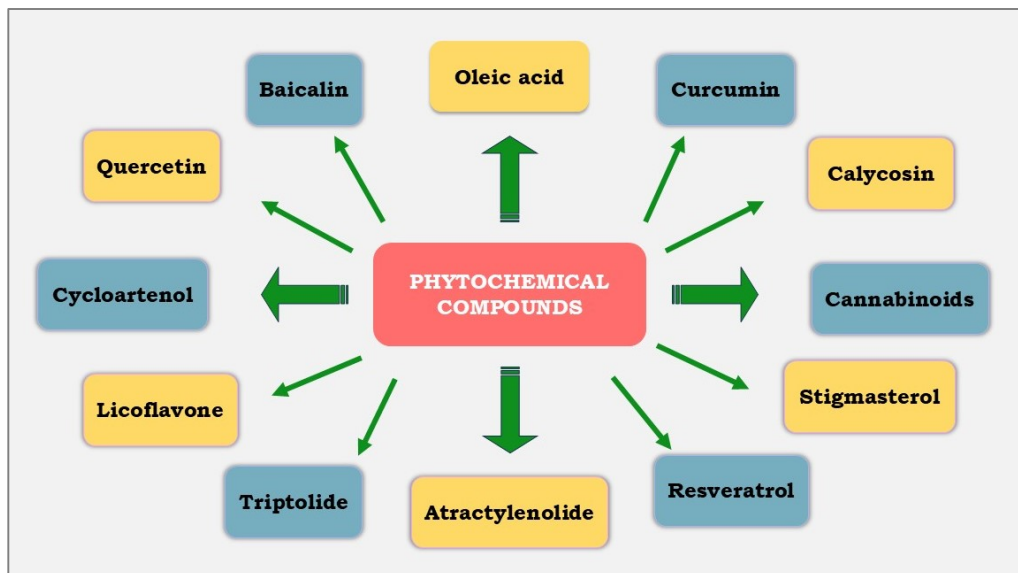


Figure 2 Some major phytoconstituents that show anticancer effect against pancreatic cells

Regional Variations in Pollen Germination of Vinca Species: A Comparative Study From Urban, Roadside, and Rural Environments

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Abstract

Pollen germination is a crucial process for plant reproduction, as it is directly linked to successful fertilization and the maintenance of biodiversity. This process involves the growth of the pollen tube, which facilitates the delivery of male gametes to the ovule. Various environmental factors can influence pollen viability and germination. This study examines the effects of environmental stressors on the pollen germination of *Vinca* species collected from three locations in Karnataka, India: Mangalore Urban Area, Kalladka Roadside, and Panemangalore Village. Pollen samples were harvested from mature flowers and subjected to in vitro germination assays using a standard sucrose-based medium. The results showed significant variations in pollen germination rates among the locations. Pollen from Panemangalore Village, a rural area with minimal pollution, exhibited the highest germination rate (33.33% at 15 minutes), followed by Mangalore Urban (22.22%), while Kalladka Roadside, exposed to moderate traffic-related pollution, showed the lowest rate (9.87%). The reduced germination rates in polluted areas are speculated to be due to factors such as oxidative stress, nutrient deficiencies, and physical damage caused by pollutants. These findings suggest that environmental stressors may adversely affect plant reproductive success, highlighting the importance of environmental conservation to support plant biodiversity.

Key words: Pollen Germination, *Vinca* Species, Environmental Stressors, Pollution Impact, Plant Reproductive Success

Introduction

Vinca species, commonly known as periwinkles, are widely distributed in tropical and subtropical regions. They are valued not only for their ornamental appeal, with vibrant flowers that enhance garden landscapes, but also for their significant medicinal properties, particularly in the treatment of various diseases due to the presence of alkaloids like vincristine and vinblastine. The reproductive success of these plants depends heavily on the process of pollen germination, which is a critical step in sexual reproduction. Pollen germination involves the emergence and growth of the pollen tube from the pollen grain, which ultimately delivers male gametes to the ovule for fertilization. This process ensures genetic diversity and the continuation of plant populations. [1]

Pollen germination is highly sensitive to various environmental factors, including humidity, temperature, nutrient availability, and air quality. [2] Adverse environmental conditions can reduce pollen viability, affecting fertilization success and seed production. In urban and roadside environments, pollutants such as nitrogen oxides (NO_x), sulfur dioxide (SO₂), and particulate matter can significantly disrupt plant physiological processes, including pollen viability and tube growth. Additionally, habitat fragmentation and altered microclimates in rural areas can expose plants to unique stressors, potentially influencing their reproductive efficiency compared to those in more stable, less disturbed ecosystems.

This study investigates pollen germination in Vinca species collected from three distinct locations in Karnataka, India: Mangalore urban area, Kalladka roadside, and Panemangalore village. The objective is to compare pollen germination rates in these varied environments and assess how environmental stressors, such as air pollution, temperature fluctuations, and soil conditions, affect the reproductive success of Vinca species.

Materials and Methods

Study Area: The study was conducted across three locations:

1. **Mangalore Urban Area:** Characterized by high population density and significant air pollution due to vehicular emissions and industrial activities.

2. **Kalladka Roadside:** A semi-urban area with moderate pollution levels from traffic and limited green spaces.
3. **Panemangalore Village:** A rural area with minimal pollution, where vegetation is relatively undisturbed by human activities.

Collection of Samples

Pollen samples from *Vinca* species were collected from mature flowers of plants growing in the selected areas. For each location, five plants were chosen, and pollen grains were harvested at peak flowering time. [3]

Pollen Germination Assay

Pollen germination was tested in vitro using a standard germination medium consisting of 10% sucrose, 0.1% boric acid, and 0.1% calcium nitrate. Pollen grains were placed on the medium, and the germination was observed under a light microscope at regular intervals (0, 5, 10, and 15 min). The pollen grains were considered germinated if the pollen tube length exceeded the diameter of the pollen grain.[4]

Results

1. **Initial Pollen Germination (0 minutes):** At the initial time point (0 minutes), only pollen from Mangalore Urban showed a slight germination rate of 5.5%, while pollen from Panemangalore Village and Kalladka Roadside showed no germination. This could indicate that pollen from Mangalore Urban is exposed to environmental conditions that prematurely trigger the germination process.
2. **Early Germination Phase (5 minutes):** By 5 minutes, pollen from Panemangalore Village exhibited a significant increase in germination rate to 9.09%, whereas pollen from Mangalore Urban maintained the same germination rate (5.5%), and Kalladka Roadside pollen showed a germination rate of 5.26%. The higher germination rate in Panemangalore Village could be attributed to a more favorable environment with fewer pollutants.

- 3. Mid Germination Phase (10 minutes):** At 10 minutes, pollen from Panemangalore Village showed a substantial increase in germination rate to 25%, indicating robust pollen viability. Pollen from Mangalore Urban also exhibited an increase to 11.11%, while Kalladka Roadside pollen had a germination rate of 8.64%. The lower germination rates in Mangalore Urban and Kalladka Roadside suggest that pollutants in these areas may impede pollen germination.
- 4. Late Germination Phase (15 minutes):** By 15 minutes, the differences in germination rates among the three localities became more pronounced. Panemangalore Village pollen showed the highest germination rate at 33.33%, followed by Mangalore Urban pollen at 22.22%. Kalladka Roadside pollen had the lowest germination rate at 9.87%. These results suggest that pollen from less polluted areas (Panemangalore Village) has higher viability and germination potential compared to pollen from more polluted areas (Mangalore Urban and Kalladka Roadside).

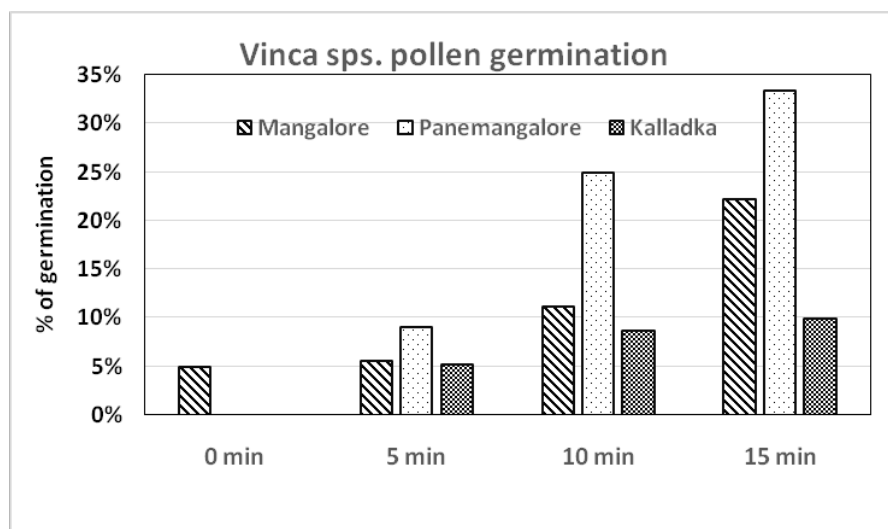


Fig 1. Vinca Pollen Germination percentage

Discussion

The results of this study indicate that environmental pollutants have a significant impact on pollen germination rates in *Vinca* species. Pollen collected from the polluted traffic area (Kalladka Roadside) consistently showed lower germination rates compared to pollen from

the normal garden (Panemangalore Village) and urban area (Mangalore Urban). Several factors could contribute to these differences:

1. **Oxidative Stress:** Pollutants such as vehicular emissions and industrial pollutants can generate reactive oxygen species (ROS) in pollen grains, leading to oxidative stress and impairing pollen viability.[5]
2. **Nutrient Availability:** Environmental pollutants can interfere with nutrient uptake in plants, leading to deficiencies in essential nutrients required for pollen germination and tube growth.[6]
3. **Physical Damage:** Pollutants can cause physical damage to pollen grains, including deformation and impaired exine layers, which can hinder the germination process.[7]
4. **Impact of Temperature and Humidity:** Temperature and humidity also play critical roles in pollen germination. The higher temperatures and lower humidity in the urban area could have contributed to the reduced germination rates observed in *Vinca* pollen from that region. Pollen grains are particularly sensitive to desiccation, and low humidity can significantly reduce their viability. In contrast, the more stable and favorable climate conditions in Panemangalore village provided an optimal environment for pollen germination.[8]

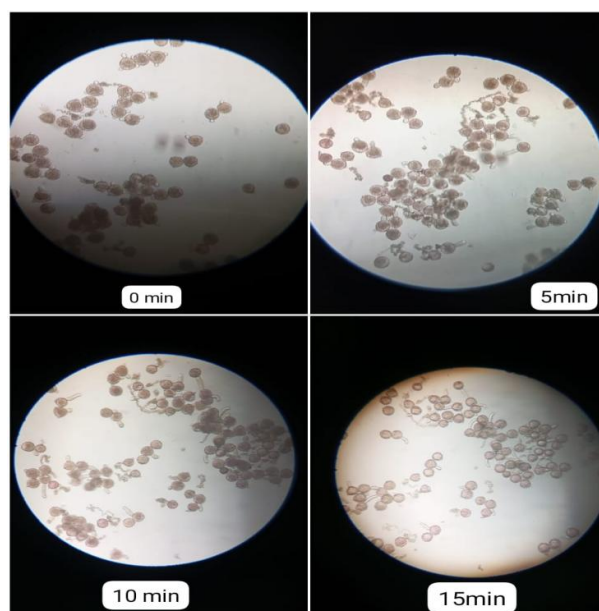


Fig 2. Representative images of *Vinca* Pollen Germination from Panemangalore area

- 5. Ecological Implications:** The findings of this study highlight the potential consequences of urbanization and pollution on plant reproduction. Reduced pollen germination in urban and roadside environments may lead to lower seed production, which can, in turn, impact the regeneration of *Vinca* populations. The effects of environmental stressors on reproductive success may result in decreased biodiversity and could limit the ability of plants to adapt to changing environmental conditions.[9]

The significantly higher germination rates observed in Panemangalore Village underscore the vital role of a cleaner and more stable environment in promoting pollen viability and successful germination. This finding emphasizes the critical need for maintaining clean and healthy environments to support plant reproduction and biodiversity. Rural areas, characterized by lower pollution levels and more consistent environmental conditions, provide an ideal setting for plants to thrive. In such environments, plants are likely to exhibit enhanced reproductive success, leading to healthier and more robust populations. This, in turn, contributes to increased biodiversity, which is essential for ecosystem resilience and sustainability. The study highlights the broader ecological implications of environmental pollution and underscores the importance of environmental conservation efforts to protect and preserve plant biodiversity for future generations.

Conclusion

This study demonstrates the significant impact of environmental pollutants on the pollen germination of *Vinca* species. Pollen from the polluted traffic area (Kalladka Roadside) showed consistently lower germination rates compared to pollen from the normal garden (Panemangalore Village) and urban area (Mangalore Urban). The results underscore the importance of addressing environmental pollution to safeguard plant health and reproductive success. Further research is needed to explore the long-term effects of pollutants on plant reproduction and to develop strategies for mitigating their impact.

Acknowledgement

Authors thank the Principal and Head of the Department, Botany, University College Mangalore and Mangalore University.

