Oxidation Communications 47, No 4, 732–742 (2024)

Antibacterial, antioxidative, biological, antifungal activity, etc.

SYNERGISTIC EFFECT OF PLANT EXTRACTS FROM DELONIX ELATA, HYGROPHILA AURICULATA, AND DRYNARIA QUERCIFOLIA AGAINST INFLAMMATION

HARIPRIYA MANI[a], ANU BAISEL[b], M. RAVIKUMAR[c], R. M. SARAVANAKUMAR[d], PALED MAHESHWARI[e], P. VIVEK[a], S. S. MEENAMBIGA[a], S. BASKAR[f], S. IVO ROMAULD[a]*

[a]*Department of Bioengineering, School of Engineering, Vels Institute of Science Technology and Advanced Studies (VISTAS), 600 117 Pallavaram, Tamil Nadu, India*
E-mail: *ivoromauld@gmail.com*
[b]*School of Social Sciences and Languages, Vellore Institute of Technology, 632 014 Vellore, India*
[c]*New Horizon College of Engineering, Outer Ring Road, 560 103 Bellandur, Bangalore, India*
[d]*Department of Civil Engineering, Veltech Rangarajan Dr. Sagunthala R &D Institute of Science and Technology, Chennai, India*
[e]*Government First Grade College, Naubad Bidar, 58 540 Karnataka, India*
[f]*Department of Automobile Engineering, Vels Institute of Science and Technology, Chennai, India*

ABSTRACT

Inflammatory diseases including different types of rheumatics are a major cause of morbidity worldwide. This study addresses the global burden of inflammatory diseases, focusing on safer alternatives to synthetic anti-inflammatory drugs. Extracts from ethnomedicinal plants, *Delonix elata*, *Hygrophila auriculata*, and *Drynaria quercifolia*, were combined to enhance their anti-inflammatory properties. The ethanolic extract was obtained from the leaves of *Delonix elata*, seeds of *Hygrophila auriculata*, and rhizomes of *Drynaria quercifolia*, showing the existence of phenolics, flavonoids, and alkaloids in preliminary phytochemical screening. Two combinations, D (1:1:1) and E (10:5:1), were prepared. *In vitro* studies revealed that combination D shown maximum total antioxidant activity the strongest capacity to scavenge DPPH radicals. Antibacterial assays demonstrated varying zones of inhibition for *E. coli* and *S. aureus* among the combinations. Notably, combination D showed the largest zone of inhibition for *E. coli*, while sample C was most effective against *S. aureus*. Protein denaturation

* For correspondence.

assay for anti-inflammatory activity highlighted combination D's remarkable inhibition of BSA denaturation (97.37% at 1mg/ml concentration). This research explores potential natural remedies and holistic healthcare solutions through the synergistic effects of plant extracts, aligning with traditional medicinal knowledge.

Keywords: *Delonix elata*, *Hygrophila auriculata*, *Drynaria quercifolia*, anti-inflammatory effect, antioxidant, phytochemicals, synergy.

AIMS AND BACKGROUND

In a global context, increasingly dominated by synthetic pharmaceuticals, the resurgence of interest in natural remedies has gained traction within healthcare, driven by recognition of the limitations and adverse effects of conventional drugs[1,2]. Two essential physiological reactions that severely reduce quality of life when they are chronic or dysregulated are inflammation and pain[3]. The need for creative and comprehensive ways to treating non-communicable illnesses is highlighted by the state of healthcare today, which is characterised by their increasing prevalence. Finding safer and more effective management strategies is made more important by the rise in chronic inflammatory diseases like rheumatoid arthritis, osteoarthritis, and inflammatory bowel diseases, as well as concerns about opioid misuse[4]. This has led to a reevaluation of alternative therapies. Various societies have long utilised local flora for medicinal purposes, making plant-derived treatments an essential part of human healthcare[5]. Plants possess essential nutritional importance for growth and development in man and animals[6]. Plants are one of the essential sources of medicines[7]. The use of herbal medicines and phytonutrients is quickly spreading around the world due to their effectiveness and lack of adverse effects[8]. Traditionally used for their therapeutic qualities, plant species such as *Drynaria quercifolia*, *Hygrophila auriculata*, and *Delonix elata* have been shown in indigenous medical systems to have analgesic and anti-inflammatory qualities, indicating the continuing knowledge of traditional healers. It is appropriate to investigate plant extracts from these species with an emphasis on their synergistic effects in the treatment of inflammation and pain[9]. It has the potential to reveal the pharmacological gems hidden in botanical resources and provide new perspectives on holistic medical practices. This study aims to clarify the complex relationship between plant extracts and human health, offering an insight into the field of complementary and alternative medicine and its potential benefits[10].

