ABSTRACT

and pesticidal activity (45% mortality rate) while found in the habitat of forests [4]. Among these, nearly 90% of the plants are considered to be medically important as they are widely used in primary healthcare need is reported among 80% of the population based medicine is found to be cost effective [2] and do not pose any 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, India.

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 of 1,410 m above MSL, the hills spread across 30 sq. km.

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

N.K. UDAYA PRAKASH1, S. BHUVANESWAR1*, N. SRIPRIYA2, R. ARULMOZHI3, K. KAVITHA3, R. ARAVITHA3, B. BHARATHIRAJA3

Vel Tech Dr. RR Dr. SR Technical University, Avadi-Alamadhi Road, Avadi, Chennai 600062 MARINA LABS, 40, Anna Nedum Pathai, Choolaimedu, Chennai 600094 2Department 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 (EC50 = 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.

Physicochemical 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 100 µg/mL was used as the initial concentration. This was serially diluted to obtain the dilutions of 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 [12]. The antibiotic Streptomycin was used as the positive control. Each concentration was inoculated with 0.01 mL 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 µg/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:

\[
\text{EC}_{50}\text{ value} = \frac{\text{Control Absorbance} - \text{Test Absorbance}}{\text{Control Absorbance}} \times 100
\]

**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 paraehaemolyticus*. 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 EC50 value when compared to all the species studied. The EC50 value recorded for this species was 15.23 µg/mL. None of the plants except *Chrysanthemum* sp. has shown 100% inhibition against DPPH even at the concentration of 50 µg/mL. The results of the percent inhibition of DPPH by different plants are tabulated in Table 3.

**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: Phytochemicals in Aromatic plants of Yelagiri Hills

<table>
<thead>
<tr>
<th>Species</th>
<th>Flavonoids</th>
<th>Tannins</th>
<th>Saponins</th>
<th>Cardiaglycosides</th>
<th>Terpenoids</th>
<th>Steroids</th>
<th>Phlobatannins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus citriodora</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lantana camara</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chrysanthemum sp.</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cinnamomum verum</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Callistemon citrinus</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**RESULTS**

**Phytochemical Analysis**

The plants studied for 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 for *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.
Table 2: Antibacterial property of leaves of aromatic plants of Yelagiri Hills

<table>
<thead>
<tr>
<th>Plants</th>
<th>E. coli</th>
<th>B. subtilis</th>
<th>K. pneumonia</th>
<th>P. fluorescens</th>
<th>V. parahaemolyticus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus citriodora</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>Cinnamomum verum</td>
<td>50</td>
<td>50</td>
<td>12.5</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Lantana camara</td>
<td>50</td>
<td>50</td>
<td>12.5</td>
<td>Nil</td>
<td>25</td>
</tr>
<tr>
<td>Chrysanthemum sp.</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>12.5</td>
<td>50</td>
</tr>
<tr>
<td>Callistemon citrinus</td>
<td>50</td>
<td>25</td>
<td>Nil</td>
<td>12.5</td>
<td>25</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Plants</th>
<th>10 mg/mL</th>
<th>20 mg/mL</th>
<th>30 mg/mL</th>
<th>40 mg/mL</th>
<th>50 mg/mL</th>
<th>EC₅₀ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus citriodora</td>
<td>0</td>
<td>72</td>
<td>80</td>
<td>85</td>
<td>88</td>
<td>16.94</td>
</tr>
<tr>
<td>Lantana camara</td>
<td>17</td>
<td>33</td>
<td>61</td>
<td>69</td>
<td>72</td>
<td>26.07</td>
</tr>
<tr>
<td>Chrysanthemum sp.</td>
<td>0</td>
<td>37</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>22.06</td>
</tr>
<tr>
<td>Cinnamomum verum</td>
<td>28</td>
<td>70</td>
<td>78</td>
<td>82</td>
<td>84</td>
<td>15.23</td>
</tr>
<tr>
<td>Callistemon citrinus</td>
<td>0</td>
<td>71</td>
<td>72</td>
<td>72</td>
<td>79</td>
<td>17.04</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Species</th>
<th>10 mg/mL</th>
<th>20 mg/mL</th>
<th>30 mg/mL</th>
<th>40 mg/mL</th>
<th>50 mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus citriodora</td>
<td>55</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Lantana camara</td>
<td>80</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Chrysanthemum sp.</td>
<td>50</td>
<td>75</td>
<td>75</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td>Cinnamomum verum</td>
<td>55</td>
<td>70</td>
<td>75</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>Callistemon citrinus</td>
<td>55</td>
<td>65</td>
<td>85</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Species</th>
<th>% Inhibition 50mg</th>
<th>% Inhibition 100mg</th>
<th>% Inhibition 150mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus citriodora</td>
<td>0</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Lantana camara</td>
<td>0</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Chrysanthemum sp.</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cinnamomum verum</td>
<td>35</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Callistemon citrinus</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

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., Cinnamomum 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.

REFERENCES