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Evaluation of Antibacterial Properties of Copper Nanoparticles Synthesized using the Leaf Extract of *Ruellia patula*

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Abstract

The present study explores the green synthesis of copper nanoparticles (CuNPs) using *Ruellia patula* leaf extract as a natural reducing and stabilizing agent. This eco-friendly approach eliminates the need for hazardous chemicals, making it a sustainable alternative to conventional synthesis methods. The formation of CuNPs was confirmed by UV-Visible spectroscopy, which exhibited a characteristic absorption peak at 260 nm. Further characterization using FTIR spectroscopy identified various functional groups responsible for the reduction, capping, and stabilization of CuNPs. The synthesized CuNPs were evaluated for antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas sp.* The results demonstrated significant antibacterial potential, with the highest zone of inhibition observed against *Staphylococcus aureus* (15 mm). These findings suggest that plant-mediated CuNPs possess promising antimicrobial properties, making them suitable for applications in biomedicine, pharmaceuticals, and environmental remediation. This study highlights the cost-effective, non-toxic, and sustainable synthesis of CuNPs using medicinal plants, paving the way for future research into their therapeutic and industrial applications.

Key Words: *Ruellia patula* , CuNPs, FTIR, *Escherichia coli*

Introduction

The field of nanotechnology is the foremost energetic locale of investigate in fabric sciences and the amalgamation of nanoparticles is picking up altogether all through world. Nanoparticles appear completely novel or enhanced properties taking under consideration

specific characteristics shape, structure. Nanoparticles can be categorized broadly as inorganic and natural nanoparticles. Inorganic nanoparticles join semi-conductor nanoparticles like (like ZnO, ZnS, CdS), metallic nanoparticles (like Au, Ag, Cu, Al), and attractive nanoparticles (like Co, Fe, Ni), whereas natural nanoparticles consolidate carbon nanoparticles (like fullerenes, quantum specks, carbon nanotubes).

CuNPs are exceedingly harmful to microorganisms, shows a solid bactericidal impact on numerous species of microorganisms, to act antifungal, hostile to- fiery specialists and works in avoiding contaminating wound recuperating. The most advantage of the green blend of CuNPs is that they are effectively accessible, secure to handle and have a wide inconstancy of metabolites. The essential phytochemicals capable have been recognized as terpenoids, flavones, ketones, aldehyde, amides and carboxylic acids. (Raju *et al.*, 2022).

A wide range of industries, including agriculture, industrial engineering, and technology, have made use of copper nanoparticles (CuSO₄). Compared to other NPs, CuNP synthesis has drawn special attention because of its practical qualities, which may be obtained at a lower cost than copper and silver. (Chung *et al.*, 2017). When compared to other methods including chemical reduction, photochemical reduction, electrochemical reduction, heat evaporation, etc., the green synthesis approach was determined to be the most effective. This green synthesis approach has a number of benefits over other approaches, including economic effectiveness, ease of use, lower temperature requirements, the use of less hazardous ingredients, and compatibility with food and medical applications. Green synthesis was utilized to create nanoparticles from a variety of plants. (Vijila *et al.*, 2016).

Ruellia patula, a medicinal herb, is sometimes referred to as "Kiranthinayagam" or "Kayappacchilai." It is a traditional medicinal plant used to cure wounds. Itching, bug bites, sores, eye conditions, tumors, skin conditions, rheumatism, renal affections, dental issues, and insect bites are all treated with the leaves. Pharmacological research revealed its antinuclear, antioxidant, wound-healing, and cardio tonic properties. Analyzing the antibacterial activity of the medicinal plant *Ruellia patula* against various species is the goal of the current study. Products made from herbs are a reliable source of both conventional and modern medicine, and they are widely utilized to treat a variety of medical issues. (Ramadevi *et al.*, 2016). Whole plant leaf extract is also used to cure syphilis, gonorrhea, and renal infection (Mounika *et al.*, 2023). The wound healing properties of the leaves of *Ruellia patula* has not been scientifically evaluated so

far (Saroja *etal* ., 2009).This study reports a green route for the synthesis of CuNPs using *Ruellia patula* leaf extract and evaluation of its antibacterial activity against certain pathogenic bacteria.

Methodology

Collection of plant materials:

Fresh leaves of *Ruellia patula* were collected from South Thittangulam village, Thoothukudi district . Collected leaves were transported to the laboratory.

Preparation of Leaf Extract:

Ten grams of freshly harvested *Ruellia patula* leaves were cleansed and rinsed with tap water to get rid of any contaminants on the surface. After adding the leaves to a 500 ml beaker with 300 ml of distilled water, the mixture was heated to 60⁰C for one hour. After allowing the solution to cool at room temperature, what man filter paper no. 1 was used to filter it. For use in subsequent experiments, the filtrate was collected in a conical flask and kept at 40°C.

Synthesis of copper nanoparticles (CuNPs)

To prepare copper nanoparticles, 100ml of 0.1M copper sulphate solution was added drop wise to 20 ml *Ruellia patula* leaf extract under constant stirring at 80⁰C for 3 hrs .The reaction mixture became dark green, and green precipitate developed. For further precipitation, the reaction mixture was kept overnight to allow complete reaction. The precipitate was obtained by centrifugation at 10,000 rpm for 15 min at room temperature. The precipitate (containing copper compound) was oven dried at 80⁰ C for 24 hrs.

Characterization of CuNP:

After synthesis of CuNPs the dried fine power was used for characterization with UV-Vis, FTIR.

UV-visible spectroscopy:

The UV-visible absorption spectrum of the biosynthesized CuNPs was scanned within the range of 200-600 nm in an UV-visible spectrometer for determining the maximum absorbance (λ_{max}).

Fourier Transform Infrared Spectroscopic (FT-IR) analysis:

The FT-IR analysis of the biosynthesized CuNPs was performed by preparing KBr discs and corresponding subjected in Thermo Nicolet is 50 with inbuilt ATR in the absorption range between 4000-30 cm^{-1} with a detector at the resolution of 0cm^{-1} .

Test pathogen:

The test pathogens such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas* sp, and *Escherichia coli* were obtained from Department of Microbiology K.R. College of Arts & Science. Further, the culture inoculums of individual pathogens were prepared by using sterile nutrient broth and incubated at 37°C for 24 hr. After incubation, the individual culture suspensions were used as seed inoculums for further studies.

In vitro assay antibacterial activity of CuNPs:

Antibacterial activity of biosynthesized CuNP's was determined by Agar –well diffusion assay using sterile Muller Hinton (MHA) agar. Wells of 5mm diameter was made by using sterilized cork borer on Muller Hinton agar. 0.1 ml each of pathogenic bacteria cultures was spread on the plates, followed by the addition of 50 μl of CuNPs into the well. Simultaneously, 25 μg / ml of tetracycline was used as positive control for *Staphylococcus aureus* , *Escherichia coli*, *Pseudomonas* sp and *Streptococcus pyogenes* . DMSO was used as negative control. The culture plates were incubated at 37°C for 24 hr and zone of inhibition was measured.

Results:

Synthesis of CuNP

The formation of Cu nanoparticles was initially confirmed visually, the change in color of the reaction mixture from blue to dark green indicates formation of copper nanoparticles (Fig.1)

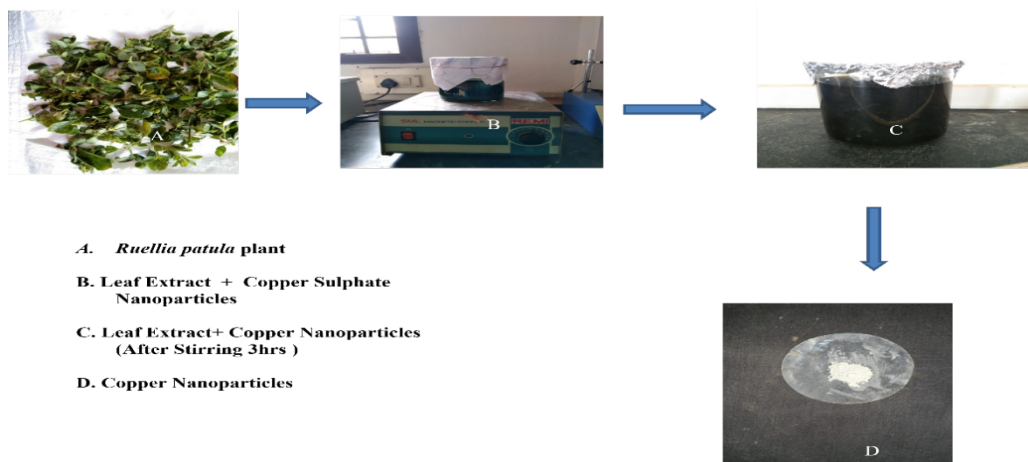


Figure 1: Synthesis of Copper Nanoparticles

UV-Vis Spectroscopy

The UV-Visible absorption spectrum of copper nanoparticles (CuNPs) was recorded in the wavelength range of 235–800 nm, as shown in the figure 2. The spectrum exhibits a strong absorption peak around ~260 nm, which is indicative of interband transitions of Cu nanoparticles.

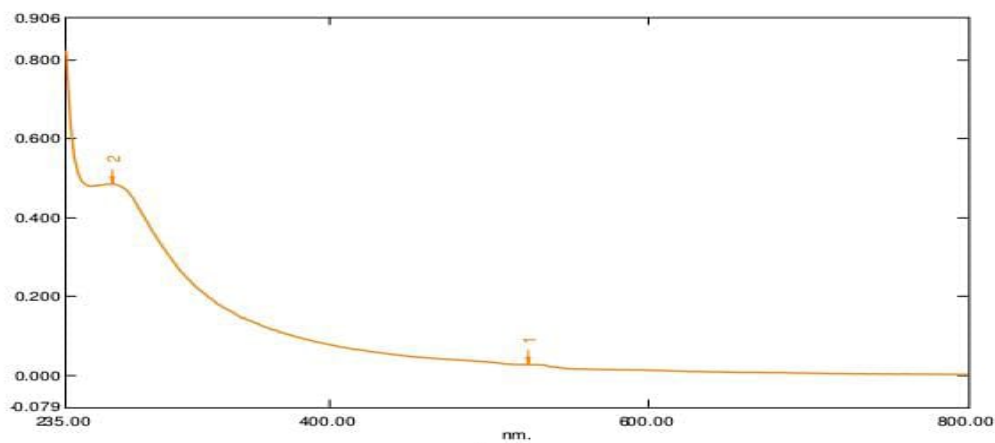


Figure: 2 UV-Visible Spectral peak

Fourier Transform Infrared spectroscopic (FT-IR) Analysis:

FTIR analysis of the synthesized CUNP's was obtained and studied which ranges from 400 to 4000nm. The functional groups are represented in table 1 and figure 3. The bands of biosynthesis CuSO_4 NPs were seen at 603.13cm⁻¹, 662.77 cm⁻¹, 779.71 cm⁻¹, 961.84 cm⁻¹, 995.72 cm⁻¹, 1097.83 cm⁻¹, 1164.23cm⁻¹, 1198.45 cm⁻¹, 1622.12 cm⁻¹, 1745.19 cm⁻¹, 2854.46 cm⁻¹, 2925.76 cm⁻¹, 3259.56 cm⁻¹, 3345.99cm⁻¹. The bands of FTIR of CuSO_4 NPs at 3345.99 corresponds to =O-H (belonging to H bonded Alcohol, Phenol), 3259.56 corresponds to =N-H(belonging to 1⁰2⁰Amines, Amides), 2925.76 corresponds to =C-H(belonging Alkyne), 2854.46 corresponds to =C-H (belonging to Aldehyde), 1745.19 corresponds to =C-O(belonging to ester), 1622.72 corresponds to = C=C(belonging to Alkenes), 1198.45 corresponds to = C-X(belonging to Fluoride), 1164.23 corresponds to = C-X (Fluoride), 1097.83 corresponds to =C-X(belonging to Fluoride), 995.72 corresponds to =O-H bend (belonging to Carboxylicacids), 961.84 corresponds to =C-H bend (belonging to Alkenes), 779.71 corresponds to =C-X (belonging to chloride), 662.77 corresponds to =N-H WAG (belonging to 1⁰2⁰Amines, Amides), 603.13 corresponds to =C-X (belonging to Bromide , Iodide).

