ANTIMICROBIAL ASSAY OF ELAEOCARPUS SPECIES OF WESTERN GHATS OF KARNATAKA

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ABSTRACT
The efficacy of six species of Elaeocarpus found in the Western ghats belt of Karnataka namely, E. munronii, E. serratus var. serratus, E. serratus var. wiebelii, E. tuberculatus, E. variabilis and Elaeocarpus sp. (unidentified) was studied for their antimicrobial activity against five clinically important bacterial and four phytofungal pathogens using Disc diffusion method and Poisoned food technique. All the species of Elaeocarpus showed varying degrees of antimicrobial activity in 50% aqueous extracts. Extracts of E. tuberculatus and E. variabilis inhibited all the pathogens except E. coli and R. solani. E. serratus var. serratus and E. serratus var. wiebelii were found to be more effective against bacterial pathogens than fungal pathogens. Phytochemical analysis of aqueous leaf extracts of six Elaeocarpus species was also carried out.

KEYWORDS
Antimicrobial activity, Aqueous extracts, Elaeocarpus and Phytochemicals.

INTRODUCTION
The genus Elaeocarpus of the family Elaeocarpaceae is found in the evergreen and semi evergreen forests of the Indo-Pacific region of the world. In Karnataka, E. munronii (Wl.) Masters, E. serratus var. serratus Zmarzy, E. serratus var. wiebelii Zmarzy, E. tuberculatus Roxb., E. variabilis Zmarzy and Elaeocarpus sp., are distributed in the districts which lie in the Western ghats. Traditionally different species of Elaeocarpus are used in folk medicine as antidote for poison, antiseptic, biliousness, carries of teeth, diarrhea, dysentery, epilepsy, gonorrhea,
hemorrhages, leprosy, liver complaints, piles, pneumonia, rheumatism, scabies, tooth ache, typhoid, ulcers, as mouth wash, appetizer etc. Different pharmacological activities viz., analgesic, antifungal, antiinflammatory, antimicrobial, antidiabetic, antioxidant, antiviral, antitumor, antihypertensive, antianxiety and antidepressant are studied using various species of *Elaeocarpus*.

World Health Organization estimated that about 80% of the world population depends on traditional medicine for primary health care in which plants are the main source of medicine. Plants are the important source of modern pharmaceutical drugs, nearly 25% of the pharmaceutically important drugs prescribed worldwide are derived from plants. Plants showing antimicrobial activity possess healing property. This property is attributed to the secondary metabolites such as alkaloids, flavonoids, steroids, tannins, terpenoids, saponins etc. present in the plants. The antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. Scanty information is available on the above six species of *Elaeocarpous* regarding their antimicrobial activities. The effect of *Elaeocarpus* plant extracts against Phytopathogenic fungi is not reported so far. In the present communication, antimicrobial effect of the aqueous leaf extracts of the above mentioned *Elaeocarpus* species against clinical bacteria and phytopathogenic fungi is reported.

**MATERIALS AND METHODS**

**Preparation of aqueous leaf extract**

Aqueous extracts were obtained from the healthy leaves of different species of *Elaeocarpus* collected from their natural habitat. Aqueous leaf extracts were prepared by blending leaves and sterile distilled water in 1:2 (weight/volume) proportion and were autoclaved at 121°C for 15-20 minutes. The extracts were centrifuged at 8000rpm for 10 minutes. These aqueous leaf extracts were considered as 50%.

**Antimicrobial assay**

**Test Microorganisms**

Human pathogenic bacterial isolates used for the current study were *Bacillus subtilis*, *Escherichia coli* (MTCC 40), *Salmonella typhi* (MTCC 733), *Shigella flexneri* (MTCC 1457) and *Staphylococcus aureus*. Phytopathogenic pathogens *Fusarium oxysporum* (IARI 6464.06), *Phytophthora arecae*, *Magnaporthe grisea* (MTCC 1477) and *Rhizoctonia solani* were obtained from infected Tobacco, Areca nut, Paddy and Tobacco plants respectively.

**Antibacterial test (Disc diffusion method)**

Sterilized Himedia susceptibility test discs of 5mm diameter were loaded with 50 µl of 50% concentration of aqueous leaf extracts and were placed on the solidified nutrient agar plates equidistantly. The standard drug gentamicin and sterile distilled water discs served as positive and negative controls respectively. The plates were incubated at 37°C for 24-48 hours. The zone of inhibition was measured in mm at 6 hours intervals. Percentage of Relative Inhibition Zone Diameter (% RIZD) was calculated by applying the expression

\[
\% \text{RIZD} = \frac{\text{IZD sample} - \text{IZD negative control}}{\text{IZD antibiotic standard}} \times 100
\]

(Where IZD is the inhibition zone diameter (mm)).

**Antifungal test (Poisoned food technique)**

The antifungal activity of aqueous leaf extracts obtained as above was incorporated into PDA medium. The PDA medium prepared using distilled water served as control. The sterilized molten and cooled agar medium were poured into the labeled Petri plates and allowed for solidification. 5mm diameter fungal discs were inoculated. The plates were incubated at 25±2°C for 7 days (*F. oxysporum, R. solani and P. arecae*) and 14 days (*M. grisea*). The diameter of fungal growth was measured in mm. Antifungal activity was calculated as per cent inhibition (I) = C-T/C x 100, where C = growth of test fungus in control in mm, T=growth of test fungus in treatment in mm.

