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In vitro Antibacterial Activity of different crude leaves extracts of *Sterculia foetida* Linn

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ABSTRACT:

Sterculia foetida is a soft handsome woody tree with various pharmacological properties and they are most prevalently found in India, Thailand, Indonesia, Ghana, and Australia. The biochemically active compounds present in the plant possess good medicinal properties which have been already reported in several research papers. The present study was designed to screen the biochemically active compounds present in the leaves of *Sterculia foetida.* Qualitative analysis were performed using five different solvents (hexane, chloroform, methanol, ethyl acetate and aqueous) by using various standard protocol for each specific metabolite. The phytochemical screening revealed the presence and absence of fifteen secondary metabolites (carbohydrates, tannins, saponin, flavonoids, alkaloids, quinones, terpenoids, glycosides, triterpenoids, phenols, coumarins, proteins, cardiac glycosides, steroids, phytosterols) from all the five extracts in varying concentrations. Screening studies finally revealed the presence of high content of five secondary metabolites in methanol leaves extract when compared with other four solvent leaves extracts.

KEYWORDS: *Sterculia foetida Linn,* Leaves Extracts, Agar well diffusion method, Zone of inhibition, anti-bacterial activity.

INTRODUCTION:

Worldwide use of traditional medicine as an alternative therapy is gaining more popularity among the people.[1] The World Health Organization (WHO) declared that any part of plant constitutes could act as the precursor for the production of natural drug.[2,3] Approximately 80% of people in the world strongly believe on plant based natural drugs for their treatment than the modern synthetic drug due to awareness of its toxicity.[4-6] Moreover, scientists and researchers started working on identification of natural compounds from the traditional plants which bears various pharmacological properties.[7,8]

Even the pharmaceutical industry turned their attention towards manufacturing the natural products as drugs because of its higher efficiency and non-toxicity.[9,10]

The medicinal significance of plants lies in their phytochemicals like flavonoids, alkaloids, tannins, phenol, steroids and phenol compounds which exhibits better inhibitory activity towards infectious diseases rather than synthetic drugs.[11-17] The WHO reported that , severe pathogenic diseases are caused by especially bacteria and viruses.[18-19] At present, numerous researches are in progress; involving various medicinal plants, for the isolation of bioactive molecules which possess good antibacterial activity against various bacterial diseases.[20-22] The isolation of small molecules depends on the extraction of crude extracts of various parts of plants using different solvents.[23-29]

In the present study, the plant Sterculia foetida Linn, which has an important role from ancient times in ayurvedic traditional system of India has been examined. The raw leaves of the tree were reported for various medicinal properties like antioxidant[30], anticonvulsant[31], antidermatophytic[32], antiinflammatory[33] activities. Different parts of the plants such as bark, leaves, roots, flowers and seed have been used as herbal medicine without knowing it's pharmacological characteristics scientifically.[34-37] The generic name of the plant, 'Stercus' means 'manure'; species name foetida means 'stinking'; and the

tamil name is Pinnari.[38] In Tamil Nadu, the new leaves appears in branches after flowering during the month of March-April.[39] Hence in the current work, an attempt was made to identify its efficacy against few different bacterial organisms.

At present in India, a sudden increase in the emergence of bacterial disease among the population of poor socioeconomic level due to lack of public awareness is noticed. Though the Government on India is taking necessary steps either to control or eradicate the prevalence of these diseases, they are still a problem to overcome. Therefore, natural medicines which are very effective with less side effects and low cost may be considered for future treatment.[40-41]

The objective of the present investigation is to assess the potentiality of five different (hexane, methanol, chloroform, ethyl acetate and aqueous) leaves extract of Sterculia foetida Linn for antibacterial activity against five different bacterial strain (Escherichia coli, Salmonella typhi, Salmonella paratyphi, Micrococcus luteus, Bacillus subtilis)

MATERIALS AND METHODS:

Collection and Identification of leaves of *Sterculia foetida:*

The fresh and mature leaves of *Sterculia foetida* were collected in the month of April, 2014 from Pallavaram, Tamilnadu. The collected leaves were further identified and authenticated by the Department of Botany, Madras Christian College, Tambaram.

