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Review Article

Studies on bio activity and phytochemistry of leaves of common trees

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ABSTRACT

Plants are well known for their medical importance. Among the plant habits, trees produce large biomass of leaves and are found to be wasted. In this study, the methanolic extracts of leaves of few trees were evaluated for their bactericidal, free radical scavenging ability and insecticidal activity. Further, their phytochemical constituents were studied. Leaves of *Annona squamosa, Citrus limon, Delonix regia, Millingtonia hortensis* and *Thespesia populnea* belonging to different families were selected. The crude extracts were studied for antibacterial efficacy through Micro broth dilution, free radical scavenging ability using DPPH, and their insecticidal activity by using the larvae of *Artemia salina* and insect pest of *Sitophilus oryzae.* Further, the aqueous extract of the leaves were screened for phytoconstituents. The phytochemical studies revealed that Saponins and Steroids are present in all 5 tree leaves. The plant, *Milingtonia hortensis* showed wider antibacterial spectrum. *Citrus limon* recorded better free radical scavenging activity followed by *Annona squamosa* and *Thespesia populnea. Annona squamosa* showed better insecticidal activity with both the larvae of *Artemia salina* and against the rice weevil *Sitophilus oryzae.*

Keywords: Bactericide; Free radical scavenging; Insecticidal; Phytochemistry; Tree Leaves

INTRODUCTION

Plants are well known for their medical importance. Rajasekaran (2002) estimated that over 6000 plants are in use in traditional, folk and herbal medicine in India representing around 75 % of medicinal need of third world countries. Screening medical potency of plants is recommended by Shihabudeen et al, (2010) as plants are found to possess natural chemicals with novel mechanism of action and effective treatment. Traditional medical system in different countries like China, Japan, Korea, Indonesia and India are widely using plants as their medical source. Plants are classified into herbs, shrubs and trees based on their habit. Among these, trees produce larger biomass as leaves. If these leaves are found to possess effective bioacti vity, trees are more preferable when compared with herbs and shrubs as they posses higher biomass of leaves. With that as an intention, studies on Bactericidal, free radical scavenging ability and insecticidal activity of few trees was conducted.

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Tree is defined as a perennial plant, having single woody stem or trunk growing to a considerable height and bearing lateral branches at some distance from the ground. Trunks support the spreading branches above which are protected by a covering or bark (Mukherjee, 1983). They are found to possess leaves in large number with greater biomass and these constitute a large percent that are wasted as litter (Udayaprakash et al, 2013a). Hence, usage of these leaves of trees as an alternate to that of herbs or shrubs will be a greater advantage in sourcing them. In this study, the leaves of few common trees in Tiruvallur district of the state of Tamil Nadu in India were studied for their biological importance. Leaves of 5 different trees i.e. *Annona squamosa, Citrus limon, Delonix regia, Millingtonia hortensis* and *Thespesia populnea* belonging to different families were selected and studied.

MATERIALS AND METHODS

Plant Source

The leaves of the trees belonging to five different families, i.e. *Annona squamosa* (Annonaceae)*, Citrus limon* (Rutaceae)*, Delonix regia* (Fabaceae)*, Millingtonia hortensis* (Bignoniaceae) and *Thespesia populnea* (Malvaceae) were collected from Tiruvallur District of State of Tamil Nadu in India. The collected plant materials were identified using the manuals of Gamble (1915) and Mathew (1983). The specimen of plant herbariums are

deposited with the Research and Development, Veltech Dr. RR Dr. SR Technical University, Chennai.

The leaves of the plants 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 25g of each dried pulverized sample 250ml of Methanol was added and stirred in temperature-controlled shaker at 30 ± 2°C. After 48 hours, the extracts were filtered and concentrated using rotary evaporator and stored for further usage. These extracts were reconstituted for evaluating antibacterial, free radical scavenging activity and insecticidal properties when and where required.

Phytochemical analysis

The dried pulverized plant materials (5g) 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 methods (Evans (1994); Udayaprakash et al, (2013b)).

