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Original Article

STUDIES ON PHYTOCHEMISTRY, ANTIOXIDANT, ANTIBACTERIAL, LARVICIDAL AND PESTICIDAL ACTIVITIES OF AROMATIC PLANTS FROM YELAGIRI HILLS

N.K. UDAYA PRAKASH[1]. S. BHUVANESWARI[2*]., N. SRIPRIYA[2]., R. ARULMOZHI[3]., K. KAVITHA[3]., R. ARAVITHA[3], B. BHARATHIRAJA[3]

Vel Tech Dr. RR Dr. SR Technical University, Avadi-Alamadhi Road, Avadi, Chennai 600062 MARINA LABS, 40, Anna Nedum Pathai, Choolaimedu, Chennai 600094 [3,]Department of Biotechnology, Vel Tech High Tech Dr. RR Dr. SR Engineering College, Avadi-Alamadhi Road, Chennai 600062, Email: bhuvaneswari8@yahoo.co.in

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ABSTRACT

Objectives: To study the bioactivity potential and phytochemistry of few of the aromatic plants, i.e. *Cinnamomum verum* of Lauraceae, *Chrysanthemum* sp. of Asteraceae, *Lantana camara* of Verbenaceae and *Eucalyptus citriodora* and *Callistemon citrinus* of the family Myrtaceae, from Yelagiri Hills of Tamil Nadu, India.

Methods: The Aqueous ethanol (3:1 ratio) extracts of the leaves were studied for their bioactivities like, antibacterial efficacy (MIC) through micro broth dilution, antioxidant property using DPPH, larvicidal activity using *Artemia salina* and pesticidal potency using *Sitophilus oryzae* along with their phytochemistry.

Results: The plant *Chrysanthemum* sp. has showed better antibacterial activity. *Cinnamomum verum* showed better antioxidant (EC_{50} = 15 mg/mL) and pesticidal activity (45% mortality rate) while *Lantana camara* is found to possess high larvicidal potency. The phytochemical detection revealed that all the 5 species possess Terpenoids whereas none of them showed the presence of Phlobatannins and Steroids as their constituents.

Conclusion: The study suggests the need of proper selection of plants for their specific application with specified biological functions.

Keywords: Aromatic plants, Yelagiri Hills, Antibacterial, Antioxidant, Larvicidal, Pesticidal, Phytochemistry.

INTRODUCTION

Plants serve as a major curative tool for Indian system of medicine and the information on this is found in ancient literature [1]. Plant based medicine is found to be cost effective [2] and do not pose any side effects [3]. Usage of locally available medicinal plants for the primary healthcare need is reported among 80 % of the population in the world (WHO). Among these, nearly 90 % of the plants are found in the habitat of forests [4].

Thus, scientific community has paid wide attention to gain knowledge on ethnobotanical information about medicinal plants from different geographical locations including forests [5]. Mostly, the plants are utilized as crude extracts or mixtures in all medical preparations [6]. Although these plant extracts are widely used, they lack scientific validation [7-8]. Hence, the present study was conducted on few of the plants from Yelagiri hills of the state of Tamil Nadu. The plants were selected based on their prevalence and their use as industrial crops.

The plants which emit fragrance are simply termed as Aromatic plants. The fragrance is due to the secondary metabolites synthesized and stored in vital organs. The secondary metabolites are considered to be medically important as they are widely used in the system of medicine for various ailments. They are extracted using wide range of solvents and are used as ingredients in medical preparations. In this study, the Aqueous Ethanol (3:1 ratio) extracts of few of the aromatic plants from Yelagiri hills were studied for their phytochemical constituents and their bioactivities which include antibacterial potency, antioxidant capability, larvicidal and pesticidal activities.

MATERIALS AND METHODS

Plant Source

The plants were collected from Yelagiri Hills with rich biodiversity belonging to Eastern Ghats in the state of Tamil Nadu, India. Located at 12.57° N, 78.64° E with an altitude is of 1,410 m above MSL, the hills spread across 30 sq. km.