Processing of Plant leaves:

The leaves were collected and washed with the running tap water, dried under shades for two weeks. The dried leaves were blended into coarse powder using electric blender and later stored at room temperature.

Extraction of Plant Material by Soxhlet Apparatus

The 100 grams of powdered leaves were subjected to successive extraction with five different solvents- hexane, ethyl acetate, chloroform, methanol, and aqueous using soxhlet apparatus by continuous percolation process. The extracts were collected and dried under reduced pressure and temperature using rotary vacuum evaporator. The crude extracts were stored in refrigerator for the studies of antibacterial activity.

Test Organism:

The antibacterial activity was carried out for Gram negative bacteria - *Escherichia coli* (MTCC 443)*, Salmonella typhi* (MTCC 734)*, Salmonella paratyphi* (MTCC 735) and Gram positive bacteria - *Micrococcus luteus* (MTCC 1538), *Bacillus subtilis* (MTCC 441). The bacterial pathogens *E. coli, S. typhi, S. paratyphi, M. luteus* and *B. subtilis* were obtained from CAS in Botany, University of Madras, Chennai-25. The strains were maintained in nutrient agar slants at 4°C.

Screening for antibacterial activity:

The antibacterial activity of hexane, ethyl acetate, chloroform, methanol, and aqueous extracts of *Sterculia foetida* was performed using Agar well diffusion method. Using sterile cotton swab, the Mueller–Hinton agar plates were swabbed with the freshly prepared diluted culture. A 6mm hole was bored aseptically with a sterile cork borer. The well were filled with three different concentrations (25µl, 50µl, 100µl) of plant extracts and allowed to stand for 1 hr for the perfusion. The plates were incubated at 30°C for 24hrs. Dimethyl sulfoxide (DMSO) was used as a negative control and the antibiotics- Imipenem were used as positive control for the studies. The plates were observed for zone of inhibition of anti-bacterial activity.

Statistical Analysis:

All the analysis was conducted in triplicates. The statistical analysis of the data was presented as mean values ± standard deviation (SD).

RESULTS AND DISCUSSIONS:

The antibacterial activity of five different (Hexane, Chloroform, Methanol, Ethyl Acetate and Aqueous) leaf extracts of *Sterculia foetida* were determined using Agar well diffusion technique by measuring the diameter of the zone of inhibition.[42, 43] The negative control (DMSO) does not showed inhibitory activity against all bacterial strains. The results are displayed in table 1.

The Hexane leaf extracts showed the inhibitory activity as concentration dependent. The antibacterial activity of hexane extract was maximum of 16.4mm for *Micrococus luteus* followed by moderate inhibition for *B. subtilis*-14.3mm, *E. coli and S.paratyphi* - 10.2mm, *S. typhi*- 9.40mm.

The leaf extracts of chloroform solvent exhibited the concentration dependent activity. The best inhibitory activity was observed for *Micrococus luteus* (15.32mm), *S.typhi* (15.06mm), *B.subtilis* (14.33mm), *S.paratyphi* (13.36mm) and moderate activity was observed for *Escherichia coli* (8.33mm).