Antibacterial assay – Determination of Minimum Inhibitory Concentration (MIC)

The antibacterial potency of extracts of leaves of common trees were screened against 6 bacterial strains, i.e. *Bacillus subtilis* (MTCC 121)*, Escherichia coli* (MTCC 443)*, Klebsiella pneumoniae* (MTCC 1320)*, Pseudomonas aeruginosa* (MTCC 424)*, Staphylococcus aureus* (MTCC 96) and *Streptococcus epidermidis* (MTCC 435) procured from Microbial Type Culture Collection and Gene Bank, Chandigarh, India.

The initial concentration of the plant extract at 100mg/ml was used and this was serially diluted (Sule and Agbabiaka, 2008) by transferring 5ml of the sterile plant extract into 5ml of sterile Nutrient broth to obtain 50mg/ml concentration. This was repeated to obtain the dilutions of 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml and final ly 1.6 mg/ml (Udayaprakash et al, 2013c). This was compared with Streptomycin as control. Each concentration was inoculated with 0.1ml of 24 hours bacterial cell suspension and incubated at 37°C for 24 hours. The growth is indicated by cloudiness or turbidity of the broth. The lowest concentration of plant dilution which inhibits the bacterial growth was taken as Minimum Inhibitory Concentration (MIC).

Free radical scavenging assay

The extracts obtained from tree leaves were studied for their Free radical scavenging activity using DPPH (2, 2 diphenyl-1-picryl hydrazyl). Half ml of extracts prepared at various concentrations (15, 7.5, 3.75, 1.87, 0.93 mg/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 sol ution was read at 517 nm. The percent inhibition was calculated using the following formula:

$$Effective\ concentration\ \% = \frac{Control\ absorbance\ -\ Test\ absorbance}{Control\ absorbance} \times 100$$

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 mg of dry material per ml (mg/ml) that inhibits the formation of DPPH radicals by 50 Percentage (Seal, 2011).

Insecticidal activity

Insecticidal activity of the plant leaves were analyzed using larvae of *Artemia salina* and rice pests, *Sitophilus oryzae.*

The seeds of *Artemia salina* were procured from Philadelphia, USA. The seeds were incubated in sea water for 48 hours in a small water tank provided with aeration for hatching. Light was provided with 40 Watts lamp for 12 hours cycle. After 48 hours, the hatched larvae at nauplii stage were removed and used for the experiment.

Sitophilus oryzae adults were collected from naturally infested rice grains from a local market in Chennai. The insects were collected and reared on clean and uninfested rice grains in a jar with sufficient rice grains with muslin cloth as cover to ensure ventilation. After 48 h, the adults were removed and used.

Bioassay

For evaluation of larvicidal activity, test tubes contai ning leaf extracts of trees at five different concentrations (2, 4, 6, 8 and 10 mg/ml) were used. To each test tube containing 10ml of sea water, around 20 larvae were added and maintained in triplicates. The viability of the larvae was recorded after 24hours. Nauplii were considered dead when they are non motile and stayed at the bottom of the tubes.

For evaluation of pesticidal activity, 2 ml. of the plant extract constituted using 2mg, 4mg, 6mg, 8 mg and 10 mg/ml was poured onto sterile Petridish and allowed to dry. A plug of cotton was used to wipe the extract from the plate. The cotton plug on which the extract was adsorbed was placed in a Petridish along with adult *Sitophilus oryzae* (20 numbers) and few grains of rice to feed on. The observations were recorded after 24 hours period.

Table 1: Presence of phytochemicals in leaves of different trees

Sample	Tannins	Phloba tannins	Saponins	Flavonoids	Terpenoids	Cardiac Glycosides	Steroids
Annona squamosa	+	-	+	-	-	-	+
Citrus limon	-	-	+	+	-	-	+
Delonix regia	+	-	+	-	+	+	+
Millingtonia hortensis	+	-	+	-	+	-	+
Thespesia populnea	+	-	+	-	-	-	+

+ Positive, - Negative

Table 2: Antibacterial property of leaf extracts (MIC recorded – mg/ml)

