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Research Article

STUDIES ON THE PHYTOCHEMISTRY AND BIOACTIVITY OF LEAVES OF FEW COMMON TREES IN CHENNAI, TAMIL NADU, INDIA

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ABSTRACT

Objective: To know the importance of tree leaves wasted as litter in larger biomass, the study was conducted to evaluate their bio efficacy and phytochemistry.

Methods: Leaves of 5 common trees in Chennai, i.e. *Mangifera indica, Phyllanthus acidus, Psidium guajava, Tectona grandis* and *Terminalia catappa* belonging to different families were selected. The methanol extract of the leaves were studied for their bioactivities like, antibacterial efficacy through Disc diffusion method, antioxidant property using DPPH, larvicidal activity using *Artemia salina* and pesticidal potential using *Sitophilus oryzae* along with their phytochemistry.

Results: The phytochemical studies revealed that Steroids is present in all 5 tree leaves and phlobatannin is absent in all. The plant, *Psidium guajava* has showed maximum activity against the bacteria, *Escherichia coli* followed by *Proteus mirabilis* and *Bacillus subtilis.* None of the plants showed 100 % Inhibition against DPPH even at high concentration studied. The EC_{50} value recorded for *Phyllanthus acidus* was 61µg/ml and for *Terminalia catappa* it was around 68µg/ml. At 48 hours of exposure 100 % mortality of the larvae, *Artemia salina* was observed for all plants. The pesticidal activity revealed that the maximum mortality (100 %) was recorded at the 24th hour treatment for the plant, *Phyllanthus acidus*.

Conclusion: The study revealed that the leaves of different species exhibit different bio activities and thus, proper scientific study and selection is needed to identify the plants for specific application along with certain biological properties.

Keywords: Common trees, *Phyllanthus acidus*, Bio efficacy, Phytochemistry, Chennai

INTRODUCTION

Plants are the nature's "chemical factories" providing rich source of organic chemicals on earth [1]. Plants are extensively used in the management and treatment of diseases and ailments since pre-historic age. More recently it has been found that many plants have medicinal values [2]. According to World Health Organization (WHO), more than 80 % of the world's population relies on traditional medicine for primary healthcare needs and the reason is the broader degree of chemical diversity and novelty found than any other source [3]. Hence research on plant products is rapidly rising to meet the growing demand for complementary and alternative medicine.

Natural products from higher plants may possess bioactive compounds with novel mechanism of action. They may be effective in the treatment of infections and diseases while the side-effects are minimal. Therefore, it is of great importance to carry out the screening of these plants to validate their use for medicinal purposes [4]. More so herbal medicines have received much attention as sources of lead compounds since they are considered as time tested and safe for both human use and environment friendly. They are also cost effective, easily available and affordable. Remedies through plants are used since ancient times even if the mechanisms of action, toxicity and efficacy of very few of them have been evaluated scientifically. Therefore there is the need to look inwards to search for plants with the aim of validating the ethno-medicinal use and subsequently the isolation and characterization of compounds which will be added to the potential list of drugs [5].

Tree is defined as a woody perennial plant, typically having a single stem or trunk growing to a considerable height and bearing lateral branches at some distance from the ground. All trees have trunks with which it supports the spreading branches above and protected by a covering or bark, which is essential for the well-being of the tree [6]. Trees are found to produce leaves of larger biomass when compared to shrubs or herbs. Arboreal leaves constitute a large percent of the biomass that are wasted as litter. To know the bioactivity, i.e. defined as the given agent's interaction with or effect on the living organism or tissue, the present study is conducted. Leaves of 5 common trees in Chennai, i.e. *Mangifera indica, Phyllanthus acidus, Psidium guajava, Tectona grandis* and *Terminalia catappa* belonging to five different families were selected and evaluated for their bioactivity.

MATERIALS AND METHODS

Plant Source

The leaves of the trees belonging to five different families, i.e. *Mangifera indica* (Anacardiaceae), *Phyllanthus acidus* (Phyllanthaceae), *Psidium guajava* (Myrtaceae), *Tectona grandis* (Verbenaceae) and *Terminalia catappa* (Combretaceae) were collected from Chennai city of Tamil Nadu State in India. The leaves were chosen in a way that they are not infected or damaged and are healthy. The healthy leaves of the collected trees were cleaned thoroughly in running tap water and shade-dried for 4 days. The dried leaves were made into powder using electric blender and stored.

Preparation of plant extracts

The plant extracts were prepared using cold-percolation method. To 15g of each dried pulverized sample 150ml of Methanol was added and stirred in temperature-controlled shaker at 30 ± 2^{o}C. After 48 hours the extracts were filtered and concentrated using rotary evaporator. These extracts were used for evaluating antibacterial, larvicidal, pesticidal and anti-oxidant properties of trees.

