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Studies on the Phytochemistry and Bioactivity of Leaves of Trees in Chennai - I

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Abstract : In continuation of our previous studies, the bio-efficacy and the phytochemical constituents of leaves of 5 more common trees of Chennai was evaluated. Methanolic extracts of the leaves of 5 common trees in Chennai, i.e. *Ceiba pentandra* (Malvaceae), *Couroupita guianensis* (Lecythidaceae), *Lannea coromandelica* (Anacardiaceae), *Mimusops elengi* (Sapotaceae) and *Plumeria obtusa* (Apocynaceae) were studied for their bio activities. Antibacterial efficacy was studied through Micro-Broth Dilution method, antioxidant property using free radical scavenging assay with DPPH, larvicidal activity using the larvae of *Artemia salina* and pesticidal potential using the rice weevil, *Sitophilus oryzae.* The phytochemical studies revealed that saponin is present in all 5 tree leaves and phlobatannin is absent in all. The plant, *Lannea coromandelica* has showed significant activity against the bacteria studied. The plants *Ceiba pentandra* and *Mimusops elengi* have recorded EC_{50} value at lower concentration of 15 µg/mL. At 48 hours of exposure, 100 % mortality of the larvae, *Artemia salina* was observed only for *Lannea coromandelica.* The pesticidal activity revealed that the maximum mortality (100 %) was recorded at 24th hour treatment for all the leaf extracts of the plants studied. The study revealed that the leaves of different species exhibit different bio activities. The leaves of *Lannea coromandelica* has recorded better bactericidal and larvicidal activity. However, *Ceiba pentandra* and *Mimusops elengi* has recorded better antioxidant ability. Thus, proper scientific validation is needed to study the importance of individual plant in specific application. This results in identification of suitable plant material with certain biological properties.

Key Words: Trees, *Ceiba pentandra*, *Couroupita guianensis, Lannea coromandelica*, *Mimusops elengi*, *Plumeria obtusa*, Chennai.

Introduction

Plants are widely used treatment of diseases in all ancient system of medicine. Due to the chemical diversity and novelty found in plants [1] more than 80 % of the world's population relies on traditional medicine[2]. Herbal medicines are considered as time tested and safe[3]. Their effectiveness in cost, availability and

affordability provides more advantage in usage of herbal medicine among the population. Although wide knowledge on plants and their application in different diseases and the way of treatment were available with the tribal source or through the traditional knowledge with elders, the scientific validation on the same is missing. Thus, the study on validating the plants for their biological efficacy is needed. Plants found to differ according to their habit as herb, shrub and trees. Among them the trees produce larger biomass as leaves and in this study, the leaves of few common trees available in Chennai city of Tamil Nadu, India is evaluated for their bio-efficacy.

Previously the bioefficacy of the leaves of the following trees, *Mangifera indica, Phyllanthus acidus, Psidium guajava, Tectona grandis, Terminalia catappa*[3], *Alangium salvifolium*[4], *Annona squamosa, Citrus limon, Delonix regia, Millingtonia hortensis* and *Thespesia populnea*[5] were studied. In continuation of the studies, 5 more trees belonging to different families were selected and evaluated for their bioefficacy against bacteria, *Artemia salinia* and *Sitophilus oryzae.* Further, their phytochemical constituent and their antioxidant ability were evaluated.

Materials and Methods

Plant Source:

The leaves of the trees belonging to five different families, i.e. *Ceiba pentandra* (Malvaceae), *Couroupita guianensis* (Lecythidaceae), *Lannea coromandelica* (Anacardiaceae), *Mimusops elengi* (Sapotaceae) and *Plumeria obtusa* (Apocynaceae) were collected from Chennai city of Tamil Nadu State in India. The leaves collected were cleaned in tap water and shade-dried for 4 days. The dried leaves were pulverized into powder using electric blender and stored.

Preparation of plant extracts:

To 15g of each dried, pulverized sample of leaves of different trees, 150ml of Methanol was added to make up the ratio of 1:10 w/v and stirred in temperature controlled shaker at 30 ± 2^{o}C. The plant extracts were prepared using cold-percolation method. After 48 hours the extracts were filtered and concentrated using rotary evaporator.

Phytochemical analysis:

The dried, pulverized leaves (10g) were extracted with double distilled water (100ml) by boiling and filtered. The presence of cardiac glycosides, flavonoids phlobatannins, saponins, steroids, tannins and terpenoids were detected as stated [6-8].