Species	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*	*Streptococcus epidermidis*
Annona squamosa	100	50	3.12	12.5	25	50
Citrus limon	25	100	100	25	25	25
Delonix regia	100	50	100	25	50	100
Millingtonia hortensis	25	25	12.5	12.5	25	12.5
Thespesia populnea	100	12.5	-	-	-	3.12
Streptomycin	6.25	25	50	12.5	100	25

The insecticidal activity against the brine shrimp and weevil is provided in percent basis depending upon the mortality of the number of insects.

$$\%\ Mortality = \frac{No\ of\ brine\ shrimp/weevil\ dead}{No\ of\ brine\ shrimp/weevil\ introduced} \times 100$$

RESULTS

Phytochemistry

The studies on the presence of phytochemicals, r e-vealed that Saponins and Steroids are present in all 5 tree leaves studied and Phlobatannin was absent. Similarly, Tannin was recorded in all the plants except that of *Citrus limon*. Flavonoid was detected only in *Citrus limon* as Cardiac glycosides in *Delonix regia.* The presence and absence of different phytochemicals in leaves of individual trees are given in Table 1.

Antibacterial efficacy

The tree, *Millingtonia hortensis* has showed antibacterial potency at lower concentration with all bacteria studied with wide spectrum of antibacterial activity. *Annona squamosa, Citrus limon* and *Delonix regia* has showed positivity to all the bacteria studied, but, with higher concentration. *Thespesia populnea* showed antibacterial potency only against *Escherichia coli, Klebsiella pneumonitis* and *Streptococcus epidermidis.* It has showed no activity against other bacteria studied. The details on the Minimum inhibitory concentration (in mg/ml) recorded for each bacteria against leaves of individual tree is presented in Table 2. For comparison, MIC against the control Streptomycin (in µg/ml) is also provided

Anti-oxidant ability

The plant, *Citrus limon* alone recorded around 99 % of inhibition at the concentration of 20mg/ml. None of the plants showed 100 % inhibition against DPPH even at higher concentration of 20 mg/ml. Other than this, *Annona squamosa* and *Millingtonia hortensis* has recorded more than 75 % inhibition. The EC_{50} value recorded for *Citrus limon* was 6.27 mg/ml and for *Millingtonia hortensis* it was around 8.33 mg/ml. However, the positive control, i.e. BHA has recorded the EC_{50} value at 25.78µg/ml. The results of the percent inhibition of DPPH by leaves of different trees are tabulated in Table 3.

Insecticidal activity

Larvicidal potency

The larvicidal potency of plant studied showed that the following plants, *Citrus limon* and *Millingtonia hortensis* has recorded 100 % mortality of the larvae at 24 hrs time interval at the lowest concentration (2.5mg/ml) studied. The plants, *Delonix regia* and *Thespesia populnea* has recorded 70 % mortality rate of the larvae at 24 hrs time interval and it recorded 100 % mortality only at higher concentration. The % mortality of the larvae against the leaf extracts of trees studied are presented in Table 4.

Pesticidal activity

The study revealed that the maximum mortality (100 %) was recorded at the 24th hour treatment for the plant, *Annona squamosa* even at the lower concentration of 2mg/ml. Other than this plant, none of the oth-

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

Conc. mg/ml	Percent Inhibition against DPPH				
	Annona squamosa	*Citrus limon*	*Delonix regia*	*Millingtonia hortensis*	*Thespesia populnea*
5	33.33	44.44	22.22	44.44	-
10	44.44	66.66	33.33	55.55	33.33
15	66.66	77.77	44.44	66.66	44.44
20	77.77	99.99	55.55	77.77	55.55
EC_{50} Values	11.27	6.27	17.88	8.33	17.88

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

Species	2.5mg/ml		5mg/ml		7.5mg/ml		10mg/ml	
	24 Hrs	48 Hrs	24 Hrs	48 Hrs	24 Hrs	48 Hrs	24 Hrs	48 Hrs
Annona squamosa	70	100	100	100	100	100	100	100
Citrus limon	100	100	100	100	100	100	100	100
Delonix regia	70	100	80	100	90	100	100	100
Millingtonia hortensis	100	100	100	100	100	100	100	100
Thespesia populnea	60	100	60	100	70	100	100	100