Table 1; Antibacterial activity of different leaf extracts of *Sterculia foetida* Linn

Organism	Hexane			Chloroform			Methanol			Positive control Imipenem
Conc. (µg/ml	25	50	100	25	50	100	25	50	100	
E. coli	8.06±	9.46±	10.26±	5.46±	6.2±	8.33±	8.2±	14.2±	18.53±	28.5±

	0.11	0.11	0.30	0.11	0.20	0.23	0.00	0.17	0.58	0.55
M. luteus	9.20± 0.19	14.40± 0.34	16.40± 0.52	11.93± 0.11	14.13± 0.11	15.32± 0.10	9.6± 0.0	14.86± 0.11	17.73± 0.23	22.9± 0.83
S.,typhi	7.20± 0.20	8.20± 0.2	9.40± 0.40	12.06± 0.11	13.53± 0.46	15.06± 0.11	11.3± 0.23	12.93± 0.11	18.20± 0.0	29.43± 0.15
S.paratyphi	8.20± 0.2	9.03± 0.05	10.26± 0.46	5.13± 0.11	11.26± 0.23	13.26± 0.46	12.26± 0.11	14.46± 0.46	16.20± 0.34	27.23± 0.32
B.subtilis	12.16± 0.2	13.26± 0.30	14.33± 0.30	12.13± 0.23	13.73± 0.11	14.33± 0.41	13.80± 0.0	14.83± 0.11	16.26± 0.46	25.06± 0.94

Table 1 Continued

Organism	**Ethyl acetate**			**Aqueous**			**Positive control Imipenem**
Conc. (µg/ml	**25**	**50**	**100**	**25**	**50**	**100**	
E. coli	8.06±0.11	9.13±0.11	10.33±0.11	7.40± 0.40	9.13±0.11	10.20±0.26	28.5±0.55
M. luteus	8.33±0.30	8.03±0.96	8.80± 0.72	2.80± 1.05	3.73±0.64	4.20±0.20	22.9±0.83
S.,typhi	7.20±0.20	8.26±0.23	8.66± 0.30	5.36±0.47	5.30±1.13	5.86±0.23	29.43± 0.15
S.paratyphi	1.46±1.28	4.46±0.80	6.06± 0.11	1.73±0.90	3.0±0.20	3.73±0.64	27.23± 0.32
B.subtilis	0.80±1.38	3.33±1.30	5.93± 0.30	1.46± 0.46	3.16±1.04	4.3± 1.26	25.06± 0.94

Figure -1; Antibacterial effect of Leaf extracts of *Sterculia foetida* Linn in different Concentration against Bacterial Strains

The crude methanol extracts of *Sterculia foetida* showed a significant antibacterial activity against all the five organism *Escherichia coli, S.typhi, S.paratyphi, Micrococus luteus* and *Bacillus subtilis* at the concentration 100µl. The zone of Inhibition was 18.53±0.58, 18.20±0.0, 16.20±0.34, 17.73±0.23, 16.26±0.46 respectively. While moderate degree of activity was showed at the concentration of 50 µl, 25µl of methanol leaf extract (Figur-1).

The Ethyl acetate leaf extracts showed maximum bacterial inhibitory activity on *Escherichia coli* (8mm, 9mm and 10mm) at all concentrations. The moderated activity was observed in *M.luteus* (8mm), *S-typhi* (7.2mm – 8.66mm) at all concentration. The poor zone of inhibition was observed in *S.paratyphi, B.subtilis* at all concentration of extract.

Among the five extracts, aqueous leaf extracts showed least zone of inhibition. *Escherichia coli* had 10.20mm zone of inhibition at 100µl concentration were considered to be moderate inhibitory activity. For the organism *S.typhi, S.paratyphi, Micrococus luteus* and *Bacillus subtilis* the zone of inhibition was 3.7mm - 5.8mm and is considered to be very poor for the aqueous extract.

The standard drug Imipenem showed high degree of inhibition against all the five organism *Escherichia coli, S.typhi, S.paratyphi, Micrococus luteus* and *Bacillus subtilis*.