Antibacterial Assay:

The methanol extracts of all 5 trees were screened against 5 bacterial strains. *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 1320), *Salmonella typhi* (MTCC 531), *Staphylococcus aureus* (MTCC - 96) and *Streptococcus epidermidis* (MTCC - 435) were procured from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. The stock cultures were maintained on slants of nutrient agar in 4˚C. Active cultures for screening their susceptibility were prepared by transferring loop full of cells from stock cultures to test tubes containing Mueller Hilton Broth which were incubated at 37˚C for 24 hours. Micro Broth dilution method was performed as stated by Tamilarasan [9].

The plant extract of 100 µg/mL as initial concentration was taken in the first well of 96 well titre plates. This was serially diluted which results in 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.125 µg/mL, 1.6 µg/mL and finally 0.8 µg/mL in series. The study was conducted using 96 well titre plates. Each well with respective concentration of plant extract was inoculated with 0.01mL of 24 hours bacterial cell suspension. This was incubated at 37^{o}C for 24 hours. The presence of turbidity of the broth indicates positive growth.

DPPH free radical scavenging assay:

The leaf extracts of all 5 trees were studied for their free radical scavenging assay using DPPH (2, 2 diphenyl-1-picryl hydrazyl) [10,11].

Percentage inhibition was calculated using the formula,

$$\text{Effective concentration \%} = ((\text{Control Absorbance} - \text{Test Absorbance}) / \text{Control Absorbance}) \times 100$$

The concentration that inhibits 50 % of DPPH activity is calculated as EC_{50} value and reported.

Brine Shrimp lethality assay:

The seeds of Brine Shrimp, *Artemia salina* were procured from Philadelphia, USA. The seeds were incubated in marine water for 48 hours for hatching in a small water tank provided with required aeration and light for 12 hours cycle. After 48 hours of hatching, the larvae at nauplii stage were removed and used for experiments. The nauplii were taken in different test tubes containing 10ml of sea water and 20 larvae. To this, extracts of leaves at different concentrations (2, 4, 6, 8 and 10 mg/ml) were added. After 24 hours and 48 hours, the viability of larvae was observed and mortality was recorded [12]. Nauplii were considered dead when they are immobile and stayed at the bottom of the test tubes. The percent mortality of brine shrimp is calculated as hereunder.

$$\text{\% Mortality} = (\text{No. of brine shrimp dead}/\text{No. of brine shrimp introduced}) \times 100$$

Pesticidal activity

The adult pests of *Sitophilus oryzae* were collected from naturally infested rice grains supplied through Public Distribution System of Chennai, Tamil Nadu. These insects were reared as stated [13,14]. One ml of the leaf extract constituting 10, 20, 30, 40 and 50 mg of the concentration of extract were poured in a dry clean Petri dish and allowed to dry. Then a plug of cotton was used to wipe the extract from the plate. The cotton plug was placed in a Petri dishe containing adult *Sitophilus oryzae* (20 numbers) along with one gram of rice and the plates were sealed. The death rate of the rice weevil was observed after 24 and 48 hours of incubation and reported as percent mortality [15].

$$\text{\% Mortality} = (\text{No. of weevil dead}/\text{No. of weevil introduced}) \times 100$$

Results

Phytochemistry:

The studies on the presence of phytochemicals, showed that saponins are present in all the 5 tree leaves studied and phlobatannin is absent in all five plants. Tannin was recorded in 4 tree samples except that of *Ceiba pentandra*. Similarly, flavonoids are not recorded in the leaf extract of *Couroupita guianensis*. The presence and absence of tannins, phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides and steroids of individual plant species are given in Table 1.

Table: 1 Presence of phytochemicals in leaves of different trees

Species	Ceiba pentandra	Couroupita guianensis	Lannea coromandelica	Mimusops elengi	Plumeria obtuse
Tannins	-	+	+	+	+
Phlobatannins	-	-	-	-	-
Saponins	+	+	+	+	+
Flavanoids	+	-	+	+	+
Terpenoids	+	-	+	-	+
Cardiac glycosides	-	+	+	-	+
Steroids	+	+	-	-	+

+ Positive, - Negative

Antibacterial efficacy:

Among the plants studied, *Lannea coromandelica* has showed better antibacterial activity when compared to all other plants. The plant extract has inhibited all the bacteria other than *Escherichia coli* at the concentration of 12.5 µg/mL. However, the growth of the bacteria, *Escherichia coli* was arrested by the plant extract of *Couroupita guianensis* at the concentration of 12.5 µg/ml. The details on minimum inhibitory concentration recorded for individual leaf extract of the plants is provided in Table 2.