Drynaria quercifolia, also known as "Pankhiraj" in Bengali and Indian languages, is one of the first medicinal plants with significant therapeutic efficacy for treating a wide range of ailments. In the natural world, it can be either epipetric (growing on rocks) or epiphytic (growing on trees). As a member of the *Polypodiaceae* family of Pteridophyta, *Drynaria quercifolia* is extensively found in tropical and subtropical regions such as Africa, North America, Bangladesh, and India. Many Indian tribal cultures utilise this plant to cure a variety of illnesses, including cholera, typhoid fever, chronic jaundice, headaches, coughs, and skin conditions. Certain tribes in

Tamil Nadu's Eastern Ghats are known to employ a soup made from the rhizomes of *Drynaria quercifolia* to treat rheumatic ailments. Screening *Drynaria quercifolia* for phytochemicals. Many Indian tribal cultures utilise this plant to cure a variety of illnesses, including cholera, typhoid fever, chronic jaundice, headaches, coughs, and skin conditions. Certain tribes in Tamil Nadu's Eastern Ghats are known to employ a soup made from the rhizomes of *Drynaria quercifolia* to treat rheumatic ailments. *Drynaria quercifolia's* phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, saponins, and other chemicals. Several *in vitro* and *in vi*vo models have demonstrated the antibacterial, analgesic, anti-inflammatory, and hepatoprotective properties of the rhizome of *Drynaria quercifolia*[11]. India's arid woodlands are home to the deciduous tree Delonix elata Linn. (family: Caesalpinaceae). The leaves, stem and flower extracts were employed by traditional healers in the villages of Karnataka, India, to treat rheumatic and bronchial issues, jaundice, and hepatic illnesses. Additionally, the plant has been linked to joint discomfort and stiffness, particularly in the knees. The leaves are used as a carminative or to cure paralysis, fever, malaria, bronchitis in newborns, and flatulence. Phytochemical analyses have identified a spectrum of compounds within *Delonix elata*, including flavonoids, alkaloids, quercetin, and catechins. The ethnomedicinal values of *Delonix elata* extend to its application in mitigating inflammatory conditions and combating microbial infections. Beyond traditional uses, scientific scrutiny has revealed its promising pharmacological potential. *In vitro* investigations have demonstrated noteworthy antioxidant and anti-inflammatory activities, signifying its potential as a therapeutic agent. In vivo studies have explored its effects on glucose metabolism, suggesting anti-diabetic properties[12]. The *Hygrophila auriculata* (K. Schum) as members of the Acanthaceae family are *Heine*, *Asteracantha longifolia* Nees, *B. longifolia* Linn*., H. schulli* (Ham.) MR and SM Almeida*, H. spinosa* T. Anders*,* and *Barleria auriculata* Schum*.* This upright, semiwoody shrub grows in India's damp regions. The plant or its components are recommended to be used as an aphrodisiac and to cure a variety of problems including edoema, gastrointestinal disorders, gallstones, kidney stones, calculi in the urine, rheumatoid diseases, leucorrhoea, Skin infections, TB, anaemia, physical pain, and constipation. According to reports, the plant contains the following: triterpenes (betulin, hentricontane, lupeol, and lupenone), minerals, fatty acids, aliphatic esters, amino acids, and essential oils; flavonoids, including quercetin, luteolin, ellagic acid, gallic acid, and apigenin; and sterols (stigmasterol and asterol). Plant extracts and bioactive compounds have been found to possess antimicrobial, anthelmintic, antitermite, nephroprotective, hepatoprotective, inhibitory, antitumor, antidiabetic, anticataract, antioxidant, hematopoietic, diuretic, anti-inflammatory, analgesic, antipyretic, antimotility, aphrodisiac, neuroprotective, anti-endotoxin, and antiurolithiatic activity[13]. These botanical species, characterised by theirs diverse phytochemical profile, stand as a subject of scientific interest, presenting avenues for further exploration in both traditional and modern medicinal contexts. The elucidation of their phytochemical constituents and the exploration of the biological activities contribute to the ongoing

discourse on their therapeutic potential. This study aims to study the synergistic effects of various combinations of these three plant extracts for antioxidant, anti-inflammatory and anti-bacterial activities.