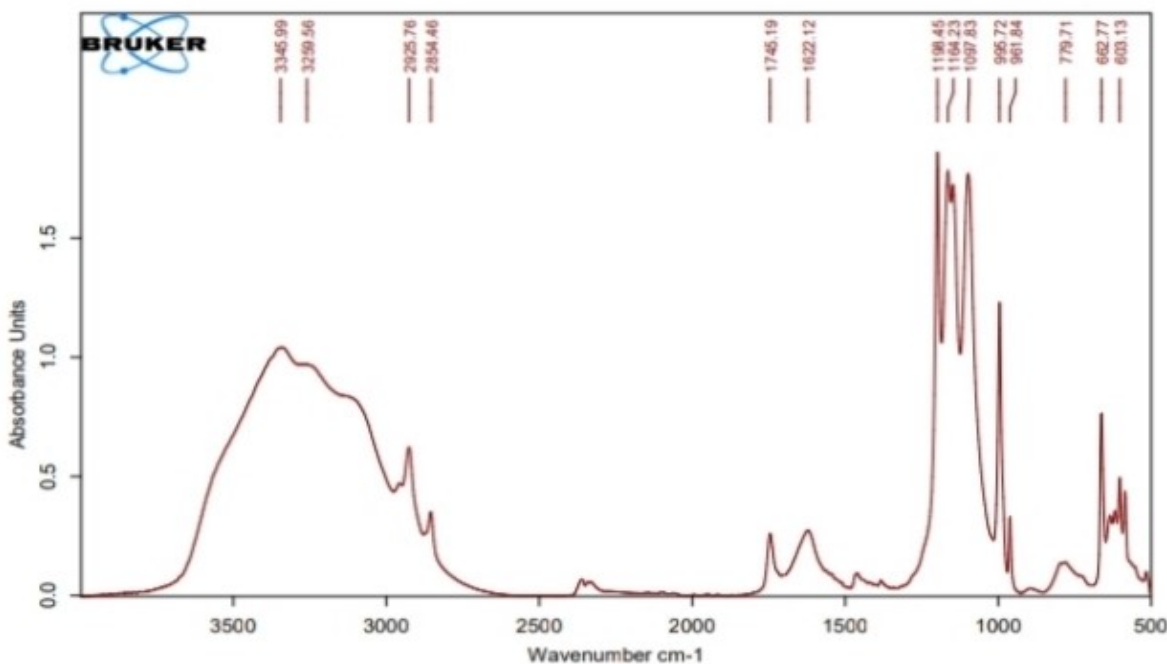


Figure: 3 FTIR Spectrum of CUNPs

Table 1 : Probable Functional Groups of CUNPs

Wavelength (cm⁻¹)	Functional groups	
	Bond type	Specific context
3345.99	O-H (bend)	Alcohol, Phenol
3259.56	N-H(stretch)	1 ⁰ 2 ⁰ Amines, Amides
2925.76	C-H(stretch)	Alkynes
2854.46	C-H(stretch)	Aldehyde
1745.19	C-O(stretch)	Ester
1622.72	C-C(stretch)	Alkenes
1198.45	C-X(stretch)	Fluoride
1164.23	C-X(stretch)	Fluoride
1097.83	C-X(stretch)	Fluoride
995.72	O-H(bend)	Carboxylic acids
961.84	C-H (bend)	Alkenes
779.71	C-X (stretch)	Chloride
662.77	N-H wag	1 ⁰ 2 ⁰ Amines
603.13	C-X(stretch)	Bromide, Iodide

Antibacterial activity of synthesized CuNP's:

The results of antibacterial activity of copper nanoparticles against the pathogenic bacteria like *Staphylococcus aureus*, *Pseudomonas* sp, *Escherichia coli* and *Streptococcus pyogenes* sp. copper nanoparticles are shown in the table 2 and figure 4.

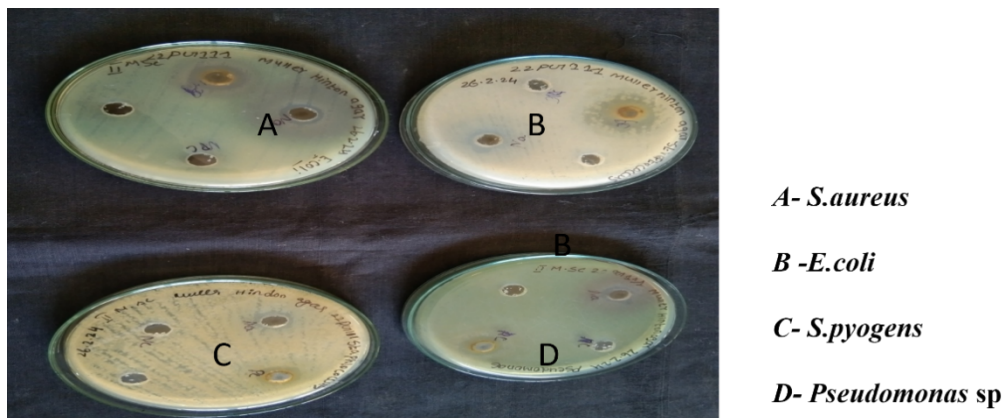


Figure 4: Antibacterial Activity of Synthesized Copper Nanoparticles

Table 2: Zone of Inhibition (mm) of CuNPs Nanoparticles against Gram positive and Gram negative bacteria

Sample	Organisms	Concentration of CuNPs	Zone of Inhibition (mm)	
			Nanoparticle	Positive control
CuNPs	<i>Staphylococcus aureus</i>	50µg/ml	13mm	14mm
	<i>E.coli</i>		13mm	16mm
	<i>Streptococcus pyogenes</i>		13mm	23mm
	<i>Pseudomonas sp</i>		13mm	14mm

Discussion and Conclusion

Various applications of nanoparticles stimulate the need for synthesizing them. But, the conventional methods are usually hazardous and energy consuming. This leads to focus on green synthesis of nanoparticles which seems to be easy, efficient, and eco-friendly approach. Copper nanoparticles are used to their large surface area by volume ratio than the bulk media.

In the present study, an absorbance peak at 260 nm in the UV-Visible spectrum indicates the presence of copper nanoparticles (CuNPs). This result is consistent with previous studies, where absorption peaks were reported at 264 nm and 524 nm (Chinnathambi *et al.*, 2021). The observed peak suggests the successful synthesis of CuNPs using *Ruellia patula* plant extract. FTIR spectroscopy was employed to identify the possible biomolecules present in the leaf extract, which acted as reducing and capping agents for the synthesis and stabilization of CuNPs. The FTIR spectra showed the presence of bands which appear at 3345.99 represents the =O-H bonded frequencies of alcohol, phenol, 3259.56 cm⁻¹ corresponds to N-H stretching belonging to 1^o2^o amines, amides, 2925.76 corresponding to C-H stretching in alkyne, 2854.76 represents the C-H stretching belonging aldehyde, 1745.19 C-O stretching belonging to ester, 1622.12 corresponds to C-C stretching belonging alkane, 1198.45 corresponds to C-X stretching fluoride, 1164.23 corresponds to C-X stretching fluoride, 1097.83 corresponds to C-X stretching fluoride, 995.72 represents the =O-H bend carboxylic acids, 961.84 corresponds to C-H bend alkenes. These functional groups confirm the presence of biomolecules responsible for nanoparticle formation and stability. Similar findings have been reported by Das *et al.*, (2019), where the broad band at 3345.99 cm⁻¹ was attributed to O-H stretching vibrations of alcohols. The results of antibacterial activity revealed that the synthesized copper nanoparticle have some antibacterial activity. The maximum zone of inhibition was observed against *Staphylococcus aureus* (15mm) *Streptococcus pyogenes* (13mm), *Escherichia coli* (13mm) and *Pseudomonas sp.* (14mm). These findings align with the study by Thakur *et al.*, (2014), where CuNPs demonstrated strong antibacterial activity against *E. coli* and *Staphylococcus sp.* However, in contrast to their findings, the nanoparticles in this study showed inhibition towards *Streptococcus pyogenes* and *Pseudomonas sp.*, suggesting variations in synthesis methods or bacterial susceptibility.

The biosynthesized copper nanoparticles are trusted to be friendly with environment, non-toxic, biosafe and biocompatible. It is desirable for biomedical application such as drug carriers, cosmetics, and filling the medical materials. Copper nanoparticles are used as fungicide, algacide, root killer, and herbicide in both agriculture and nonagricultural settings. It is also used as an antimicrobial and molluscicide.

Aknowledgement

The author is thankful to the management and principal of K.R.College of Arts & Science,Kovilpatti for their constant support and encouragement.

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The Role of Novel Gluten-Free Flours in Reducing Symptoms and Improving Quality of Life in Celiac Disease Patients

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Abstract

Celiac disease is an autoimmune disorder triggered by the ingestion of gluten, leading to intestinal inflammation and a range of debilitating symptoms. A strict gluten-free diet (GFD) is the only effective treatment, necessitating the development of novel gluten-free flours that improve both nutritional content and sensory properties of gluten-free products. This paper explores the role of alternative gluten-free flours, such as those derived from legumes, nuts, seeds, and pseudo-cereals, in enhancing the dietary experience and health outcomes of celiac patients. By reviewing recent studies and clinical trials by analyze the impact of these novel flours on digestive health, nutrient absorption, and overall quality of life. The findings suggest that innovative flour alternatives contribute to better gastrointestinal tolerance, improved gut microbiota composition, and reduced nutritional deficiencies commonly observed in celiac patients. Furthermore, consumer acceptability and market trends indicate a growing demand for high-quality gluten-free products. This study highlights the need for continued research and development in gluten-free food technology to optimize both health benefits and sensory characteristics of gluten-free diets.

Key words: Celiac disease, gluten free, gut health, symptoms, nutritional benefits.

Introduction

Celiac disease (CD) is a chronic inflammatory bowel condition triggered by consumption of gluten (Green et al,2015 & Schuppan D and Zimmer KP,2013). Its prevalence has been reported to be 1–2 % in North and South America, North Africa, Middle East and India (Accomando S, Cataldo F, 2004 & Leffler DA, Schuppan D, 2010).Celiac disease (CD) is a chronic autoimmune disorder. It is characterized by an immune response to gluten, a protein found in wheat, barley, and rye. The immune reaction leads to damage in the small intestine, impairing nutrient absorption and causing symptoms such as diarrhoea,

weight loss, fatigue, and abdominal pain. The only known effective treatment for CD is a strict gluten-free diet (GFD) for life, excluding dietary wheat, rye, barley (Ciacci et al., 2002). However, traditional gluten-free products often lack essential nutrients, dietary fiber, and have poor textural and sensory attributes. This has driven the development of novel gluten-free flours that offer superior health benefits and improve the quality of life in CD patients.

Nutritional and Functional Properties of Alternative Gluten-Free Flours

Alternative gluten-free flours, including those derived from pseudo-cereals includes quinoa, amaranth, buckwheat, legumes such as chickpea, lentil, pea, and nuts like almond, coconut, offer a range of health benefits. These flours are rich in proteins, fiber, vitamins, and minerals, addressing the common nutritional deficiencies seen in celiac patients. They also provide better glycemic control, contributing to overall metabolic health. Research has shown that using a blend of gluten-free flours can improve the texture, moisture retention, and nutritional composition of gluten-free baked goods and other food products.

Gluten-Free Foods and their Benefits

1. Rice and Rice Flour - A staple gluten-free option that provides energy and essential nutrients like iron and B vitamins. Studies suggest that fortified rice flour can address micronutrient deficiencies common in celiac disease patients. Despite the many qualities of rice flour, it was necessary to expand the raw materials used for the development of gluten-free products to diversify the product range and to enrich the nutrient content. Thus, a long list of raw materials was used in various studies to develop the gluten-free sector as much as possible. Among them are cereals (maize, sorghum), whole grains (brown rice, millet, teff, oatmeal), pseudo-cereals (amaranth, quinoa, buckwheat), legumes (pea, lentils, soybean, chickpea, gram), seeds (flax seeds, pumpkin seeds), nuts (almond, walnut, peanuts), tuberous rhizomes (tiger nut, jerusalem artichokes), and other types of raw materials (plantain, coconut) (Saturni et al., 2010 & Hosseini et al., 2018).
2. Amaranth - A nutrient-dense grain high in iron, calcium, and fiber, beneficial for bone health and digestion. Clinical studies suggest that amaranth consumption can help improve immune function and reduce oxidative stress. Additionally, its high calcium content supports bone health, which is particularly important for CD patients who are at risk of osteoporosis.

3. Quinoa - A pseudo-cereal high in protein and all essential amino acids, quinoa supports muscle development, digestive health, and provides antioxidants. Studies have shown that quinoa can help maintain a healthy gut microbiome, which is often compromised in celiac patients.
4. Buckwheat - Despite its name, buckwheat is naturally gluten-free and rich in fiber, magnesium, and antioxidants, which help reduce inflammation and support cardiovascular health.
5. Teff Flour - A staple in Ethiopian cuisine, teff is high in iron, calcium, and fiber. These nutrients promote gut health, prevent anemia, and support bone health. Additionally, teff contains resistant starch, which acts as a prebiotic, fostering beneficial gut bacteria and improving digestion.
6. Sorghum - Rich in antioxidants, fiber, and protein, sorghum supports metabolic and cardiovascular health. It is naturally gluten-free and has been shown to reduce oxidative stress, which can be elevated in individuals with CD. The presence of polyphenols in sorghum also provides anti-inflammatory properties, potentially aiding in symptom management.
7. Chickpeas - Provide plant-based protein and fiber, aiding digestion and stabilizing blood sugar levels. Research indicates that chickpea flour enhances satiety and improves gut health.
8. Almond Flour - A great alternative to wheat flour, rich in healthy fats, vitamin E, and magnesium, supporting heart health and reducing inflammation. Almond flour also has a lower glycemic index compared to traditional flours, making it beneficial for blood sugar control.
9. Coconut Flour - High in dietary fiber and beneficial fats, promoting satiety and gut health. Coconut flour has been linked to improved lipid profiles and better metabolic health.
10. Corn and Cornmeal - A versatile ingredient that adds fiber and antioxidants, beneficial for cardiovascular health. Corn-based gluten-free foods are widely accepted due to their neutral taste and ability to mimic traditional wheat-based products.
11. Lentils and Pea Flour - Excellent sources of plant protein, fiber, and essential minerals, supporting muscle and digestive health. Lentils and pea flour contain bioactive compounds that contribute to overall gut health and inflammation reduction.