Table 2. Minimum Inhibitory Concentration (MIC) (In µg/mL) recorded against the bacteria studied for different tree leaves

Species	Ceiba pentandra	Couroupita guianensis	Lannea coromandelica	Mimusops elengi	Plumeria obtusa
Bacillus subtilis	12.5	50	12.5	25	50
Staphylococcus epidermidis	25	25	12.5	25	25
Staphylococcus aureus	25	25	12.5	50	12.5
Escherichia coli	25	12.5	25	50	25
Salmonella typhi	50	25	12.5	12.5	25
Klebsiella pneumoniae	25	25	12.5	25	50

Anti-oxidant ability:

The plants *Ceiba pentandra* and *Mimusops elengi* have recorded their EC_{50} values at the lower concentration of 15 µg/mL. *Lannea coromandelica* and *Plumeria obtusa* have recorded their EC_{50} values at 25 and 35 µg/mL. The percent inhibition recorded against DPPH by different leaf extracts of the trees are presented in Table 3.

Table 3: Percent Inhibition recorded against DPPH using leaf extract of different trees

Species	10	20	30	40	50	EC_{50} µg/mL
Ceiba pentandra	44.4	66.6	88.8	94.44	100	15
Couroupita guianensis	33.3	55.5	66.6	88.8	100	18
Lannea coromandelica	22.2	33.3	66.6	77.7	88.8	25
Mimusops elengi	44.4	66.6	77.7	88.8	100	15
Plumeria obtusa	18.3	22.2	33.3	66.6	88.8	35

Larvicidal activity:

The larvicidal potency of plant studied showed that 100 % mortality was recorded only for *Lannea coromandelica* even at the lowest concentration. All other plants have not recorded 100 % mortality even at the higher concentration of 10 mg/ml. The % mortality of the larvae against the leaf extracts of the trees are presented in Table 4.

Table 4: Larvicidal activity of leaf extracts on *Artemia salina* (In %)

Species (mg/mL)	2	4	6	8	10
Ceiba pentandra	40	60	70	70	80
Couroupita guianensis	10	30	60	70	80
Lannea coromandelica	100	100	100	100	100
Mimusops elengi	10	30	60	70	90
Plumeria obtusa	10	40	60	60	60

Pesticidal activity:

Hundred percent mortality of *Sitophilus oryzae* was recorded for the leaf extracts of all the plants studied at the lower concentration of 10mg/mL.

Discussion

The phytochemical analysis of the tree species showed the absence of phlobatannin in any of the tree species. This was similar to our previous studies [3,5], where phlobatannin was not recorded from any of the tree species studied. The results on phytochemical constituents help to identify the plant possessing bioactive agents like tannins, saponins, and flavonoids which are antimicrobial in nature [16]. The study also identified *Lannea coromandelica* as a better antimicrobial agent. The plant can be exploited for its antimicrobial property when compared to other tree species studied. The protective effect of *Lannea coromandelica* against the bacteria and fungi is already reported [17]. Similarly, the study also identifies *Ceiba pentandra* and *Mimusops elengi* as better

antioxidant agent. Mostly the seeds of *Ceiba pentandra* were evaluated for their antioxidant ability when compared to leaves. Present study provides knowledge on the leaves of the plant as better antioxidant agent. The ethanolic extract of the leaves of *Mimusops elengi* was found to report IC_{50} value at higher concentration [18]. The larvicidal potency was found to be poor with most of the species studied other than *Lannea coromandelica.* However, pesticidal property is concerned all the tree species exhibited pesticidal activity at low concentration which can be harboured for removal of storage pests in granaries.

Conclusion

The studies on phytochemistry and different bio-efficacy of five different trees prevalent in Chennai city revealed that the plants exhibit different characteristics against antibacterial activity, antioxidant activity, larvicidal activity and pesticidal activity. The leaves of *Lannea coromandelica* has recorded better bactericidal and larvicidal activity. However, *Ceiba pentandra* and *Mimusops elengi* has recorded better antioxidant ability. This may be attributed to different secondary metabolites reported from the plants belonging to different families. Thus, proper scientific validation is needed to study the importance of individual plant in specific application which results in identification of suitable plant material with certain biological properties.

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