Phytochemical analysis

The dried pulverized plant materials (10g) were extracted with double distilled water (100ml) by boiling. The aqueous extracts were filtered using Whatman No.1 filter paper and the qualitative phytochemical analysis for the presence of cardiac glycosides, flavonoids phlobatannins, saponins, steroids, tannins and terpenoids was carried out immediately without storage according to standard procedures [7-9].

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) and *Proteus mirabilis* (MTCC 425) procured from Microbial Type Culture Collection and Gene Bank, Chandigarh, India.

The stock cultures were maintained on slants of nutrient agar at 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 and were incubated at 37˚C for 24 hours. Disc diffusion method was used to screen the antibacterial activity according to Udayaprakash *et al* [10]. For assay, Mueller Hinton Agar (Beef Extract - 2g; Acid Hydrolysate of Casein - 17.5g; Starch - 1.5g; Agar - 17g. was used and Final pH was maintained at 7.3 ± 0.1) (MHA) was used. Onto the sterile MHA plates 0.1mL of the saline suspension was swabbed uniformly. Different concentrations of the extract (7.5mg, 10mg and 12.5mg/disc) were loaded prior a day on 5 mm sterile discs. These discs along with the control disc Streptomycin was placed on the bacteria inoculated plates. The plates were incubated at 37˚C for 24 hours. After incubation period, the diameter of inhibition zones formed around the discs was measured in millimeter. These studies were performed in duplicates for all the bacterial samples.

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) according to Melo *et al* [11]. Half ml of extracts prepared at various concentrations (15, 7.5, 3.75, 1.87, 0.93 µg/mL) in methanol was taken in small tubes and 0.5 ml of DPPH was added. The same solution of DPPH in methanol was used as control, whereas BHA was used as reference. After 30 minutes of incubation in dark at room temperature, the absorbance of the solution was read at 517 nm. The radical scavenging activity was calculated using the following formula

Percentage inhibition was calculated using the formula,

Effective concentration % = [(Control absorbance - Test absorbance)/Control absorbance] x 100

The Effective concentration (EC_{50}) values were calculated with these different concentrations of the leaf extracts. The EC_{50} value was defined as the concentration in µg of dry material per ml (µg/ml) that inhibits the formation of DPPH radicals by 50 Percentage [12].

Brine Shrimp lethality assay

The *Artemia salina* seeds were procured from Philadelphia, USA. The seeds were incubated in marine water for 48 hours for hatching in a small water tank. Aeration was provided with an aerator pump. Required light is provided with Philips 40 Watts lamp for 12 hours cycle. After 48 hours, the larvae were removed and used for the experiments. The hatched seeds were used at the nauplii stage. The nauplii of *Artemia salina* were taken in different test tubes containing 10ml of sea water and 20 larvae. To this, extracts of leaves at different concentrations (50, 20, 10, 5 mg/ml) were added. After 24 hours and 48 hours, the viability of larvae was observed and mortality was recorded [13]. 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.

% Mortality= (No of brine shrimp dead/ No of brine shrimp introduced) x 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 on clean and uninfected rice grains in the laboratory. Nearly 600 adult pests were reared in plastic containers provided with fresh rice grains, covered with muslin cloth to allow sufficient ventilation. After 48 hours the pests were used for the experiment. One ml of the leaf extract constituting 50 mg of the extract concentration was poured into a dry clean Petri plate and allowed to dry. This plate was exposed to air and the available methanol in the extract was allowed to vaporize. Then a plug of cotton was used to wipe the extract from the plate. The cotton plug was placed in a Petri plate 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 as follows

% Mortality= (No of weevil dead/ No of weevil introduced) x 100

RESULTS

Phytochemistry

The studies on the presence of phytochemicals, showed that Steroids are present in all the 5 tree leaves studied and phlobatannin is completely absent in all five plants. Tannin was recorded in all 4 tree samples and was not recorded from the leaves of *Phyllanthus acidus.* The plant, *Psidium guajava* has recorded the presence of Saponins, Flavonoids and Terpenoids along with that of Steroids and Tannins. *Mangifera indica* has recorded the presence of Flavonoids, Terpenoids and Gardiac glycosides along with that of Steroids and Tannins. The presence and absence of cardiac glycosides, flavonoids phlobatannins, saponins, steroids, tannins and terpenoids of individual plant species are given in Table 1.