12. Sweet Potatoes - Naturally gluten-free and rich in vitamins A and C, aiding immune function and eye health. Sweet potatoes also contain resistant starch, which can act as a prebiotic and support gut microbiota balance.

Impact on Symptom Reduction and Quality of Life

A diet incorporating these novel flours has been linked to several health benefits for individuals with CD. Improved nutrient intake can reduce deficiencies commonly seen in CD patients, such as iron, calcium, and B vitamins. Moreover, higher fiber intake from these flours promotes better digestive health, reducing symptoms like constipation and bloating. Anti-inflammatory and antioxidant properties in these grains and legumes contribute to reducing systemic inflammation, a common issue in untreated or poorly managed CD.

Patients consuming alternative GF flours reported fewer gastrointestinal symptoms, increased energy levels, and improved mental well-being. Psychological benefits are particularly crucial, as many CD patients suffer from anxiety and depression due to dietary restrictions and associated health challenges. Improved diet variety and better-tasting GF products contribute to better dietary adherence, reducing the likelihood of accidental gluten exposure. Additionally, consuming nutrient-dense flours ensures that CD patients receive adequate daily nourishment, helping them maintain overall health and prevent secondary complications.

Recent studies indicate that these alternative flours can positively influence gut microbiota, reducing symptoms such as bloating, diarrhoea, and abdominal pain. Certain gluten-free flours contain prebiotic fibers that enhance gut microbiome diversity, which is often compromised in CD patients due to prolonged inflammation and intestinal damage. The inclusion of these alternative flours in the diet can help restore gut health and promote better nutrient absorption.

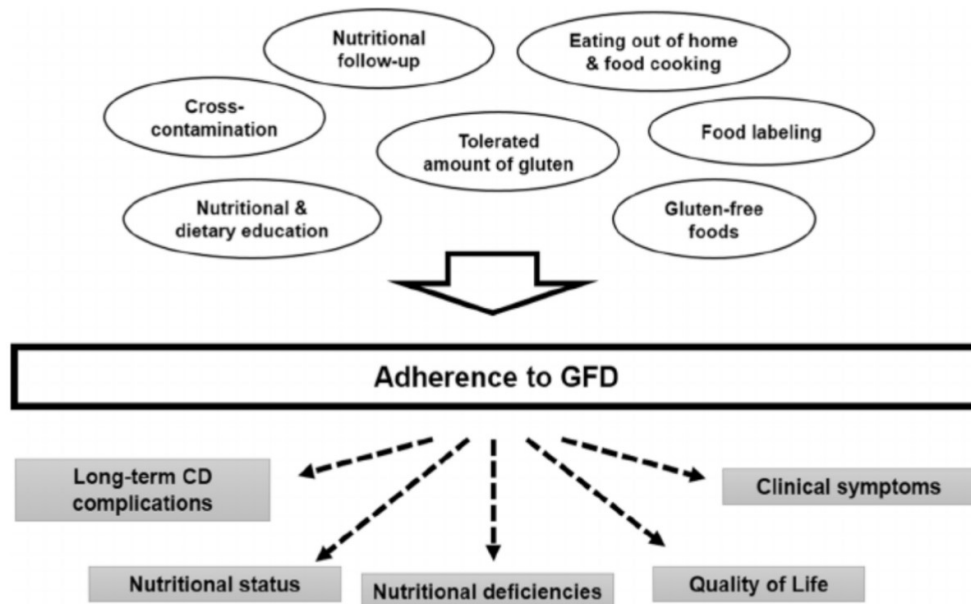


Figure: 1 Gluten-free diet, a milestone in the treatment of celiac disease (Karla A et al.,2017)

Consumer Acceptability and Market Trends

The increasing demand for high-quality gluten-free products has spurred innovations in food technology. Advances in food processing techniques, such as fermentation and hydrocolloid incorporation, have significantly improved the textural and sensory attributes of gluten-free foods. Sensory studies indicate that consumers prefer products with enhanced flavor, texture, and mouthfeel, attributes that novel gluten-free flours can provide. The global gluten-free market is expanding rapidly, with consumer awareness and dietary needs driving the development of new and improved gluten-free formulations.

Challenges and Future Directions

Despite their benefits, the adoption of novel gluten-free flours faces challenges, including higher costs, availability, and the need for optimized formulation techniques to mimic the elasticity and structure of gluten-containing foods. Additionally, contamination with gluten during processing and transportation remains a concern for manufacturers. Future research should focus on improving processing techniques, enhancing bioavailability of nutrients, and conducting long-term clinical trials to further assess health outcomes in CD patients. Emerging technologies such as enzyme treatments and genetic modification may offer solutions to enhance the texture and nutritional profile of gluten-free products.

Conclusion

Novel gluten-free flours present a promising avenue for improving the dietary experience and health outcomes of celiac disease patients. Their rich nutritional profile, combined with advancements in food science, can lead to better symptoms management and enhanced quality of life. The development of high-quality gluten-free products using alternative flours can address the nutritional deficiencies commonly observed in celiac patients. By incorporating nutrient-rich alternative flours like teff, sorghum, quinoa, amaranth, and legume-based options, etc., CD patients can benefit from a more balanced and satisfying diet. Ongoing research and innovation are essential to further optimize these alternatives and ensure broader accessibility and consumer acceptance. Continued research and innovation in GF product development can further support CD patients in maintaining a healthy and enjoyable diet while reducing the burden of strict dietary restrictions. Expanding the availability and affordability of these products will be key in ensuring that all CD patients have access to nutritious and effective dietary options. The future of gluten-free nutrition holds great potential, and further interdisciplinary research will be instrumental in advancing the well-being of individuals with celiac disease.

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Comprehensive Analysis of Semen Parameters: Insights from Routine and CASA-Based Assessments among Sudanese population.

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Abstract

Introduction: Routine semen analysis is the primary diagnostic test to evaluate male fertility. The further evaluation involved Computer Assisted Semen Analysis – CASA system, which determines sperm morphology and motion dynamics and assesses the other semen criteria like leukocyte count. **Objectives:** Determine the diversity for particular parameters of the human semen, including semen volume, pH, liquefaction time, sperm agglutination semi-quantitative rank, odor, viscosity, liquefaction status, nonspecific aggregation, and agglutination, among others. **Materials and Methods:** The study involved 566 males who had female spouses who had requested sperm analysis. Semen was examined using the CASA system and several manual laboratory procedures standardized by WHO 2010 (1). **Results:** Regarding the normal semen samples, the Mean $\pm$ SD of sperm concentrations was 63.90 ± 20.13 million per milliliter, whereas it was 36.13 ± 27.34 million per milliliter for abnormal samples respectively. The mean $\pm$ SD of semen volume was 2.652 ± 1.2677 ml. The mean $\pm$ SD of semen pH was 7.4 ± 0.4 ml. **Conclusion:** There were significant differences in semen volume and sperm concentration for normal and abnormal semen samples compared to other standards. Whereas no significant differences concerning the other parameters.

Keywords: Semen, CASA system, leukocyte, WHO,

INTRODUCTION

Understanding the semen report parameters guides the proper management, and the fertility potential of the semen can be concluded. Human semen analysis includes many parameters such as volume (ml), pH, liquefaction time (min), sperm agglutination semi-quantitative rank, odor, viscosity, liquefaction status, nonspecific aggregation, and agglutination. According to

World Health Organization – WHO (2) (Table 1), routine semen analysis is considered the primary and definitive diagnosis for evaluating male fertility. There are reference values that are set and updated every time by WHO. In addition to the routine manual semen analysis parameters, further evaluation of raw semen is involved by computer-assisted semen analysis (CASA) (3), which determines sperm morphology and motion dynamic characteristics and assesses other semen criteria like leukocyte count. recently, CASA system can assist in evaluation and detection of sperm DNA fragmentation when semen prepared with sperm chromatin dispersion (SCD) technique (4). During routine semen analysis, cellular constituents of semen such as white blood cells (WBCs), Red blood cells (RBCs), epithelial cells, and spermatogenic cells are usually seen (5). The presence of polymorph nuclear leucocytes WBCs and RBCs indicates an infection or trauma of the male reproductive or urinary tract that can be treated when using antibiotics such as doxycycline (6) or trimethoprim/sulfamethoxazole (7) as well as antioxidants nutritional supplements including zinc, vitamin E, C (8, 9), Coenzyme ubiquinol-10 (CoQ10), glutathione (10) have a big impact on sperm health and male fertility. with considering that leukocytospermia may be able to damage sperm by the generation of ROS (11). Epithelial cells from the urethral canal can usually present in normal semen (12). Spermatogenic cells and spermatids are germ cells sloughed off early in the maturation process (13). Occasionally immature sperm cells are found in normal semen, whereas an excessive number usually denotes maturation arrest (13). Maturation arrest may happen idiopathically due to varicocele, mumps orchitis, exposure to toxic constituents, or sickling disease (14)

A high number of immature germ cells in the semen is usually associated with low normal sperm counts. Also, the presence of Leukocytes in the seminal fluid is normal; however, a concentration of $>1 \times 10^6$ /ml is considered abnormal (World Health Organization 1999). In semen samples, there is a positive correlation between leukocyte count and the total count of microorganisms. An optimal sensitivity/specificity ratio appears at 0.2×10^6 WBC/mL semen (15). Excessive leukocytes may be detrimental to spermatozoa due to their excessive production of reactive oxygen species (ROS) and cytotoxic cytokines (the statement is repeated). The presence of erythrocytes indicates reproductive tract pathology, while the presence of microorganisms indicates genital tract infection (16). Lymphocytes and macrophages originate mainly from the rete testis and epididymis, but granulocytes originate primarily from the prostate and seminal vesicles (17).

The study was designed to determine diversity for general properties of the human semen, including semen volume (ml), pH, liquefaction time (min), sperm agglutination semi-quantitative rank, odor, viscosity, liquefaction status, nonspecific aggregation, and agglutination among Sudanese population. The study results will be compared with other related studies and the WHO standards.

MATERIALS AND METHODS

Study Design: A prospective case series study for infertile males who had female couples requesting semen analysis was applied. Study participants were recruited from infertile couples who came to the ULTRALAB laboratory center main campus, and ULTRALAB branch of Sudan Assisted Reproduction Center (SARC), Khartoum, Sudan. Data collection was done for 20 months, from April 1st, 2022, to December 31st, 2023.

Study Setting and study period: The study was conducted for 566 infertile men who had female couples requesting semen analysis in Khartoum, Sudan's two ULTRALAB laboratory centers. The data and sample collection were done for 20 months, from April 1st, 2022, to December 31st, 2023. We conclude a semen sample to be normal if it fulfilled all of the values given in the WHO (1, 18) handbook standards for semen analysis; nevertheless, any sample that did not meet those parameters was classed as an abnormal semen sample.

Inclusion Criteria: The study included only Sudanese men living with their partners permanently in Sudan who had complaints of infertility.

Exclusion Criteria: The study excluded infertile men who are not Sudanese and who were not living with their partners permanently in Sudan.

Data Analysis: The study procedures and instruments were standardized with maintained quality control to ensure reliable results depending on the standards of the WHO laboratory manual for the examination and processing of human semen (2010) under the operation desk, providing a constant temperature of 37°C during semen testing throughout the whole procedure. All samples were collected in a private room adjoining the andrology unit laboratory and taken after three days of sexual abstinence, obtained by masturbation, and ejaculated into clean, wide-mouthed non-toxic for the spermatozoa plastic containers (WHO, 2010). Semen analysis began with a visual inspection and odor assessment between 30 and 60 minutes after ejaculation. The pH was examined using pH paper. A phase-contrast microscope adjusted at ×200 and ×400 total magnification as a combination of a ×20 and a ×40 objective

with a $\times 10$ ocular was used to examine strands of mucus, sperm aggregation or agglutination, and the presence of other cells like epithelial cells and leukocytes. The sperm concentration (million/ml) was measured using computer-assisted semen analysis (CASA) (Mira9000 CASA system machine, Mira-Lab, Geneva, Switzerland) provided by Mira9000 special software, slide, temperature controller, soft key, Tri-ocular Microscope (Nikon E200) with plan objective lens and DCC camera (Microptic, S.L., Barcelona, Spain) of Minimum 480TV line, using Diff-Quik (Polyscience, Inc. Warrinton, PA, USA) staining system (Figure 5). CASA system considers the sample an abnormal semen sample when any examined parameters don't pass WHO criteria and vice versa for the normal semen samples. For the leucocyte evaluation, we used LEUCOSCREEN™'s Leucocyte staining method, whereas all semen pH was examined using pH indicator papers (Merck Millipore, Germany) following the manual of each reagent.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were following the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All individuals have obtained informed consent and ethical clearance from the ministry of health, Khartoum, Sudan.