EXPERIMENTAL

Different parts of the three different plants were utilised in this study. The seeds of *Hygrophila auriculata* were purchased from local markets in Chennai. The rhizomes and leaves of *Drynaria quercifolia* and *Delonix elata* respectively, were obtained from a traditional medicine shop in Coimbatore. The plant species were validated by comparing with online databases.

PREPARATION OF PLANT EXTRACT

The plant parts were rinsed in clean water and dried under shade and cool conditions to prevent from loss of volatile compounds. The dried samples were then pulverised into coarse powder form. Three conical flasks were labelled A, B, and C each representing the three plants *D. elata*, *H. auriculata*, and *D. quercifolia*, respectively. The powdered plant parts were soaked in 200 ml of 85% ethanol so that the ratio becomes 1:10. The samples were subjected to cold maceration with ethanol as a solvent for 3–5 days a under dark room. The ethanolic extracts were filtered using Whatman's filter paper twice to remove the insoluble matter from the extracted solvent. A rotary evaporator was utilised to concentrate the extracts under reduced pressure at a cooling temperature of 10°C and bath temperature. The samples were brought to a solid or semi-solid consistency. The extracts were scrapped out of the rotary flasks and weighed. A stock solution of concentration 1 mg/ml prepared with ethanol for all the three extracts and stored at 4°C. Two combination samples was then made with the ratio of A: B: C depicting 1:1:1 and 10:5:1. The combinations 1 and 2 were named D and F, respectively.

QUALITATIVE PHYTOCHEMICAL SCREENING

By using modified and standard protocols described by Roopashree et al., Obasi et al., Audu et al., and Nortjie et al.[14–17] the presence of the following secondary metabolite phytochemicals was examined in all the three whole plant extracts: alkaloids, flavonoids, tannins, saponin, glycosides, terpenoids, triterpenoids, phenols, glycosides, proteins, and carbohydrates.

DPPH RADICAL SCAVENGING ASSAY

The antioxidant activity of the three plant extracts were measured by DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay described by Kumara et al.[18] For analysis, a fresh stock solution of DPPH (0.2 mg/ml) was made. Stock solutions containing 0.1 mg/ml of L-ascorbic acid were made in ethanol and kept at 25°C. In different test tubes 1 ml the three plant extracts and the two ratios were taken each

in different concentration ranging from 200 to 1000 µg/ml. Prepared ascorbic acid standard (PC) was also taken in similar aliquots. To this, 1 ml of DPPH solution was added and the volume was made up to 3 ml with ethanol, and the flasks were given a good shake before being left at room temperature for half an hour without being exposed to light. Using a UV spectrophotometer, absorbance was measured at 520 nm right away with ethanol and DPPH as control (NC), and the experiment was carried out in triplicate. Using the log dosage inhibition curve, the standard's IC_{50} value (the concentration of the extracts required to inhibit only 50% of the DPPH free radical) was determined. Increased free radical activity was shown by the reaction mixture's lower absorbance. The percentage of inhibition for DPPH scavenging effect was determined as:

$$\text{DPPH scavenging effect (or) \% Inhibition} = (A_0 - A_1)/A_0 \times 100,$$

where A_0 is the absorbance of control and A_1 is the absorbance of the sample.

PHOSPHOMOLYBDATE REDUCTION ASSAY

The analysis for the samples' total antioxidant capacity was conducted using the phosphomolybdenum technique described by Umamaheswari & Chatterjee[19] with slight modifications. With 28 mM sodium phosphate, 4 mM ammonium molybdate, and 0.6 mM sulphuric acid, the phosphomolybdate reagent was newly prepared by combining them in equal amounts. One ml of reagent solution was shaken with a 0.1 ml aliquot of the sample solution. After being covered, the test tubes were incubated for ninety minutes at 95°C in a water bath. The absorbance was measured at 695 nm for all the samples. Ascorbic acid was used as standard. The antioxidant capacity was computed using the formula below:

$$\text{Total antioxidant capacity (\%)} = (A_1 - A_0)/A_1 \times 100.$$

ANTI-BACTERIAL ASSAY

Using the agar well diffusion technique, the plants extracts and their combinations were tested for antibacterial activity on Mueller Hinton Agar (MHA) plates. A final inoculum of 1.5 108 CFU/ml was obtained after the investigating organisms *E. coli* and *S. aureus* were inoculated in nutrient broth and cultured overnight at 37°C to bring the turbidity to 0.5 McFarland standards. Plant extracts were produced in Di-methyl Sulphoxide (DMSO) at a concentration of 50 mg/ml. The MHA medium was prepared and pH was adjusted to 7.3 using phosphate buffer. After sterilisation, they were brought under the laminar air flow chamber. The plates were divided into 2 sets (6 each) *E. coli* and *S. aureus*. Further they were labelled as A, B, C, D, and E and control. The agar medium was poured evenly into each of the petri plates and left to cool. Using standardised microbial culture broth of the *E. coli* and *S. aureus* MHA plate was inoculated on the respective Petri dishes by spread plate method. In each of the plates, three 6 mm wells were bored and a mixture of 50 µl extracts from various

plants was added to each well, with DMSO serving as the NC and amoxicillin (30 µg) serving as the PC for bacteria. After letting it diffuse for around half an hour at room temperature, it was incubated for eighteen to twenty-four hours at 37°C. After incubation, plates were examined to see whether the presence of a clean zone surrounding the well suggested the antibacterial activity of the compounds being tested. The zone of inhibition was measured and recorded in millimetres.

ANTI-INFLAMMATORY ACTIVITY BY PROTEIN DENATURATION ASSAY

A modified version of the BSA test described by Bailey-Shaw et al.[20] was used to assess the anti-inflammatory properties of the plant extracts and its combined ratios. BSA solution was made in phosphate buffered saline (0.4% w/v) and glacial acetic acid was used to bring the pH down to 6.4. Each plant extract was produced as stock solutions in ethanol at a concentration of 1 mg/ml. Test tubes containing 1 ml of 0.4%, w/v BSA buffer were filled with corresponding aliquots of 200 to 1000 µl which indicate concentrations of 200, 400, 600, 800 and 1000 µg/ml of the stock solutions. Similar protocols were followed for the assay of the PC with aspirin and NC with ethanol. After that, the solutions were heated for 10 minutes at 72°C in a water bath and then allowed to cool for 20 minin a lab setting. The turbidity of the solutions, or the level of protein precipitation, was measured at 660 nm in a spectrophotometer using an air blank. The average absorbance values were recorded after the investigations were conducted twice. The percentage inhibition of denaturation of protein with respect to the negative control was determined using the following formula:

$$\% \text{ inhibition of protein denaturation} = (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100.$$

RESULTS AND DISCUSSIONS

YIELD OF PLANT EXTRACTS

By using the cold maceration method with ethanol, the highest yield was obtained from *Delonix elata* extract of 5.26% while the lowest yield was 2.58% of *Drynaria quercifolia* (Table 1).

Table 1. Plant extract yield

Plant sample	Solvent used	Extract yield (%)
Delonix elata (A)	ethanol	5.26
Hygrophila auriculata (B)	ethanol	3.25
Drynaria quercifolia (C)	ethanol	2.58

PHYTOCHEMICAL SCREENING

The data in Table 2 show the observed phytochemical constituents' presence in the three plant extracts.

Table 2. Phytochemical profile of plant extracts

Phytochemicals	*Delonix elata*	*Hygrophila auriculata*	*Drynaria quercifolia*
Alkaloids	+	+	+
Flavonoids	++	++	++
Steroids	–	–	–
Terpenoids	++	+	+
Saponins	–	+	++
Tannins	+	+	+
Phenolics	++	+	+
Anthranol glycosides	+	+	–
Cardiac glycosides	+	+	–
Carbohydrates	–	–	–
Proteins	–	–	–

ANTIOXIDANT ACTIVITY

DPPH radical scavenging assay. DPPH scavenging activity was performed for all the five samples in six different concentrations. The percentage inhibition was calculated from the recorded absorbance value for these concentrations. Table 3 shows the inhibition percentages for the five samples. IC_{50} values for samples A, B, C, D, and E were obtained to be 214.54, 759.76, 194.28, 211.78, 214.54, respectively. Maximum inhibition percentage was obtained for sample D. Figure 1 represents the graphical representation of percentage inhibition of DPPH free radicals by all the five samples between the concentrations of 50 to 300 µg/ml.