Table 1: Presence of phytochemicals in leaves of different trees

Sample	Tannins	Phloba tannins	Saponins	Flavanoids	Terpenoids	Cardiac Glycosides	Steroids
Terminalia catappa	+	-	+	-	-	-	+
Psidium guajava	+	-	+	+	+	-	+
Phyllanthus acidus	-	-	-	-	-	-	+
Mangifera indica	+	-	-	+	+	+	+
Tectona grandis	+	-	-	-	+	+	+

+ Positive, - Negative

Antibacterial efficacy

The antibacterial efficacy of *Phyllanthus acidus* and *Tectona grandis* against the bacteria studied was almost negligible. The plant, *Psidium guajava* has showed maximum activity against the bacteria, *Escherichia coli* followed by *Proteus mirabilis* and *Bacillus subtilis.* The plant, *Mangifera indica* has demonstrated its maximum antibacterial efficacy against, *Escherichia coli* however; it has not recorded any zone of inhibition against the pathogenic bacteria, *Salmonella typhi. Terminalia catapa* is the only tree species which showed maximum zone of inhibition against the bacteria, *Salmonella typhi.* Similarly, *Klebsiella pneumonia* showed zone of inhibition at the maximum against the leaf extract of *Mangifera indica.* The details of the zone of inhibition recorded for different bacteria against the extracts of leaves of different tree species is presented in Table 2.

Anti-oxidant ability

None of the plants showed 100 % Inhibition against DPPH even at high concentration studied. The plant, *Phyllanthus acidus* alone recorded nearly 97 % of inhibition at the concentration of 250µg/ml. Other than this, *Terminalia catappa* and *Tectona grandis* has recorded more than 80 % inhibition. The EC_{50} value recorded for *Phyllanthus acidus* was 61µg/ml and for *Terminalia catappa* it was around 68µg/ml. However, the positive control, i.e. BHA has showed the EC_{50} value at 25.78µg/ml. The results of the percent inhibition of DPPH by different plants are tabulated in Table 3.

Larvicidal activity

The larvicidal potency of plant studied showed that the following plants, *Phyllanthus acidus, Tectona grandis* and *Psidium guajava* has

recorded 100 % mortality of the larvae, *Artemia salina* at 24 hrs time interval at the lowest concentration (5mg/ml) studied. The plant *Mangifera indica* has recorded 70 % mortality rate of the larvae at 24 hrs time interval and it recorded 100 % mortality at 48 hrs at the lower concentration. The plant *Terminalia catappa* has recorded 30 %, 60 % and 70 % of mortality at 24 hours exposure at the concentration of 5mg/ml, 10mg/ml and 25mg/ml respectively. However at 48 hours of exposure 100 % mortality of the larvae was observed for the plant at lower level of concentration. The % mortality of the larvae against the leaf extracts of the trees are presented in Table 4.

Table 2: Zone of inhibition recorded (in mm) for leaves of different Trees

Bacteria	*Bacillus subtilis*			*Escherichia coli*			*Salmonella typhi*			*Klebsiella pneumoniae*			*Proteus mirabilis*		
0.1	**7.5**	**10**	**12.5**	**7.5**	**10**	**12.5**	**7.5**	**10**	**12.5**	**7.5**	**10**	**12.5**	**7.5**	**10**	**12.5**
P. guajava	10	10	10.5	12	13	13	-	10	10	9	9	9	10	10.5	10.5
P. acidus	7.5	8	8	6	8	8	7	7	7	-	-	8	6	7	7.5
M. indica	8	10	11	10	11	13	-	-	-	10	10	10.5	10	10.5	11
T. grandis	7	7	8	6.5	7	7.5	6.5	6.5	7.5	-	-	6.5	7.5	8	8
T. catappa	8.5	9	10	10	11	12	10	11	12	7	8	9	8	9	10

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

Conc. µg/ml	Percent Inhibition against DPPH					
	Terminalia catappa	*Psidium guajava*	*Phyllanthus acidus*	*Mangifera indica*	*Tectona grandis*	BHA (control)
15.62	-	-	-	-	-	35.294
31.25	-	-	-	-	-	64.706
62.5	45.45	-	51.51	-	15.15	82.353
125	57.57	18.18	75.75	42.42	78.78	88.235
250	87.8	63.63	96.96	60.6	81.81	94.118
EC_{50} Values	68.75	196.44	60.66	206.27	79.33	25.78