On analyzing the above results of antibacterial activity, it was confirmed that methanol leaf extracts possess best antibacterial activity against all the five organisms when compared with other leaf extracts[44] and at the same time growth media is also responsible for antibacterial activity.[45] Therefore it was revealed that the compound present in the methanol leaf extract showed good inhibitory activity against the organism of *Escherichia coli, S.typhi, S.paratyphi, Micrococus luteus, Bacillus subtilis.* The antibacterial effect of crude methanol extract may be due to the presence of phytochemical like flavonoids, tannins, coumarins, saponin, trepenoids etc. The presence of each bioactive molecules exhibit different mechanism on the microorganism. The most effective bioactive components are present in flavonoid compounds which has the ability to form a complex with cell wall of bacteria and extracellular proteins.[46] Compounds under tannin family has the capacity to arrest the cell wall synthesis of bacteria.[47] The small molecules belonging to terpenoids group , have the ability to weaken the membrane tissue by terminating the cell wall of bacteria.[48] Coumarins have the ability to accumulate in the cell adjacent to infected cell.[49] Saponin compounds act as the inhibitor for the growth of bacteria.[50] Further best antibacterial compound can be identified from the above phytochemicals and can be further used for the treatment of bacterial diseases.

CONCLUSION:

Nowadays the use of traditional method as an alternative medicine has been increased. The researchers turn their attention towards the medicinal plants for treating various infections. The present study clearly proved that *Sterculia foetida* Linn showed better antibacterial activity in crude methanol extract. Further isolation, purification and identification procedure is going on for the identification of the particular bioactive compound which is responsible for the antibacterial activity. Later, the isolated bioactive natural compound may serve as leads for the development of new pharmaceuticals against bacterial diseases.

CONFLICT OF INTEREST:

The authors declare they have no competing interests.

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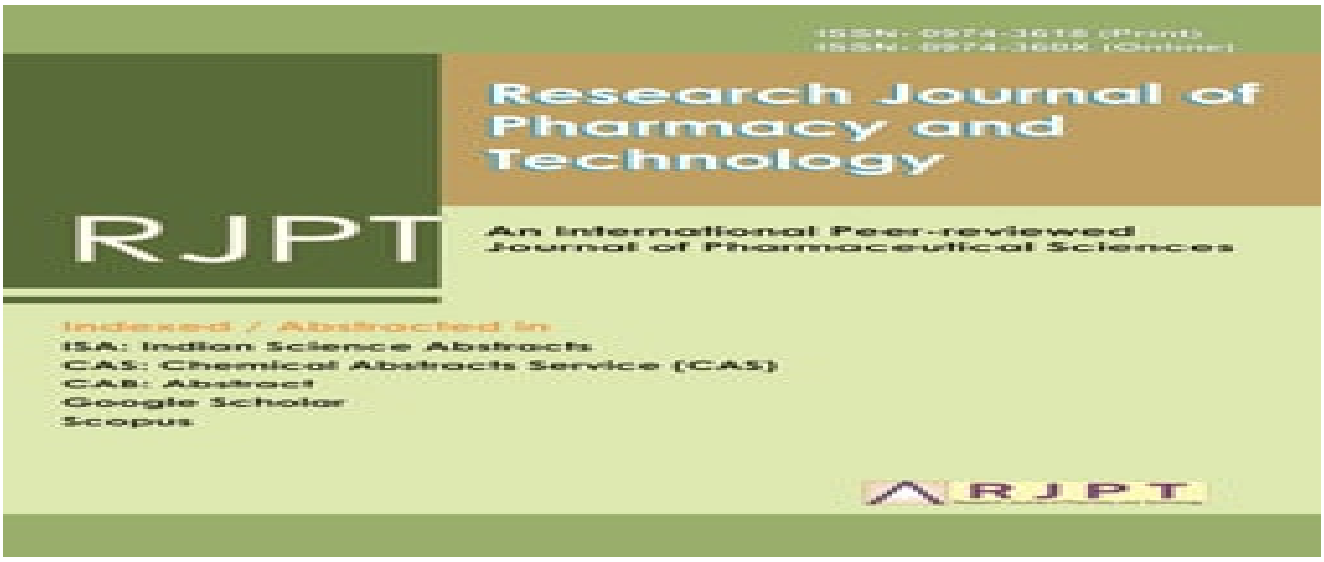

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