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

Species	2mg/ml		4mg/ml		6mg/ml		8mg/ml		10mg/ml	
	24 Hrs	48 Hrs	24 Hrs	48 Hrs	24 Hrs	48 Hrs	24 Hrs	48 Hrs	24 Hrs	48 Hrs
Annona squamosa	100	100	100	100	100	100	100	100	100	100
Citrus limon	40	45	55	75	75	90	100	100	100	100
Delonix regia	20	50	35	55	30	65	35	75	100	100
Millingtonia hortensis	25	35	30	40	50	55	70	85	100	100
Thespesia populnea	-	-	40	50	50	75	65	100	100	100

ers showed any pesticidal activity at significant level. The mortality rate recorded for *Sitophilus oryzae* against the leaf extracts of the trees are presented in Table 5.

DISCUSSION

Although, previous study on few of the Bi oactivities of the plant was already reported, current study provides an extended study report on, antibacterial, antioxidant, larvicidal, pesticidal and phytochemistry of the 5 di f-ferent tree species. Among these, larvicidal and pesti-cidal studies on few of the species are reported for first time. Previous study includes the study on phytochem-istry and pharmacology of *Annona squamosa* (Pandey and Barve, 2011), review on *Millingtonia* (Ramasubra-maniaraja, 2010). Chemical and biological investiga-tions on *Delonix regia* was conducted by Jahan et al, (2010). Muthiah et al, (2012) studied the antioxidant activity of leaves, fruit and peel extract of *Citrus limon*. Sarma et al, (2011) has studied the antioxidant and anti-inflammatory activity of *Thespesia populnea.*

The phytochemical studies on the tree leaves showed the presence of Saponins, Steroids and Tannins. Phlo-batannins are generally termed as condensed tannins due to the cell damage or enzymatic action. This is ab-sent in the leaves studied which is similar to the results obtained on other 5 tree members (Udayaprakash et al, 2013a). Because of plant kingdom representing a reservoir of biologically active compounds (Udayapra-kash et al, 2012a) nearly 80 % of world population r e-lies on this traditional medicine for their primary health care (WHO). Although phytochemicals are non-nutritive, they are found to possess several medicinal attributes to it. Further, they reduce the cost and time for pharmaceutical industries, which are looking for such compounds. The biological importance of plant secondary metabolites was discussed by Udayaprakash et al, (2013c).

The plant compounds may provide natural source of novel or lead compound which may be employed in controlling microbial diseases worldwide (Udayapra-kash et al, 2012b). The antimicrobial studies showed that except that of *Thespesia populnea* all other plants possess wide spectrum antibacterial acti vity. Among the plants, *Millingtonia hortensis* alone showed signifi-cant bactericidal activity when compared with others. Antibacterial activities of the plants are attributed to the presence of Tannins (Peteros and Uy, 2010) and Terpenoids (Prabhuseenivasan et al, 2006). Among the antioxidant activity, *Citrus limon* has showed better activity compared with other plants. Antioxidant ability of Saponins (Francis et al, 2002) and Flavonoids (Ghasemzadeh and Jaafar, 2011) are already reported.

The leaf extracts of *Citrus limon* and *Millingtonia hor-tensis* showed toxicity for *Artemia salina* nauplii at lower concentration of 2mg/ml at 24 hours of expo-

sure. In the investigation of the biological activity of plant extracts and natural products, the assay on *Artemia salina* is a valuable tool for establishing toxicity and cytotoxicity parameters (Udayaprakash et al, 2013d). Thus, these plants can be used for the investigation of cytotoxicity on tumor cells. Nowadays, plants are considered as biological control agents to protect stored products from pests including rice weevils (Buatone and Indrapichate, 2011). The study on pesticidal activity against *Sitophilus oryzae* revealed that, *Annona squamosa* has shown significant activity when compared to other plants.

CONCLUSION

Through this study, it is concluded that different plant species are effective against different biological parameters studied. The plant *Millingtonia hortensis* has showed better antibacterial potency, *Citrus limon* possess, better antioxidant potency, and they both towards larvicidal activity and the plant, *Annona squamosa* has showed better pesticidal activity. Thus, proper scientific study and selection is recommended to identify the plants for their application towards specific biological activity.

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