The leaves of five different aromatic plants, i.e. *Cinnamomum verum* (Lauraceae), *Chrysanthemum* sp. (Asteraceae), *Lantana camara* (Verbenaceae), *Eucalyptus citriodora* and *Callistemon citrinus* (Myrtaceae) were collected. Healthy, uninfected leaves were chosen and were cleaned thoroughly in running water.

The washed leaves were shade dried for 4-5 days until they developed crispy nature when crushed through hands. The dried, crispy leaves were pulverized using blender and stored for further use [9].

Preparation of plant extracts

The crude from the plants were extracted through cold percolation method. Aqueous Ethanol mixture of 3:1 ratio was used as the solvent. To 20 g of each dried pulverized sample, 200 mL of solvent was added and stirred in a temperature controlled shaker at 30 ± 2°C for a period of 48 h. The extracts were filtered and concentrated for evaluating the antibacterial, larvicidal, pesticidal and antioxidant properties of the aromatic plants.

Phytochemical analysis

The dried pulverized plant materials (5 g) were extracted with double distilled water (100 mL) by boiling. The aqueous extracts were filtered using Whatman No.1 filter paper and the detection of phytochemicals like cardiac glycosides, flavonoids phlobatannins, saponins, steroids, tannins and terpenoids was done according to standard procedures [10-11].

Antibacterial assay

Minimum Inhibitory Concentration (MIC)

The antibacterial efficacy of aromatic plant extracts were studied against 5 bacterial strains, i.e. *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 1320), *Pseudomonas fluorescens* (MTCC 2421) and *Vibrio parahaemolyticus* (MTCC 451) procured from Microbial Type Culture Collection and Gene Bank, Chandigarh, India.

The plant extract of 100µg/mL was used as the initial concentration. This was serially diluted to obtain the dilutions of 50µg/mL, 25µg/mL, 12.5µg/mL, 6.25µg/mL, 3.125µg/mL, 1.6 µg/mL and finally 0.8 µg/mL [12]. The antibiotic Streptomycin was used as the positive control. Each concentration was inoculated with 0.01mL of 24 hours bacterial cell suspension and incubated at 37°C for 24 hours. The presence of cloudiness or turbidity of the broth indicates positive growth. The concentration which inhibits the bacterial growth is considered as the Minimum Inhibitory Concentration (MIC).

DPPH free radical scavenging assay

The aqueous ethanol extracts obtained from the leaves of aromatic plants of Yelagiri hills were studied for DPPH (2, 2 diphenyl-1-picryl hydrazyl) free radical scavenging activity. Extracts of various concentrations (10, 20, 30, 40 and 50 mg/mL) were taken in small tubes.

The extracts were made up to 1 mL using methanol and 1 mL of 0.01 mM DPPH was added to each of the tube. Similar solutions of DPPH in Butylated Hydroxyanisole (BHA) were used for reference and methanol was used as the blank. After half an hour of incubation in dark at room temperature, the absorbance was read at 517 nm. The percent inhibition was calculated using the formula:

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

EC_{50} value is defined as the concentration in mg of dry material per mL of solvent (mg/mL) that inhibits the formation of DPPH radicals by 50 % [13].

Larvicidal activity

The eggs of *Artemia salina* were procured from Philadelphia, USA. In a small water tank containing sea water, the eggs were incubated for 48 hours for hatching. Required light was provided with Philips 40 Watts lamp for 12 hours cycle. After 48 hours, the larvae were used for the experiments. The nauplii of *Artemia salina* were challenged in different test tubes containing 10 mL of sea water and 20 larvae. To this, extracts of leaves at different concentrations (10, 20, 30, 40 and 50 mg/mL) were added. After 48 hours, the viability of larvae was observed and mortality was recorded [14]. Nauplii were considered dead when they were immobile and stayed at the bottom of the test tubes. The percent mortality of brine shrimp was 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. The pests were reared in the laboratory, in plastic containers with fresh rice grains. The containers were covered with muslin cloth to allow sufficient ventilation. Leaf extracts of 0.5 mL, 1 mL and 1.5 mL volume constituting 50 mg, 100 mg and 150 mg concentration were poured into a clean Petri plate and allowed to dry.