RESULTS

Generally, the result of the study indicated that out of 566 samples of semen, there were 87 (15.4%) normal and 479 (84.6%) abnormal semen samples. Out of all the normal semen samples, sperm concentrations (million/ml) were observed to be within 25.40 to 118.80 million per milliliter, with a mean and standard deviation of 63.90 million per milliliter and 20.13 million per milliliter, respectively. The abnormal semen samples revealed a sperm concentration (million/ml) within a range of 0.0 to 145.70 million per milliliter, having a mean and standard deviation of 36.13 million per milliliter and 27.34 million per milliliter, respectively. Out of 566 samples, 563 (99.5%) had a normal odor. The study results also indicated normal viscosity, complete liquefaction (Figure 3, 4), and no agglutination for the majority of samples with a frequency of 561 (99.1%), 556 (98.2%), and 565 (99.8%), respectively (Table 3). The study results of overall semen samples (566 semen samples)

(Table 2) indicated that the majority of the semen samples had a volume of semen ranging between 1 to 5 ml with a mean value and standard deviation of 2.652 ml and 1.2677 ml, respectively (Figure 1). The mean semen volume was 3 ml for the normal semen and 2.6 ml for abnormal semen samples (Figure 2). The mean pH results for overall semen samples were 7.4 with a standard deviation of 0.4 and a range of 6.4 between a minimum pH of 1.0 up to a maximum of 7.4. Out of the 566 semen samples, the majority (96.28%) had a liquefaction time of 30 minutes. The study results didn't show a significant difference in liquefaction between the normal and abnormal semen samples, 30 and 31 minutes.

DISCUSSION

Out of all the normal semen samples, sperm concentration (million/ml) (Table 2) was observed to be within 25.40 to 118.80 million per milliliter, which is much higher than the lower limit of the WHO standard having a mean of 63.90 million per milliliter. The abnormal semen samples revealed a sperm concentration (million/ml) that ranges between 0.0 to 145.70 million per milliliter, having a mean value of 36.13 million per milliliter. According to the WHO standards (15 million/ml (range: 12 to 16 million/ml)), the values are enough to make a male fertile in the absence of other determinants of infertility (WHO, 2010). Evaluating an accurate quantity and quality of semen is essential because it helps calculate the total number of spermatozoa and non-sperm cells in the ejaculate; a volume greater than or with a lower limit of 1.5 ml is considered normal (WHO,2010). The study results indicated that the majority of the semen samples had a volume ranging between 1 to 5 ml with a mean value of 2.652 ml which is higher than the WHO standard documented in 2010. The results of this study indicated that the pH of normal and abnormal semen samples was 7.4. Still, other studies on human semen revealed that in most patients with oligospermia or/and asthenospermia, a lower pH of less than 7.2 is evident (19). Similar to the above studies, the WHO standards for current pH references documented 7.2 as a lower threshold value (WHO,2010). Zhou et al (19) also revealed that pH 7.2 and 8.2 are the optimal condition for total motility and PR (abbreviation should follow the entire term) of human sperm. In addition, velocity parameters (VSL, VAP, and VCL (abbreviation should follow the entire term)) were significantly decreased at pH 6.2 and 5.2, which was consistent with the result of the motility.

The study results also didn't show a significant difference in liquefaction between the normal and abnormal semen samples, as the majority (96.28%) had a liquefaction time of 30 minutes. Based on the WHO (2010), an observation of semen that includes inspection should begin after waiting 30 minutes after liquefaction and no later than 1 hour after ejaculation; if it stays for longer than an hour, its quality will be affected. According to Cui, D. et al study (20), there was a significant negative effect of anti-sperm antibody (ASA) on sperm concentration, sperm motility (A + B), and sperm liquefaction. The appearance of a typical liquefied semen sample is homogeneous and grey-opalescent in color (1). A study that was conducted by Plessis et al (21) indicated that the liquefaction time could be affected by semen hyperviscosity (SHV), which is caused by multiple contributing factors, such as infection, inflammation, leukocytospermia, hypofunction of the male sex accessory glands, oxidative stress (OS), and genetic factors.

Conflict of Interest (COI): The Authors declare that there is no conflict of interest.

Funding: None

Acknowledgment

The authors wish to acknowledge the help of Dr. Mohammed Dfa Allah for his support in statistical analysis and professor. Mamon Haj Ali provided the study with many academic ideas.

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Legends of figures

Figure 1. Shows semen volume (ml) in overall semen samples

Figure 2. Comparison of mean seminal fluid volume between the normal and abnormal semen samples.

Figure 3. Incidence of different liquefaction times in 556 semen samples.

Figure 4. Comparison of the mean liquefaction time between the normal and abnormal semen samples (n=556)

Figure 5. Mira9000 CASA system machine, Mira-Lab, Geneva, Switzerland, shows an azoospermic sample with many pus cells

Legends of Tables

Table 1. Indicates the history of such average semen values revealed by the WHO

Table 2. Random samples of semen properties (Volume, pH, Liquefaction Time – Liq. Time & Sperm Agglutination) taken from infertile couples of unknown root of infertility were attended fertility clinic and laboratory (Ultra Laboratory – Khartoum, Sudan) (n=566)

Table 3. General properties of the human semen samples that were taken from infertile couples of unknown root of infertility were attended to the fertility clinic and laboratory (Ultra Laboratory – Khartoum, Sudan) (n=566)

FIGURES

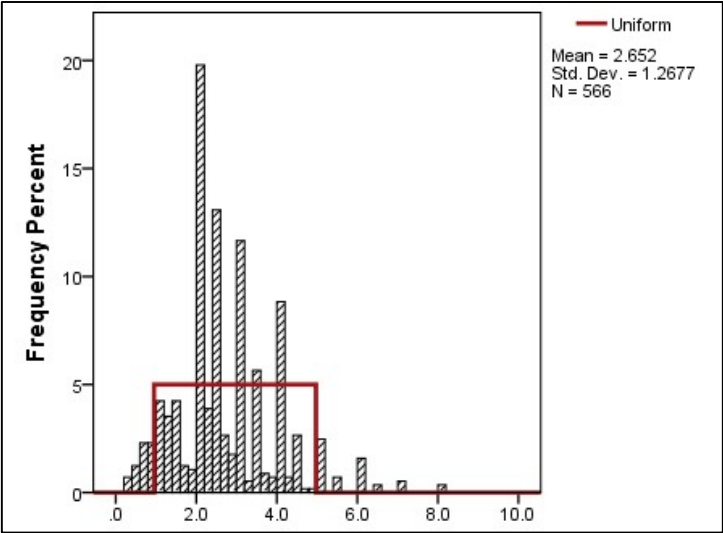


Figure 1.

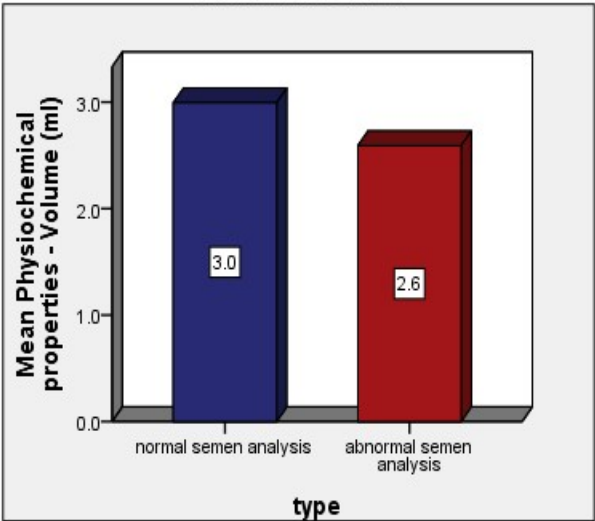


Figure 2.

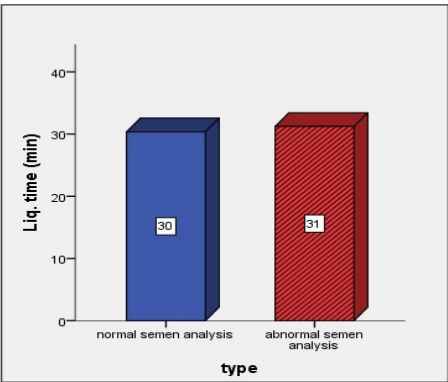
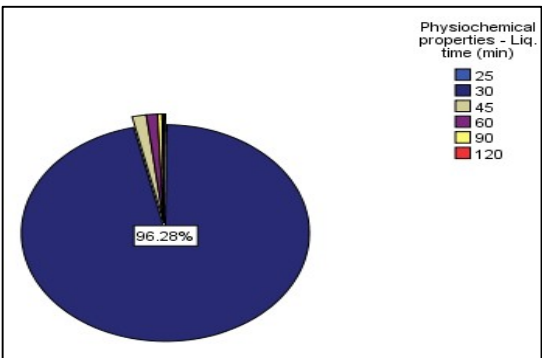


Figure 3. Figure 4.

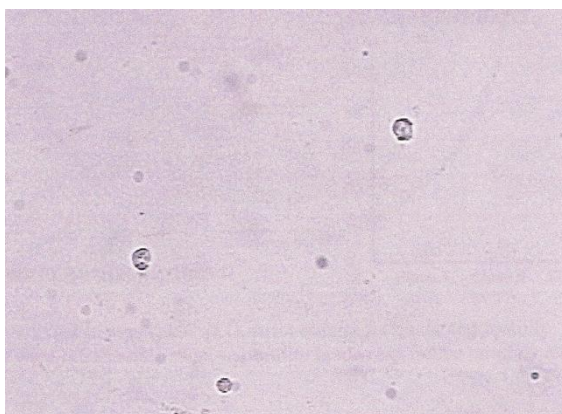


Figure 5.

TABLES

Table 1.

Semen Parameters	Year				
	1980	1987	1992	1999	2010
Volume (ml)	-	>2.0 ml	>2.0 ml	>2.0 ml	1.5 (1.4-1.7)
Sperm concentration (million/ml)	20 -200	≥ 20 million/ml	≥ 20 million/ml	≥ 20 million/ml	15 (12 - 16)
Leucocyte count	<4.7	<1.0	<1.0	<1.0	<1.0

Source: 1.WHO (2); 2010, p 224; and Sonia and Ashok, 2012, p 117

Table 2.

Semen properties	Mean	Standard Deviation	Range	Minimum	Maximum	Percentile 25	Percentile 75
Volume (ml)	2.7	1.3	7.7	0.3	8.0	2.0	3.4
pH	7.4	0.4	6.4	1.0	7.4	7.4	7.4
Liq. time (min)	31	7.0	95	25	120	30	30
Sperm Agglutination semi-quantitative rank	1.0	0.0	1.0	0.0	1.0	1.0	1.0

Table 3

Physiochemical properties		N	Percent (%)
Odor			
	Normal	563	99.5
	Abnormal	0	0.00
	Missed	3	0.5
Viscosity			
	Normal	561	99.1
	Abnormal	0	0.00
	Missed	5	0.9
Liquefaction status			
	Complete	556	98.2
	Incomplete	0	0.00
	Missed	10	1.8
Nonspecific aggregation			
	Null	564	99.6
	Present	0	0.00
	Missed	2	0.4
Agglutination			
	Null	565	99.8
	Present	0	0.00
	Missed	1	0.2
Total		566	100%

NUTRACEUTICAL POTENTIAL AND BENEFITS OF PHYTOCHEMICALS IN PREVENTING NON-COMMUNICABLE DISEASES

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Abstract

Non-communicable diseases (NCDs), such as cardiovascular diseases, diabetes, cancer, and neurodegenerative disorders, pose significant global health challenges. Phytochemicals, bioactive compounds found in plants, have gained attention for their potential in preventing and managing NCDs. This article explores the nutraceutical potential of various phytochemicals, their mechanisms of action, and their efficacy in mitigating the risk of NCDs. The review also discusses recent scientific findings and clinical implications, emphasizing the importance of dietary interventions for global health improvement.

Key words: NCD, phytochemicals, bioactive, nutraceutical

Introduction

The rise in NCDs is a major public health concern, accounting for approximately 71% of global deaths (World Health Organization [WHO], 2021). Lifestyle factors such as poor diet, sedentary habits, and environmental influences contribute significantly to NCD prevalence. Increasing evidence suggests that phytochemicals, naturally occurring compounds in fruits, vegetables, whole grains, spices, condiments, and medicinal plants, play a crucial role in disease prevention. These bioactive compounds exert antioxidant, anti-inflammatory, anti-carcinogenic, and cardioprotective effects, making them valuable in nutritional therapy and preventive healthcare. They have demonstrated promising roles in improving metabolic health, enhancing immune responses, and reducing the risk of chronic illnesses (Liu et al., 2020). This paper examines the classification, biochemical mechanisms, and health benefits of phytochemicals in NCD prevention. Additionally, it highlights current

research and discusses the potential incorporation of phytochemicals into dietary recommendations and nutraceutical formulations.

Classification of Phytochemicals and Their Health Benefits

Phytochemicals are broadly classified into flavonoids, phenolics, carotenoids, alkaloids, and organosulfur compounds. Each class exhibits unique properties beneficial for human health.

Flavonoids

Flavonoids, found in berries, tea, citrus fruits, and cocoa, possess strong antioxidant and anti-inflammatory properties. They modulate cell signaling pathways, inhibit oxidative stress, and support cardiovascular health (Liu et al., 2020). Studies indicate that quercetin, a flavonoid, reduces blood pressure and improves endothelial function (Egert et al., 2019). They exhibit antioxidant, anti-inflammatory, and cardioprotective properties (Kumar & Pandey, 2021).