Table 3. DPPH radical scavenging activity

S. No	Concentration (µg/ml)	% Inhibition				
		Delonix elata	*Hygrophila auriculata*	*Drynaria quercifolia*	Combination-1 (1:1:1)	Combination-2 (10:5:1)
1	50	44.46	66.42	25.28	59.96	46.13
2	100	53.69	73.99	31.37	65.87	50.55
3	150	62.92	76.38	42.44	72.69	62.55
4	200	68.63	80.63	56.46	81.55	69.74
5	250	74.72	86.16	66.61	85.42	78.6
6	300	81.73	88.75	73.06	91.14	84.87

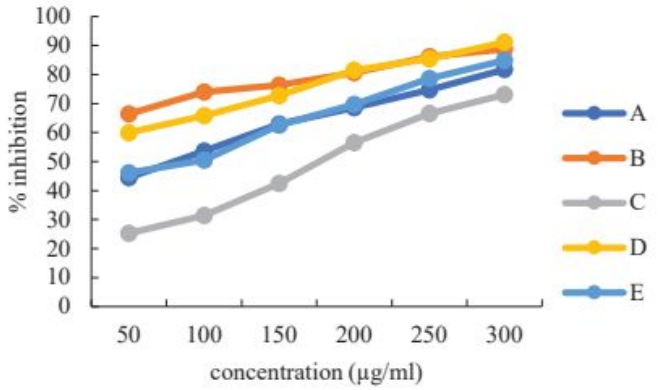

Fig. 1. Concentration versus percentage inhibition of DPPH assay

Phosphomolybdate assay. Table 4 shows the absorbance value and total antioxidant activity of all the plant extracts at same concentrations with phosphomolybdate assay. At concentration of 1 mg/ml, the maximum percentage reduction or total antioxidant activity was obtained for sample D, which was equal to 48.46% followed by sample E and then the others. This shows that the combination of the three extracts had made a significant effect on the antioxidant activity to get increased.

Table 4. Phosphomolybdate assay

Plant extracts	Concentration (mg/ml)	Absorbance	Total antioxidant activity (%)
A	1	0.446	31.38
B	1	0.398	38.77
C	1	0.641	1.38
D	1	0.335	48.46
E	1	0.367	43.54

ANTIBACTERIAL ASSAY

The antibacterial assay was performed for two different bacteria, gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus*. Table 5 depicts the zone of inhibition of plant extracts on the growth of the selected bacteria. For *E. coli*, the zone was higher in case of sample D whereas for *S. aureus*, sample C formed the largest zone out of other plant extracts. This shows that the combination could have both additive and subtractive effects. However, the positive control amoxicillin formed the maximum zone of inhibition for both the bacteria.

Table 5. Zone of inhibition for antibacterial assay

Sample	Concentration (mg/ml)	Zone of inhibition (mm)	
		E. coli	*S. aureus*
A	1	10±0.82	11.3±1.7
B	1	9.3±1.25	9.3±1.25
C	1	12.67±0.94	12.67±1.15
D	1	13±1.63	12±0.82
E	1	11.3±1.80	9.67±0.94
Amoxicillin (positive control)	1	16.3±1.70	24±0.82

In vitro ANTI-INFLAMMATORY ASSAY

The anti-inflammatory activity of the plant extracts and combinations were studied by their role in inhibition of protein denaturation. The inhibition percentages for various concentrations of plant samples are listed in Table 6. The IC_{50} values for samples A, B, C, D and E were found to be 583.49, 685.75, 403.01, 473.30, and 3000.24, respectively. Sample D has exhibited a maximum percentage inhibition of 97.37%. Again, this explains that there has been a synergistic effect of the combination over individual extracts. The graphical representation of concentration versus percentage inhibition of protein denaturation is shown in Fig. 2.