A plug of cotton was used to wipe the extract from the plate and placed in a Petridish containing adult pests (20 in numbers) along with one gram of rice and sealed. The mortality rate of the rice weevil was observed after 48 h of incubation and reported [15-17].

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

RESULTS

Phytochemistry

The studies on the presence of phytochemicals showed that Terpenoids are present in all the aromatic plant species studied. However, Phlobatannin and Steroids are completely absent in all the plants studied. Saponin was detected in all species except that of *Chrysanthemum* sp. Flavonoids were recorded in *Lantana camara, Chrysanthemum* sp. And *Cinnamomum verum* while Tannin showed its presence in *Eucalyptus citriodora, Lantana camara* and *Callistemon citrinus.* The presence and absence of cardiac glycosides, flavonoids phlobatannins, saponins, steroids, tannins and terpenoids of individual plant species are presented in Table 1.

Antibacterial efficacy

The antibacterial efficacy of aqueous ethanol extracts of the aromatic plants is not so significant. The lowest concentration of MIC recorded was 25 µg/mL against *Escherichia coli, Bacillus subtilis* and *Vibrio parahaemolyticus*. The plants *Cinnamomum verum* and *Lantana camara* have recorded their lowest MIC range at the concentration of 12.5 µg/mL against *Klebsiella pneumoniae.* Similar results were observed with *Chrysanthemum* sp. and *Callistemon citrinus* against *Pseudomonas fluorescens.* The details of MIC recorded for each species against the studied bacteria is presented in Table 2.

Antioxidant ability

The plant species, *Cinnamomum verum* has recorded the lowest EC_{50} value when compared to all the species studied. The EC_{50} value recorded for this species was 15.23 mg/mL. None of the plants except *Chrysanthemum* sp. has showed 100% inhibition against DPPH even at the concentration of 50 mg/mL. The results of the percent inhibition of DPPH by different plants are tabulated in Table 3.

Larvicidal activity

The plants, *Eucalyptus citriodora* and *Lantana camara* have recorded 100 % mortality rate against *Artemia salina* at the concentration of 20 mg/mL. The other plant species, *Chrysanthemum* sp. and *Cinnamomum verum* have recorded 100 % mortality at the concentration of 50 mg/mL only. Percent mortality rate recorded for the larvae against the aqueous ethanol leaf extracts of the aromatic plants from Yelagiri hills, Tamil Nadu is presented in Table 4

Pesticidal activity

The study revealed that none of the aromatic plants studied showed significant pesticidal activity. The species, *Cinnamomum verum* alone has induced 45 % mortality of the storage pest, *Sitophilus oryzae.* The percent mortality recorded for the pest against individual aromatic plant species is given in Table 5

DISCUSSION

India is one among the nations which possesses historical record on medicinal plants and has contributed to the knowledge on world's traditional medicine. The traditional knowledge on India's ethnic diversity is rich. However, the lack of validation, analysis and method of replication has prevented real engagement of this knowledge in different applications [18]. More than 8000 species and 40,000 herbal formulations are widely used in India. Thus, conservation of these plants contributes self reliance, for the nation's own health needs [19]. WHO emphasizes the need for wider use of traditional medicine to promote their medicinal systems with many resolutions [20]. The current study on scientific validation of few aromatic plants from Yelagiri hills may provide data on Bioefficacy and the importance of these species.