Phenolic Compounds

Phenolics, such as resveratrol (found in grapes and red wine) and curcumin (from turmeric), demonstrate anticancer and neuroprotective effects. Curcumin inhibits tumor growth, while resveratrol enhances mitochondrial function, reducing the risk of neurodegenerative diseases (Singh et al., 2022). Polyphenols are abundant in coffee, cocoa, and grapes, polyphenols modulate oxidative stress, regulate glucose metabolism, and promote gut microbiota health (Scalbert et al., 2019).

Carotenoids

Carotenoids, including beta-carotene, lycopene, and lutein, are abundant in carrots, tomatoes, and leafy greens. These compounds prevent macular degeneration and reduce the risk of cardiovascular diseases through their potent antioxidant effects (Johnson, 2019). They function as potent antioxidants and play a role in cancer prevention and immune enhancement (Tanaka et al., 2020).

Alkaloids

Alkaloids such as caffeine and capsaicin are known for their metabolic and thermogenic effects. They regulate glucose metabolism, enhance insulin sensitivity, and contribute to weight management, reducing the risk of obesity-related diseases (Szkudelski, 2021). Alkaloids have shown anti-inflammatory, neuroprotective, and anti-cancer properties (Zhou et al., 2022).

Organosulfur Compounds

Sulfur-containing compounds, found in garlic and onions, exhibit cardioprotective and anticancer properties. Allicin, a bioactive sulfur compound, lowers cholesterol levels and prevents platelet aggregation, reducing the risk of atherosclerosis (Ried, 2020). These compounds have strong antimicrobial, detoxifying, and cardiovascular benefits (Rahman, 2021).

Role of Spices, Condiments, and Herbs in NCD Prevention

Traditional Indian spices, condiments, and herbs contain bioactive phytochemicals with potent health benefits:

- **Cinnamon (*Cinnamomum verum*):** Rich in cinnamaldehyde, cinnamon has been shown to improve insulin sensitivity and reduce fasting blood glucose levels in diabetic patients (Ranasinghe et al., 2019).
- **Black Pepper (*Piper nigrum*):** Contains piperine, which enhances nutrient bioavailability and exhibits anti-inflammatory properties that help reduce metabolic syndrome risks (Meghwal & Goswami, 2012).
- **Cloves (*Syzygium aromaticum*):** High in eugenol, cloves have demonstrated antimicrobial, anti-inflammatory, and cardioprotective effects (Cortés-Rojas et al., 2014).
- **Ginger (*Zingiber officinale*):** Gingerol, the bioactive component of ginger, has been shown to lower cholesterol levels, improve digestion, and possess anticancer properties (Shukla & Singh, 2007).
- **Tulsi (*Ocimum sanctum*):** Also known as holy basil, tulsi has adaptogenic, antimicrobial, and anti-diabetic effects (Pattanayak et al., 2010).
- **Coriander (*Coriandrum sativum*):** Exhibits antihyperglycemic and lipid-lowering properties beneficial for diabetic and heart disease patients (Chithra & Leelamma, 2000).

- **Cardamom (*Elettaria cardamomum*):** Known for its antihypertensive and lipid-lowering effects, cardamom has shown promise in reducing oxidative stress and improving metabolic health (Verma et al., 2022).
- **Fenugreek (*Trigonella foenum-graecum*):** Rich in saponins and fiber, fenugreek helps in glycemic control and lipid metabolism, benefiting diabetic patients (Sharma et al., 2023).
- **Turmeric (*Curcuma longa*):** Beyond curcumin, turmeric contains other bioactive compounds like turmerones that contribute to anti-inflammatory and neuroprotective effects (Jurenka, 2021).

Mechanisms of Action

Phytochemicals exert their health benefits through multiple mechanisms, including:

- **Antioxidant Activity:** Neutralizing free radicals to reduce oxidative stress. Phytochemicals scavenge free radicals, reducing oxidative stress-induced damage to cellular components (Williamson, 2018).
- **Anti-inflammatory Effects:** Modulating inflammatory cytokines and signaling pathways. Many phytochemicals inhibit pro-inflammatory cytokines, reducing chronic inflammation linked to NCD progression (Wang et al., 2020).
- **Modulation of Enzymatic Pathways:** Some compounds modulate metabolic enzymes, improving lipid metabolism and insulin sensitivity (Hertog et al., 2021).
- **Regulation of Metabolic Processes:** Improving insulin sensitivity and lipid metabolism.
- **Gene Expression Modulation:** Influencing epigenetic modifications for cancer prevention.
- **Gut Microbiome Interaction:** Enhancing gut flora for improved metabolic health. Polyphenols and other bioactive compounds influence gut microbiota composition, enhancing metabolic health and immune function (Zhou et al., 2022).
- **Epigenetic Modifications:** Certain phytochemicals influence gene expression related to cellular repair, detoxification, and cancer suppression (Scalbert et al., 2019).

Role of Phytochemicals in Preventing Specific NCDs

Cardiovascular Diseases (CVDs)

Flavonoids and polyphenols improve vascular function by reducing endothelial dysfunction, lowering LDL cholesterol, and preventing platelet aggregation. Studies have shown that increased consumption of polyphenol-rich foods, such as berries and dark chocolate, is associated with a reduced risk of heart disease (Kumar & Pandey, 2021).

Diabetes and Metabolic Syndrome

Polyphenols enhance insulin sensitivity by modulating glucose transporters and reducing postprandial glycemic response. Resveratrol, found in grapes and red wine, has been reported to improve pancreatic beta-cell function and glycemic control (Liu et al., 2020).

Cancer Prevention

Carotenoids, flavonoids, and alkaloids exhibit anti-carcinogenic properties by inducing apoptosis, inhibiting cell proliferation, and modulating inflammatory pathways. Cruciferous vegetables contain sulforaphane, which has been linked to a reduced risk of prostate, breast, and colorectal cancers (Tanaka et al., 2020).

Neurodegenerative Diseases

Neuroprotective effects of phytochemicals such as curcumin and catechins include the reduction of amyloid plaque formation in Alzheimer's disease and enhancement of cognitive function. Polyphenols also protect neuronal integrity by reducing oxidative damage and inflammation (Williamson, 2018).

Recent Advances and Future Perspectives

Recent research has explored the bioavailability and delivery systems of phytochemicals to enhance their efficacy. Encapsulation techniques, nanoformulations, and synergistic combinations with probiotics are being investigated to improve absorption and stability. Functional foods enriched with phytochemicals, such as fortified teas, bioactive dairy products, and plant-based supplements, are gaining prominence as viable preventive strategies against NCDs (Wang et al., 2020).

Conclusion

Phytochemicals offer substantial health benefits in preventing and managing NCDs through their antioxidant, anti-inflammatory, and metabolic-regulating properties. Integrating these bioactive compounds into daily diets through functional foods and nutraceuticals could significantly reduce the global burden of chronic diseases. However, further clinical research is necessary to determine optimal dosages and interactions with conventional medications.

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Isolation and Screening of Rhizosphere Bacteria for Industrially Important Extracellular Enzyme

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Abstract

The Rhizosphere, the region surrounding plant roots, harbors diverse microbial communities that play a crucial role in plant growth and ecosystem functioning. This study aimed to isolate and screen Rhizosphere bacteria for industrially important extracellular enzymes. Soil samples were collected from the Rhizosphere of various plants, and bacteria were isolated using selective media. A total of 10 bacterial isolates were obtained and screened for their ability to produce extracellular enzymes such as amylase, cellulase, lipase, and protease. Four isolates showed positive results for one or more enzymes, with 10 isolates exhibiting high enzyme activity. Our results highlight the potential of Rhizosphere bacteria as a source of industrially important enzymes, which could be used in various biotechnological applications such as bio fuel production, food processing, and in the textile and paper-pulp industries. Further studies on enzyme optimization and strain improvement are recommended for commercial utilization.

Keywords: Rhizosphere, Enzyme Activity, soil bacteria, Industrial application

Introduction

Agricultural soil is a rich source of microbial diversity, making it an ideal location for isolating industrial enzymes. These enzymes have various industrial applications, including food, pharmaceutical, and biofuel production (Adinarayana et al., 2014). The use of agricultural soil for enzyme production offers several advantages, including low cost, sustainability, and environmental friendliness (Brainard et al. 2013). Microorganisms in agricultural soil have evolved to adapt to different environmental conditions, such as temperature, pH, and nutrient availability (Javaid and Adrees 2009). This adaptability enables them to produce enzymes that are active in a wide range of conditions, making them useful for industrial applications. The bacteria isolated from rhizosphere soils, have proved beneficial to the plants by directly having an effect on nitrogen fixation (Han et al., 2005), solubilization of nutrients (Rodriguez and Fraga 1999), production of growth hormones, laminocyclopropane-1-carboxylate (ACC) deaminase (Correa et al., 2004) and indirectly by antagonizing pathogenic fungi by the production of siderophores, -1,3-glucanase, antibiotics, fluorescent pigments and cyanide (Pal et al., 2001). IAA producing microorganisms are

known to promote root elongation and plant growth (Patten and Glick 2002). IAA-production by root associated bacteria is a major mechanism of plant growth promotion has important implication for the development of the root system under the influence of rhizosphere microbes (Anton Hartmann et al., 2009). IAA-biosynthesis of the plant growth promoting Rhizo bacterium, *Azospirillum brasilense* was shown to be induced in rhizoplane colonizing bacteria (Rothballer et al., 2005). agricultural soil is a valuable source of industrial enzymes due to its rich microbial diversity, adaptability of microorganisms, low cost of production, sustainability, and environmental friendliness (Saranom et al., 2021). The use of agricultural soil for enzyme production offers several advantages, including the elimination of synthetic chemicals and genetically modified organisms. As the demand for enzymes continues to grow, researchers and industries must explore new sources of enzymes, including agricultural soil, to meet the increasing demand (saja ali and zainab Ali,2022).

Materials and Methods

Collection of Rhizosphere Samples:

Three rhizosphere soil samples were collected from Ragi field of Paravathur village Arakkonam ranipet District, Tamilnadu India. The soil samples were collected aseptically in a clean and sterilized container from three places. The collected samples were immediately transferred to the laboratory in an ice box for bacteriological examination.

Isolation of Rhizosphere Bacteria:

One 1g of soil sample was suspended in 99 ml of water and these suspensions were considered as 10⁻¹ dilution. Nutrient medium (NA) prepared in water was used for isolation of bacteria from 10⁻¹ suspension, 1ml was transferred to 9ml of sterile distilled water and subsequently serially diluted to 10⁻³, 10⁻⁴, etc. from the required dilution, 1 ml suspension was drawn and plated in the Petriplates then add NA medium. All the plates were incubated at 37°C for 7 days. After incubation for 1 day at room temperature, clear colony forming bacteria were selected pure culture inoculation of nutrient broth maintain the culture at 37°C.

Morphological Characteristics of Rhizosphere Bacteria :

All the isolated soil bacteria isolates were sub-cultured on NA medium the pigmentation for aerial and colony morphology produced was observed for all the isolates and the results were documented.

Growth Pattern of Rhizosphere Bacteria:

All the isolated soil Bacteria were studied for their growth on Nutrient Agar medium, among 10 isolates, most of them showed fast growth, few exhibited slow growth and rest of the isolates showed moderate growth.

Biochemical Characterisation:

Gram Staining

Clean glass slides were taken. Using a sterile needle, a loopful of isolated microorganisms was smeared on glass slide. The smear is allowed to air dry and then heat fixed. The smear was flooded with crystal violet left for one minute. Then the slide was washed with running tap water. The smear was flooded with Gram's iodine and kept for one minute and washed with running tap water. The smear was decolorized with ethyl alcohol. The slide was counter stained with safranin for one minute and washed with tap water. The smear was air dried and observed under light microscope. The cell morphology and Gram's reaction were observed.

Microscopic Examination

Cell shape and motility were examined on freshly prepared wet mounts by light microscopy of exponentially growing liquid culture. Gram nature of the isolated was determined by gram staining performed after fixing and simultaneously desalting the samples with 2% acetic acid for 5 min (Oren *et al.*, 1997).

Motility Test

Hanging Drop Method

Place a small drop of liquid bacterial culture in the centre of a cover slip place a small drop of water at each corner of the cover slip will stick to the slide with a central depression over the cover slip the cover slip will stick to the slide and when the slide is inverted the drop of bacteria culture will be suspended in the well and examine in microscope. A darting, zig zag tumbling or other organized movement indicates- positive reaction. Absence of movement or Brownian motion only indicates- negative reaction.

Catalase

Tube Or Bottle Method

Place approximately 0.2 ml of hydrogen peroxide solution in a test tube and carefully pick a colony to be tested with/loop or disposable alter actives. Rub the colony on the inside wall of the bottle above the surface of the hydrogen peroxide solution cab the tube to bottle and tilt it to allow the hydrogen peroxide solution to cover the colony. Look for vigorous bubbling occurring within 10sec.

Oxidase Test

The oxidase test was performed using an oxidase disk purchased from Himedia. A 10 μ L aliquot of the bacterial culture was carefully spotted onto the sterile disk. The development of a blue color indicated a positive reaction, while the absence of a blue color indicated a negative reaction.