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

Species	5mg/ml		10mg/ml		20mg/ml		50mg/ml	
	24 Hrs	**48 Hrs**	**24 Hrs**	**48 Hrs**	**24 Hrs**	**48 Hrs**	**24 Hrs**	**48 Hrs**
Mangifera indica	70	100	100	100	100	100	100	100
Phyllanthus acidus	100	100	100	100	100	100	100	100
Tectona grandis	100	100	100	100	100	100	100	100
Psidium guajava	100	100	100	100	100	100	100	100
Terminalia catappa	30	100	60	100	70	100	100	100

Pesticidal activity

The study revealed that the maximum mortality (100 %) was recorded at the 24th hour treatment for the plant, *Phyllanthus acidus*. *Psidium guajava* has revealed 80% mortality at the end of 24th hour treatment. The other leaf extracts *Terminalia catappa* has recorded 20% mortality and *Mangifera indica* recorded 15% mortality at the 24th hour treatment against rice weevil, *Sitophilus oryzae*. *Tectona grandis* showed no activity at all against the weevil at the 24th hour treatment. At the end of 48 hours of treatment, increase in the mortality was observed for the leaf extracts. The increase in the mortality rate of rice weevil was recorded for *Psidium guajava* from 80 to 95 %, 20 to 35 % for *Terminalia catappa* and 15 to 20 % for *Mangifera indica. Tectona grandis* recorded 20 % mortality at the 48th hour treatment against rice weevil, *Sitophilus oryzae*. The mortality rate recorded for *Sitophilus oryzae* against the leaf extracts of the trees are presented in Table 5.

Table 5: Effect of the leaf extracts on adult mortality (in %) of *Sitophilus oryzae*.

Sample (50 mg)	After 24 hrs	After 48 hrs
Mangifera indica	15	20
Phyllanthus acidus	100	100
Tectona grandis	0	20
Psidium guajava	80	95
Terminalia catappa	20	35

DISCUSSION

Isolation of pure and pharmacologically active constituents from plants remains a tedious process and hence chemical screening was performed to allow localization of useful constituents with potential activities. The results obtained revealed that the plants contained bioactive agents which are of antimicrobial nature which includes saponnins, flavanoids and tannins [14]. The present study revealed that Steroids are recorded in all the 5 tree species and Phlobatannin were not present on the same. It was expected the presence of flavanoids from the leaves of *Phyllanthus acidus* however, the same is not recorded.

The antimicrobial activities of the plant species studied, showed that they are not possessing strong activity according to Harper and Cawston [15] except that of *Psidium guajava* to the bacteria, *Escherichia coli.* The plants, *Psidium guajava, Mangifera indica* and *Terminalia catappa* has showed weak activity and the activities shown by *Phyllanthus acidus* and *Tectoca grandis* are of no significant value. Flavonoids are regarded as one of the widespread groups of natural constituents that show antioxidant property through scavenging or chelating process [12]. Flavanoids and Steroids are known to be synthesized by plants in response to microbial infections [4]. Tannins are known to possess general antimicrobial and antioxidant properties [16].

The antioxidant studies showed that *Phyllanthus acidus* has shown EC_{50} value at lower concentration (60.6µg/ml) when compared to other plants. The anti-oxidant property of the fruits of the plant *Phyllanthus acidus* was already reported [17]. Similarly, 58.9µg/ml was recorded for the methanol extract of the leaves of *Phyllanthus acidus* leaves from Tripura, India [18]. The overview on the utilization of Cicca acida (*Phyllanthus acidus*) in various areas of biological importance was reviewed by Saraju and Paul [19].

Brine shrimp nauplii have been used in various bioassays and a number of novel antitumor and pesticidal natural products have

been isolated. *Artemia salina* nauplii were used as a reference organism mainly due to their survival rate for several days without food [20]. Present study revealed that the larvae showed mortality at even lower concentration of the plant extracts. Presently, plants are more favorably exploited as biological control agents to protect stored grain products from insect pests, including rice weevils [21]. The study of Pesticidal activity revealed that maximum mortality was recorded at 24th hour in *Phyllanthus acidus* and at 24th and 48th hour for *Psidium guajava.* Other plant extracts showed moderate mortality rate. The pesticidal nature of the plants is due to the presence of monoterpenoids which are highly volatile and they have fumigant activity that might be of importance in controlling pests. Reported biological activities of plant terpenoids include repellence and deterrence, reduced palatability, growth inhibition, protein availability, enzyme inhibition and direct toxicity [22]. The leaves of the plants studied possess certain bioactive components which are of pesticidal nature. Plants species with high amount of flavonoids and alkaloids are reported as having interesting anti *Artemia salina* property [23].

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. This is also due to the presence of different types of secondary metabolites reported from the plants belonging to different families. Thus, proper scientific study and selection is needed to identify the plants for specific application along with certain biological properties.

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