Table 1: Presence of phytochemicals in Aromatic plants of Yelagiri Hills

	Phytochemical Analysis						
Species	**Flavanoids**	**Tannins**	**Saponins**	**Cardiacglycosides**	**Terpenoids**	**Steroids**	**Phlobatannins**
Eucalyptus citriodora	-	+	+	-	+	-	-
Lantana camara	+	+	+	+	+	-	-
Chrysanthemum sp.	+	-	-	-	+	-	-
Cinnamomum verum	+	-	+	+	+	-	-
Callistemon citrinus	-	+	+	+	+	-	-

Table 2: Antibacterial property of leaves of aromatic plants of Yelagiri Hills

	Antibacterial Activity (µg/ml)				
Plants	**E. coli**	**B. subtilis**	**K. pneumoniae**	**P. fluorescens**	**V. parahaemolyticus**
Eucalyptus citriodora	25	25	50	50	25
Cinnamomum verum	50	50	12.5	25	50
Lantana camara	50	50	12.5	Nil	25
Chrysanthemum sp.	25	25	25	12.5	50
Callistemon citrinus	50	25	Nil	12.5	25

Table 3: Free radical scavenging (DPPH) activity of Aromatic plants of Yelagiri Hills

Plants	**% Inhibition**					**EC_{50} (mg/mL)**
	10 mg/mL	**20 mg/mL**	**30 mg/mL**	**40 mg/mL**	**50 mg/mL**	
Eucalyptus citriodora	0	72	80	85	88	16.94
Lantana camara	17	33	61	69	72	26.07
Chrysanthemum sp.	0	37	100	100	100	22.06
Cinnamomum verum	28	70	78	82	84	15.23
Callistemon citrinus	0	71	72	72	79	17.04

Table 4: Larvicidal activity of leaves of Aromatic plants from Yelagiri Hills

Species	**% Inhibition**				
	10 mg/mL	**20 mg/mL**	**30 mg/mL**	**40 mg/mL**	**50 mg/mL**
Eucalyptus citriodora	55	100	100	100	100
Lantana camara	80	100	100	100	100
Chrysanthemum sp.	50	75	75	85	100
Cinnamomum verum	55	70	75	75	100
Callistemon citrinus	55	65	85	100	100

Table 5: Pesticidal activity of leaves of Aromatic plants from Yelagiri Hills

Species	**% Inhibition**		
	50mg	**100mg**	**150mg**
Eucalyptus citriodora	0	15	20
Lantana camara	0	5	10
Chrysanthemum sp.	0	0	0
Cinnamomum verum	35	45	45
Callistemon citrinus	0	0	20

Scientific evaluations on bioefficacy of plants from different geographical zone in the state of Tamil Nadu were already conducted by the same author [21-23]. The current study provides a scientific knowledge on few of the aromatic plants from Yelagiri Hills. The plants used in this study have not been mentioned in the list of traditional medicinal plants in Vellore district [4] except *Cinnamomum verum* and *Lantana camara* [24]. The Malayali tribals of Yelagiri hills stated the ethnomedicinal use of *Cinnamomum verum* as a treatment for cough and dysentery and *Lantana camara* against headache. Ethnobotanical survey of medicinal plants of Yelagiri hills was also studied by Tariq and Ifham [25].

The current study reveals that *Cinnamomum verum* possesses better antioxidant property and pesticidal property. The larvicidal potency was recorded at its best by the plant *Lantana camara.* Antibacterial property was found to differ according to the species of the aromatic plant as well as the bacterial species. The poor antibacterial activity may be attributed to the solvent system used (aqueous ethanol). However, using other solvents like Methanol may show better activity. Thus, proper scientific study and selection is needed to identify the plants for specific application along with certain biological properties.

CONCLUSION

Five different species, i.e. *Eucalyptus citriodora, Lantana camara, Chrysanthemum* sp.*, Cinnomomum verum* and *Callistemon citrinus* were studied for their bioefficacy. The study revealed differences in their bioefficacy which might be attributed to the solvent used and also due to the presence of different types of secondary metabolites reported. Usage of proper solvent system and specific use of aromatic plants according to the need of bioefficacy is recommended.

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CONFLICT OF INTEREST

No Conflict of Interest lies between Authors.

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