Extracellular Enzyme Screening on Solid Media

Screening of Rhizosphere Bacteria For Amylase Enzyme

The 10 bacterial isolates were screened for the production of amylase enzyme by streaking the isolates on Nutrient agar medium supplemented 1 % starch as a substrate and incubated at 37°C for 3 days. The iodine indicator, with 2% of iodine and 1% potassium iodide was poured and the clear zone around the growth was observed, which indicated the hydrolysis of starch (Coronado *et al.*, 2000).

Screening of Rhizosphere Bacteria for Cellulase Enzyme

The 10 bacterial isolates were screened for the production of cellulase enzyme by streaking the isolates on Nutrient agar medium supplemented 1 % carboxy methyl cellulose as a substrate and incubated at 37°C for 3 days. After incubation, the plates were flooded with 0.1% Congo red solution, and development of clear zone around the colonies was observed.

Screening of Rhizosphere Bacteria for Proteases Enzyme

The 10 bacterial isolates were screened for the production of protease enzyme by streaking the isolates on Nutrient agar medium supplemented 1 % Gelatin as a substrate and incubated at 37°C for 3 days. After incubation, the plates were flooded with saturated ammonium sulphate in 0.1 N HCl solutions and a clear zone around the colony was observed, which indicated the hydrolysis of gelatin.

Screening of Rhizosphere Bacteria for Lipase Enzyme

The 10 bacterial isolates were screened for the production of lipase enzyme by streaking the isolates on Nutrient agar medium supplemented 1 % Tween 20 as a substrate and incubated at 37°C for 3 days. After incubation, the plates were observed for opalescent zone formation around the colonies (Hasan *et al.*, 2009).

Preparation of Colloidal Chitin

The preparation of colloidal chitin involved several steps. First, crab shells were thoroughly washed with tap water and soaked in 2% KMnO₄. The shells were then boiled for 30 minutes and treated with 1% oxalic acid. Following this, they were treated with 5N HCl for 12 hours and subsequently washed successively with water, ethanol, and ether before being dried. The dried product, free from protein, was ground into a powder to obtain crude chitin. The chitin powder was then dissolved in 5–10 times its volume of concentrated HCl and kept at 4°C for 12 hours. The resulting syrupy liquid was filtered using glass wool and poured into 50% aqueous ethanol while stirring vigorously with a magnetic stirrer. The precipitate was collected by centrifugation at 10,000 rpm for 10 minutes, then suspended in distilled water and dialyzed against the same until the pH was neutral. Finally, the colloidal chitin was lyophilized and stored at 10°C.

Screening of Rhizosphere Bacteria for Chitinase Enzyme

The 10 bacterial isolates were screened for the production of chitinase enzyme by streaking the isolates on Nutrient agar medium supplemented 1 % chitin as a substrate and incubated at 37°C for 3 days. After incubation, the plates were flooded with 1.0% solution of Congo red. Then, the Congo red solution was poured off and the plates were again flooded with 1 N NaCl. The development of clear zone around the colonies was observed.

Result and Discussion

Collection of Rhizosphere Samples:

Three rhizosphere soil samples were collected from Ragi field of Paravathur village Arakkonam ranipet District, Tamilnadu India. The soil samples were collected aseptically in a clean and sterilized container from three places. The collected samples were immediately transferred to the laboratory in an ice box for bacteriological examination. (**Fig: 1**).



Fig :1 Sample Collection Site

Isolation Of Rhizosphere Bacteria:

Totally 10 Rhizosphere soil bacteria was isolated. The 10 soil bacteria were streaked separately on Nutrient Agar medium they were named as AB1 to AB10. (**Fig: 2 & 3**).

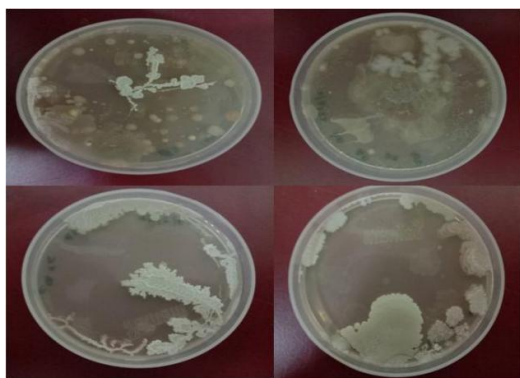


Fig 2: Isolation Plate

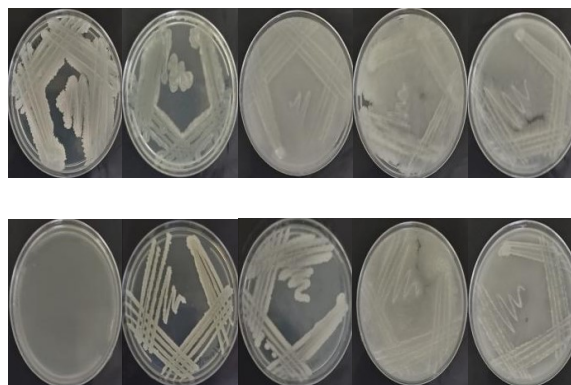


Fig 3: Pure Cultures (AB1-AB10)

Morphological Characteristics Of Rhizosphere Bacteria :

All the isolated bacteria isolates were sub-cultured on NA medium the pigmentation for aerial and colony morphology produced was observed for all the isolates and the results were documented. The colonies were distinguished by morphological characters like shape, size, color, margin, elevation and opacity. The morphologically distinct bacteria from nutrient agar were further subculture on Nutrient agar to screen them for various potential biotechnological applications (**Table: 1**)

S.NO	SIZE	SHAPE	COLOUR
AB-1	Small	Round, Raised	white
AB-2	Big	Irregular, Flat	Transparent
AB-3	Moderate	Irregular, Raised	white
AB-4	Small	Raised	Yellow creamy
AB-5	Big	Flat	Creamy white
AB-6	Small	Flat, Irregular	white
AB-7	Moderate	Flat, Dried	yellow
AB-8	Big	Raised	Creamy yellow
AB-9	Small	Round	White creamy
AB-10	Big	Irregular, Raised	Dull White

Table 1: Morphology of Soil Bacteria

Growth Pattern of Rhizosphere Bacteria:

All the isolated soil Bacteria were studied for their growth on Nutrient Agar medium, among 10 isolates, most of them showed fast growth, few exhibited slow growth and rest of the isolates showed moderate growth.

Biochemical Characterisation:

For identification of soil bacteria AB1 to AB10 the cultures were subjected for Gram staining, Motility, Oxidase and Catalase test and they were document in the **table 2**. Out of 10 Soil Bacteria 6 Bacteria were Gram Negative and 4 bacteria were Gram Positive, All the isolated soil bacteria were motile six bacteria were Oxidase positive and three were catalase positive

S.NO	GRAM STAINING	MOTILITY	OXIDASE	CATALASE
AB-1	Positive	Motile	Positive	Negative
AB-2	Negative	Motile	Negative	Positive
AB-3	Positive	Motile	Positive	Negative
AB-4	Negative	Motile	Negative	Negative
AB-5	Negative	Motile	Positive	Positive
AB-6	Positive	Motile	Positive	Negative
AB-7	Negative	Motile	Negative	Negative
AB-8	Negative	Motile	Positive	Negative
AB-9	Positive	Motile	Positive	Negative
AB-10	Negative	Motile	Negative	Positive

Table 2 : Biochemical Characterisation

Extracellular Enzyme Screening on Solid Media

Screening of Rhizosphere Bacteria for Amylase Enzyme

The 10 Soil bacterial isolates were screened for the production of amylase enzyme out of which AB1-AB 10 were utilize the starch substrate and AB 1 was found to be the highest amylase producing Soil bacteria (**Fig: 4 & Table: 3**)

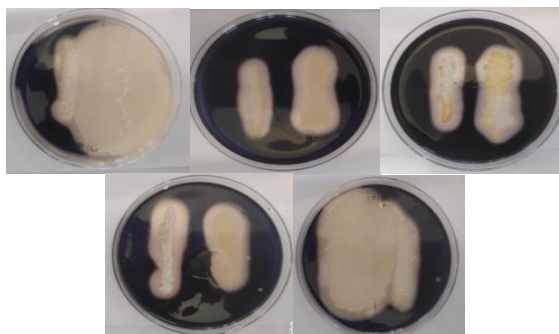


Figure 4: Amylase Plate Assay

Screening of Rhizosphere Bacteria for Cellulase Enzyme

The 10 Soil bacterial isolates were screened for the production of cellulase enzyme out of which AB2, AB3, AB5, AB6, AB8 were utilize the CM-Cellulose substrate and AB2 was found to be the highest cellulose producing Soil bacteria (**Fig: 5 & Table: 3**)

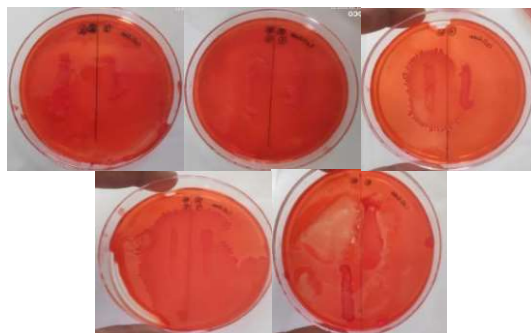


Figure 5 :Cellulase Plate Assay

Screening of Rhizosphere Bacteria for Proteases Enzyme

The 10 Soil bacterial isolates were screened for the production of protease enzyme out of which AB2, AB5, AB6, AB8 were utilize the Gelatin substrate and AB 8 was found to be the highest protease producing Soil bacteria (**Fig: 6 & Table: 3**)

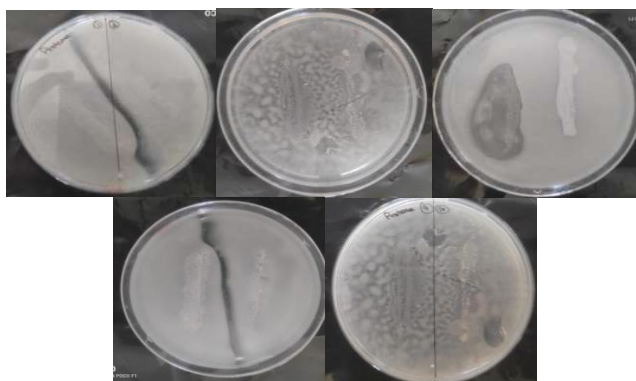


Figure 6: Protease Plate Assay

Screening of Rhizosphere Bacteria for Lipase Enzyme

The 10 Soil bacterial isolates were screened for the production of lipase enzyme out of which AB1, AB2, AB3, AB5, AB6, AB10 were utilize the Tween 20 substrate and AB10 was found to be the highest amylase producing Soil bacteria (**Fig: 7 & Table: 3**)

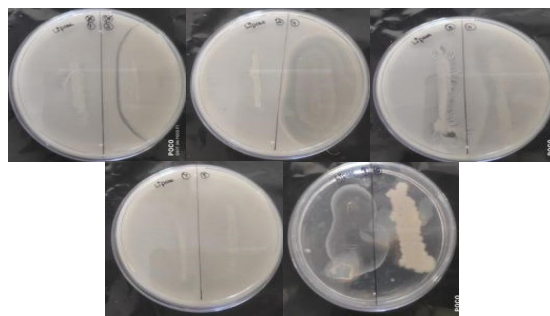


Figure 7: Lipase Plate Assay

Preparation of Colloidal Chitin

Colloidal chitin was prepared and used as a substrate for chitin enzyme screening in rhizosphere bacteria (**Fig: 9**)

SCREENING OF RHIZOSPHERE BACTERIA FOR CHITINASE ENZYME

The 10 Soil bacterial isolates were screened for the production of chitinase enzyme. AB1, AB2, AB4, AB8, AB10 were able to utilize the chitin substrate, and AB2 was found to be the highest amylase producing Soil bacteria (**Fig: 10 & Table: 3**)



Figure 9 : Colloidal Chitin

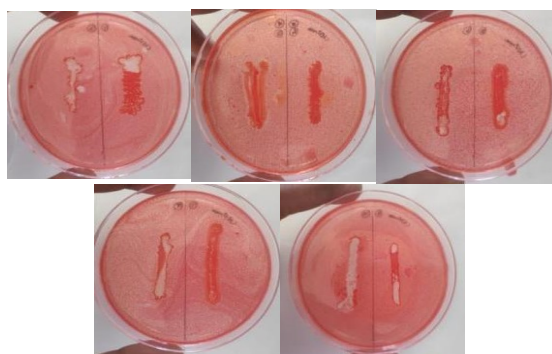


Figure 10: Chitinase Plate Assay

BACTERIA	AMYLASE	CELLULASE	PROTEASE	LIPASE	CHITINASE
AB-1	+	-	-	+	-
AB-2	+	+	+	+	+
AB-3	+	+	-	+	-
AB-4	+	-	-	-	+
AB-5	+	+	+	+	-
AB-6	+	+	+	+	-
AB-7	+	-	-	-	-
AB-8	+	+	+	-	+
AB-9	+	-	-	-	-
AB-10	+	-	-	+	+

Table 3: Extra Cellular Enzyme Production

Discussion

The isolation and characterization of rhizosphere bacteria from the Ragi field of Paravathur village, Tamil Nadu, provided valuable insights into their morphological, biochemical, and enzymatic properties. The rhizosphere, being a biologically active soil region influenced by plant roots, harbors diverse microbial communities that play a significant role in plant growth promotion, soil fertility, and biocontrol of pathogens (Bhatt et al., 2023).