Table 6. Inhibition of BSA denaturation

S. No	Concentration (µg/ml)	% Inhibition				
		Delonix elata	*Hygrophila auriculata*	*Drynaria quercifolia*	Combination-1 (1:1:1)	Combination-2 (10:5:1)
1	200	17.37	36.00	1.26	49.79	6.74
2	400	42.74	49.37	7.37	72.00	42.00
3	600	62.00	84.74	31.05	89.68	56.84
4	800	76.11	89.79	75.16	94.11	75.79
5	1000	85.79	95.05	92.95	97.37	91.58

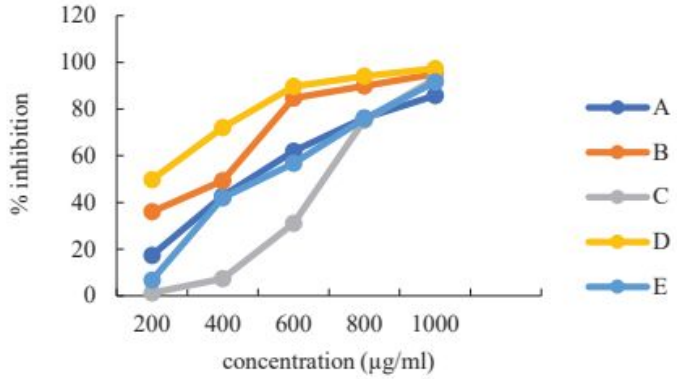

Fig. 2. Concentration versus percentage inhibition of protein denaturation

CONCLUSIONS

This research aims to provide safer alternatives to synthetic anti-inflammatory drugs by harnessing the properties of ethnomedicinal plant extracts from *Delonix elata*, *Hygrophila auriculata*, and *Drynaria quercifolia* using ethanolic extracts from leaves, seeds, and rhizomes respectively. Alkaloids, flavonoids, and phenolics (three essential bioactive compounds) were identified in these extracts based on preliminary phytochemical screening. Two different combinations of these extracts (labelled D and E) were made in predetermined ratios. These mixtures as well as individual extracts underwent extensive *in-vitro* studies to assess their anti-inflammatory, antibacterial, and antioxidant properties. The results showed that combination D had the highest overall antioxidant activity and DPPH radical scavenging. In antibacterial assays, combination D displayed significant inhibitory effects on gram-positive *E. coli*, while sample C demonstrated the largest zone of inhibition against gram-negative *S. aureus*. Importantly, in the assessment of anti-inflammatory activity using the protein denaturation assay, combination D exhibited exceptional potency with a remarkable percentage inhibition of 97.37% at a concentration of 1 mg/ml. The research findings highlight the potential of ethnomedicinal plant extracts to offer complementary therapies for inflammatory disorders, hence promoting the development of complementary and alternative medicine. It makes fresh research directions and creative therapies grounded on conventional medical knowledge possible.

ACKNOWLEDGEMENTS

The authors acknowledge the support provided by the management of Vels Institute of Science Technology and Advanced Studies (VISTAS), Chennai, Inda.

REFERENCES

1. A. CHOPRA, M. SALUJA, G. TILLU et al.: Ayurvedic Medicine Offers a Good Alternative to Glucosamine and Celecoxib in the Treatment of Symptomatic Knee Osteoarthritis: a Randomized, Double-blind, Controlled Equivalence Drug Trial. Rheumatology. **52** (8), 1408 (2013).
2. National Center for Complementary and Integrative Health (NCCIH): Complementary, Alternative, or Integrative Health: What's In a Name? [Internet]. [cited 2023 Feb 10].
3. G. SHOBHA, R. RAMADEVI, A. KHAR: Anti-inflammatory effect of the Indian Ayurvedic Herbal Formulation Triphala on Adjuvant-induced Arthritis in Mice. Phytother Res, **23** (12), 1678 (2009).
4. R. B. RAFFA, J. V. PERGOLIZZI Jr., R. TAYLOR Jr., M. H. OSSIPOV: Nature's First "Atypical Opioids": Kratom and Mitragynines. J Clin Pharm Ther, **43** (6), 874 (2018).
5. S. Y. PAN, S. F. ZHOU, S. H. GAO et al.: New Perspectives on How to Discover Drugs from Herbal Medicines: CAM's Outstanding Contribution to Modern Therapeutics. Evid Based Complement Alternat Med, **2013**, 627375 (2013).
6. J. PRIYADHARSHINI, K. KAVITHA, VIVEK PAZHAMALAI, S. IVO ROMAULD: Larvicidal Activity of *Mangifera indica* AND *Ricinus communis* against Mosquito Larvae. Oxid Commun, **45** (4), 694 (2022).
7. T. SAGITHA, S. IVO ROMAULD, P. BRINDHA DEVI et al.: Antibacterial, Antidiabetics and Larvicidal Activity of *Vitex negundo* and *Ocimum basilicum L.* extracts. Oxid Commun, **46** (1), 46 (2023).