Morphological And Biochemical Characteristics Of Rhizosphere Bacteria :

The morphological characterization of the ten bacterial isolates revealed variations in colony shape, size, elevation, and pigmentation. Such diversity is indicative of the microbial richness in the rhizosphere, which is essential for maintaining soil health and supporting plant growth (Wang et al., 2022). Among the isolates, both Gram-positive and Gram-negative bacteria were identified, with six being Gram-negative and four being Gram-positive. All isolates were motile, a characteristic often associated with beneficial plant growth-promoting bacteria (PGPB) that can efficiently colonize plant roots (Gouda et al., 2018). The oxidase and catalase tests further differentiated the bacterial isolates, with six being oxidase-positive and three catalase-positive, suggesting the presence of aerobic and facultative anaerobic bacteria.

Extracellular Enzyme Production Of Rhizosphere Production:

Extracellular enzyme production is a critical trait of soil bacteria, as these enzymes contribute to organic matter degradation, nutrient cycling, and plant growth enhancement (Kumar et al.,

2023). The screening of bacterial isolates for enzyme production revealed significant activity for amylase, cellulase, protease, lipase, and chitinase.

- **Amylase Production:** All ten isolates exhibited amylase activity, with AB1 producing the highest levels. Amylase is crucial for starch hydrolysis, making nutrients more accessible to plants and soil microbes (Chen et al., 2023).
- **Cellulase Production:** Five isolates (AB2, AB3, AB5, AB6, AB8) demonstrated cellulase activity, with AB2 being the highest producer. Cellulase is essential for decomposing cellulose, a major plant cell wall component, thereby facilitating organic matter recycling (Li et al., 2023).
- **Protease Production:** Proteolytic activity was detected in four isolates (AB2, AB5, AB6, AB8), with AB8 being the most potent producer. Proteases play a vital role in nitrogen cycling and organic matter decomposition, contributing to soil fertility (Zhang et al., 2022).
- **Lipase Production:** Lipase activity was observed in six isolates (AB1, AB2, AB3, AB5, AB6, AB10), with AB10 exhibiting the highest activity. Lipases are involved in lipid hydrolysis and can enhance the bioavailability of essential fatty acids for microbial and plant metabolism (Sharma et al., 2023).
- **Chitinase Production:** Chitinase activity was noted in five isolates (AB1, AB2, AB4, AB8, AB10), with AB2 being the highest producer. Chitinases are widely known for their role in biocontrol, as they degrade chitin, a major component of fungal cell walls, thus suppressing plant pathogens (Borah et al., 2024).

Implications for Sustainable Agriculture :

The enzymatic potential of these rhizosphere bacteria highlights their significance in agriculture. Amylase and cellulase contribute to organic matter breakdown, enhancing soil nutrient availability, while protease and lipase aid in nitrogen cycling and organic decomposition. Chitinase-producing bacteria, in particular, can act as biocontrol agents against fungal pathogens, reducing the need for chemical pesticides (Kaur et al., 2023). The diverse enzymatic activities suggest that these bacterial isolates have potential applications in biofertilizers, bioremediation, and sustainable farming practices.

Recent studies support these findings. Feng et al. (2024) reported that root exudates influence rhizosphere microbial communities, enhancing plant resistance to soil-borne pathogens. Similarly, Li et al. (2023) demonstrated that nitrogen-fixing bacteria from the rhizosphere promote plant growth and soil health. The presence of enzyme-producing bacteria in the rhizosphere is crucial for maintaining ecosystem balance and improving crop productivity.

Conclusion

The study successfully isolated and characterized ten rhizosphere bacterial strains, revealing their diverse morphological, biochemical, and enzymatic properties. The ability of these isolates to produce extracellular enzymes highlights their potential role in soil nutrient cycling, organic matter decomposition, and biological control of pathogens. These findings align with recent research emphasizing the importance of rhizosphere microbes in sustainable agriculture. Future studies should focus on the genomic characterization of these bacteria to further explore their agricultural applications.

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A study on the impact of the heavy metal Cadmium (Cd) on the growth performance of fish

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Abstract:

The impact of cadmium (Cd) on fish growth performance is significant and complex, that negatively influences physiological, biochemical, and behavioural functions. Exposure to cadmium results in notable growth inhibition, tissue buildup, and toxicity, which lead to cellular harm, oxidative stress, and weakened immune systems. These effects not only hinder the immediate health and growth of fish but also have wider ecological and economic consequences. Implementing effective mitigation strategies, such as strict environmental regulations and improved remediation efforts, is essential to combat the harmful effects of cadmium contamination and to ensure the sustainability of fish populations. Different growth parameters that are concerned with Cd toxicity in fishes includes Feed Conversion Ratio (FCR), Specific Growth Rate (SGR), Absolute weight gain (AWG), Stomasomatic index (SSI), Intestosomal index (ISI), Digestosomal index (DSI), Hepatosomatic index (HSI), Condition factor (CF) etc.

Keywords:

Channa punctata, Growth parameters, Cd toxicity

Introduction:

Aquaculture plays a crucial role in ensuring food security and providing adequate nutrition to the growing global population. Nearly half of the world's food fish supply originates from aquaculture. In recent decades, the contamination of freshwater by various substances has emerged as a significant concern. Heavy metal pollution in natural aquatic systems is often the result of human activities, such as industrial and household waste disposal. Urban industrial waste is the primary source of cadmium pollution in rivers and lakes, leading to the accumulation of this element in aquatic organisms and its movement up the food chain. Even

trace amounts of non-degradable heavy metals cause significant problems in aquatic systems by being assimilated, deposited, and incorporated at specific concentrations in abiotic substances, eventually accumulating in the bodies of aquatic organisms (Mekkawy et al., 2010). While essential metals can enhance growth and feed utilization in various species, exceeding their maximum allowable limits disrupts normal physiological and ecological functions, causing toxicity in organisms and posing a significant threat to human health. Many of these heavy metals are highly carcinogenic and can lead to serious health issues such as liver disorders, cardiovascular problems, kidney dysfunction, and even death in extreme cases. Heavy metal pollution profoundly affects the physiology of several aquatic organisms, particularly fish. Cadmium (Cd) poses a serious threat to both fish and mammals because it is toxic and remains in animal bodies for a long time. This review compiles current information on the impacts of heavy metal Cadmium (Cd) on growth performance of several fish species (Afzaal et al., 2022).

Growth parameters in fishes:

Growth parameters in fish are essential indicators of their health and development in aquaculture. The preliminary parameters to study fish growth are weight and length gain. Weight gain is the absolute increase in body weight over a specific period of time. It is a straightforward measure of growth performance. Additionally, length gain is the increase in length over time, often used alongside weight gain to assess overall growth patterns. Growth rate of fish defines the speed at which it increases in size or weight over a given period. It is often measured as specific growth rate (SGR), which is the percentage increase in body weight per day. Another important parameter to study fish growth is Feed Conversion Ratio (FCR) which is the efficiency with which fish convert feed into body mass. A lower FCR indicates better feed efficiency and more cost-effective growth. Condition Factor (K) on the other hand is a measure of the fish's health and well-being, calculated by comparing its weight to its length. It helps determine if the fish is growing properly in proportion to its length. Another important parameter includes survival rate, which is the percentage of fish that survive over a given period. Higher survival rates often correlate with better growth conditions and health. Growth efficiency is the comprehensive measure that takes into account growth rate, feed conversion, and survival rate to evaluate overall growth performance. These parameters help in assessing the effectiveness of feeding strategies, environmental conditions, and overall management practices in aquaculture (Thongprajukaew et al., 2017)

Cadmium impact on fishes:

Cadmium (Cd) exposure in fish has numerous detrimental effects, impacting their growth, health, and overall physiology. Cd exposure often leads to a lower growth rate in fish due to increased metabolic demands and decreased efficiency of energy utilization. Fish exposed to Cd may show a reduced appetite and lower feeding activity, contributing to stunted growth. Cd interferes with normal metabolic processes, leading to increased basal metabolic rates and higher energy maintenance costs. Cd can alter enzymatic activity, which disrupts various biochemical pathways essential for growth and development. Cd exposure can weaken the immune system, making fish more susceptible to diseases and infections. Cd induces oxidative stress by generating reactive oxygen species (ROS), which can damage cells and tissues. Cd exposure can negatively impact reproductive performance, including reduced fertility and altered embryonic development. It tends to accumulate in fish tissues, particularly in the liver, kidneys, and gills, that can lead to chronic toxicity. High levels of cadmium are toxic and can cause severe health issues, including liver and kidney dysfunction, cardiovascular problems, and, in extreme cases, death (Liu et al., 2022).

In summary, cadmium exposure in fish results in a wide range of adverse effects, from growth inhibition and metabolic disruption to immune suppression and reproductive impairment. These impacts underscore the importance of monitoring and regulating cadmium levels in aquatic environments to protect fish populations and overall ecosystem health.

Cadmium impact on the growth performance fishes:

Cadmium (Cd) exposure significantly impacts various growth parameters in fish, leading to compromised health and development. Cd exposure often results in a lower SGR, indicating a slower increase in body weight over time. This is due to increased metabolic demands and decreased efficiency of energy utilization. Fish exposed to Cd typically show reduced weight gain, reflecting overall poor growth performance. Cd exposure leads to a higher FCR, meaning that fish require more feed to gain the same amount of body mass. This indicates less efficient feed utilization. Fish exposed to Cd may have a lower condition factor, which is a measure of their overall health and well-being based on weight and length. This suggests that the fish are not growing in a proportionate and healthy manner. Alongside weight gain, length gain is often stunted in fish exposed to Cd, further indicating poor growth performance. Exposure to Cd can reduce survival rates due to increased susceptibility to

diseases, impaired physiological functions, and overall stress. The overall biomass production in a population of fish exposed to Cd is typically lower, reflecting the cumulative effects of reduced growth rates, poor feed conversion, and lower survival rates. Experiments consistently demonstrate that fish exposed to Cd have lower growth rates and poorer feed conversion ratios compared to unexposed fish (Heydarnejad et al., 2013).

In summary, cadmium exposure adversely affects all key growth parameters in fish, leading to slower growth rates, inefficient feed utilization, poor health, and reduced survival. These impacts highlight the importance of controlling and mitigating cadmium pollution in aquatic environments to ensure the health and productivity of fish populations. Numerous studies have demonstrated that fish exposed to metal mixtures experience reduced growth rates due to alterations in enzymatic activity and, in some cases, changes in their food sources in contaminated waters.

Table: Effect of Cd on the growth performance in fishes

Fish Species	Doses	Exposure Time (Days)	Effects	Reference
Oreochromis niloticus	50 mg/kg	84	Decrease in body weight.	(Ayyat et al., 2017)
Ctenopharyngodon idella	500 µg/l	56	Reduction in overall growth parameters.	(Taslina et al., 2022)
Danio rerio	30 µg/l	35	Decreased body weight, SGR and survival rate.	(Zheng et al., 2016)
Mystus seenghala	1/3 of LC50	112	Reduction in average wet weight, body length and condition factor.	(Fazio et al., 2021)
Pelteobagrus fulvidraco	200 µg/L	56	SGR & WG reduces.	(Xie et al., 2019)
Oncorhynchus mykiss	3 µg/L	30	Increase in FCR whereas Condition Factor (K), SGR, BWG decreases.	(Heydarnejad et al., 2013)
Ictalurus punctatus	6 µg/L	180	Body weight and length	(Jenny &

			decreases.	Brian, 2021)
Danio rerio	30 µg/l	35	Reduced growth and survival rate	(Yuan et al., 2017)

Few studies showed that, Cd exposure affects the basal metabolic rate of salmonids, potentially limiting growth by reducing energy utilization efficiency and increasing metabolic maintenance costs. Qualitative observations of fish feeding behaviour revealed that fish in the Cd-exposed groups did not feed actively, with a marked reduction in feeding activity and appetite observed at high Cd exposure levels. It has been shown that cadmium reduces food intake and assimilation, leading to decreased growth rates in fish. Indeed, the negative impact of cadmium on growth is a well-documented effect in fish and other aquatic organisms (Heydarnejad et al., 2013).

Conclusion:

The impact of cadmium (Cd) on the growth performance of fishes is profound and multifaceted, adversely affecting physiological, biochemical, and behavioral aspects. Cadmium exposure leads to significant growth retardation, tissue accumulation, and toxicity, causing cellular damage, oxidative stress, and immune suppression. These changes not only impair the immediate health and growth of fish but also have broader ecological and economic repercussions. The resultant reduced fish populations and compromised fish health can disrupt aquatic ecosystems and negatively impact commercial fisheries and aquaculture. Effective mitigation strategies, including stringent environmental regulations and enhanced remediation efforts, are crucial to addressing the adverse effects of cadmium contamination and ensuring the sustainability of fish populations.

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