8. S. IVO ROMAULD, BABU THANGAVEL, S. THIRUVENGADAM et al.: Antibaterial *in vitro* Antidiabetic and Larvicidal Activity of Plant Extracts. Oxid Commun, **46** (4), 851 (2023).
9. A. CHOPRA, P. LAVIN, B. PATWARDHAN, D. CHITRE: Randomized Double-blind Trial of an Ayurvedic Plant Derived Formulation for Treatment of Rheumatoid arthritis. J Rheumatol, **27** (10), 1365 (2000).
10. S. N. SHANKAR, T. A. DESHPANDE, N. A. RAMARAO, V. C. NAYAK, B. PATWARDHAN: A Multi-center Double-blind Randomized Controlled Trial of Guggulsterone in Osteoarthritis of the Knee. Phytomedicine, **18** (5), 343 (2011).
11. D. MODAK, S. PAUL, S. SARKAR, S. THAKUR, S. BHATTACHARJEE: Validating Potent Anti-inflammatory and Anti-rheumatoid Properties of *Drynaria quercifolia* Rhizome Methanolic Extract through *in vitro, in vivo, in silico* and GC-MS-based Profiling. BMC Complement Med Ther, **21** (1), 1 (2021).
12. P. KRISHNAPPA, K. VENKATARANGAIAH, S. K. SHIVAMOGGA RAJANNA, R. KASHI PRAKASH GUPTA: Antioxidant and Prophylactic Effects of *Delonix elata* L., Stem Bark Extracts, and Flavonoid Isolated Quercetin against Carbon Tetrachloride-induced Hepatotoxicity in Rats. BioMed Res Int, **2014**, (2014).
13. N. K. SETHIYA, N. M. AHMED, R. M. SHEKH et al.: Ethnomedicinal, Phytochemical and Pharmacological Updates on *Hygrophila auriculata* (Schum.) Hiene: an Overview. J Integr Med, **16** (5), 299 (2018).
14. T. S. ROOPASHREE, R. DANG, S. R. RANI, C. NARENDRA: Antibacterial Activity of Antipsoriatic Herbs: *Cassia tora*, *Momordica charantia* and *Calendula officinalis*. Int J Appl Res Nat Prod, **1** (3), 20 (2008).
15. L. OBASI NNAMDI, C. C. EGBUONU ANTHONY, O. UKOHA PIUS, M. EJIKEME PAUL: Comparative Phytochemical and Antimicrobial Screening of Some Solvent Extracts of *Samanea saman* (Fabaceae or Mimosaceae) Pods. African Journal of Pure and Applied Chemistry (AJPAC), **4** (9), 206 (2010).
16. S. A. AUDU, I. MOHAMMED, H. A. KAITA: Phytochemical Screening of the Leaves of *Lophira lanceolata* (Ochanaceae). Life Sci J, **4** (4), 75 (2007).
17. E. NORTJIE, M. BASITER, D. MOYO, P. NYAMUKAMBA: Extraction Methods, Quantitative and Qualitative Phytochemical Screening of Medicinal Plants for Antimicrobial Textiles: a Review. Plants, **11** (15), 2011 (2022).
18. P. KUMARA, K. SUNIL, B. ARUN KUMAR: Determination of DPPH Free Radical Scavenging Activity by RP-HPLC. Rapid Sensitive, (2018).
19. M. UMAMAHESWARI, T. K. CHATTERJEE: *In vitro* Antioxidant Activities of the Fractions of *Coccinia grandis* L. Leaf Extract. African Journal of Traditional, Complementary and Alternative Medicines (AJTCAM), **5** (1), 61 (2008).
20. Y. A. BAILEY-SHAW, L. A. WILLIAMS, C. E. GREEN, S. RODNEY, A. M. SMITH: *In-vitro* Evaluation of the Anti-inflammatory Potential of Selected Jamaican Plant Extracts Using the Bovine Serum Albumin Protein Denaturation Assay. Int J Pharm Sci Rev Res, **47** (1), 145 (2017).

Received 15 September 2024
Accepted 10 November 2024