**Statistical analysis**

Results of the experiments on antimicrobial activity of aqueous leaf extracts of *Elaeocarpus* spp. were processed statistically using SPSS version 16 software.

**Screening of Phytochemicals**

The aqueous leaf extracts were analysed for the presence of alkaloids, flavonoids, glycosides,
RESULTS AND DISCUSSION

The ethanobotanical efficacy of aqueous extracts of *E. munronii*, *E. serratus* var. *serratus*, *E. serratus* var. *wiebelii*, *E. tuberculatus*, *E. variabilis* and *Elaeocarpus* sp. against both clinical bacteria and phytopathogenic fungi showed a varied level of inhibition (Table No.1 and 2).

**Antibacterial test**

In case of bacteria, aqueous extracts of six *Elaeocarpus* species were found to be effective against four pathogens where as the result was negligible against *E. coli* which ranged from 21.84 to 37.39%. A significant inhibition was found against *S. aureus*, *S. flexneri* and *B. subtilis*. Growth of *S. typhi* was inhibited to different extent which ranged from 36.63 to 65.20 of percent RIZD.

**Antifungal test**

Aqueous extract of six species tested in the present study showed variation in percent inhibition of phytopathogenic pathogens. All the six species showed inhibition against *F. oxysporum* and *M. grisea*, which ranged from 42.58% to 90.64%. *M. grisea* was inhibited to a maximum extent of 90.64% in *Elaeocarpus* sp. (unidentified), 88.90% in *E. munronii* and minimum of 55.56% in *E. serratus* var. *serratus* aqueous leaf extract amended medium (Figure No.1). Maximum inhibition of 72.78 % was observed in *E. variabilis* and minimum of 42.58 % in *E. serratus* var. *wiebelii* against *F. oxysporum*. *P. arecae* showed varied results, significant inhibition of 80.43% was found in *Elaeocarpus* sp. (unidentified). 75.94% in *E. variabilis* and 63.56% in *E. tuberculatus*. Two species viz. *E. munronii* and *E. serratus* var. *wiebelii* showed negligible inhibition of 11.16 and 25.54% respectively (Figure No.2). *R. solani* was inhibited to an extent of 85.71% only in *E. munronii*.

**Screening of Phytochemicals**

Flavonoids and tannins were found in all the six species, glycosides were detected in five species, saponins, steroids and terpenoids were present in four species, whereas alkaloids and phlobatannins were there only in two of the *Elaeocarpus* species as given in the Table No.3.

Present *in vitro* studies revealed the antifungal and antibacterial effects of aqueous leaf extracts of six *Elaeocarpus* spp. against four phytopathogenic and five bacterial pathogens.

In *in vitro* studies were carried out to explore the fungicidal and bactericidal efficacy of aqueous leaf extracts of six *Elaeocarpous* spp. against four phytopathogenic fungi and five bacterial pathogens. All the six aqueous extracts showed variations in growth inhibition of fungi and bacteria. Similar studies made by Kumar *et al.* in *Elaeocarpus ganitrus* have shown varying degrees of growth inhibition against different clinical isolates of fungi and bacteria.

Among the six aqueous plant extracts tested, *Elaeocarpus* sp. (unidentified) was more effective against two phytopathogenic fungi viz., *M. grisea* and *P. arecae*. *E. variabilis* showed maximum effect against *F. oxysporum*. Inhibition of *F. oxysporum* by all the six species of *Elaeocarpus* is interesting, as this revelation is in contrast to the report by Rongai *et al.* where among the 500 plant extracts tested, 84% did not inhibit significantly the growth of *F. oxysporum*.

In case of *in vitro* studies on bacteria, *E. tuberculatus* was effective against *S. typhi*. *Elaeocarpus* sp.(unidentified) inhibited the growth of *S. aureus*, *E. serratus* var *wiebelii* showed maximum inhibition of *E. coli*, *B. subtilis* and *S. flexneri*. These results support the Ayurvedic and traditional use of these plants in curing many bacterial infections viz. diarrhea, dysentery, respiratory infections, dental carries, gonorrhea etc. as reported by earlier workers. Similar results were reported by earlier researchers in the fruit extract of *E. sphaericus* against bacteria, aqueous leaf extract of *E. ganitrus* inhibited bacteria and fungi isolated from clinical samples. The antimicrobial effects of *E. sphaericus* fruit extract is correlated to the presence of phytochemicals viz., alkaloids and flavonoids.
Table No.1: Effect of aqueous extracts of *Elaeocarpus* spp. on fungal plant pathogens

<table>
<thead>
<tr>
<th>S.No</th>
<th><em>Elaeocarpus</em> spp.</th>
<th>Fusarium oxysporum</th>
<th>Magnaporthe grisea</th>
<th>Phytophthora arecae</th>
<th>Rhizoctonia solani</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>E. munronii</em></td>
<td>58.81 ± 7.00⁰</td>
<td>88.90 ± 00⁰</td>
<td>11.16 ± 16.69⁷</td>
<td>85.71</td>
</tr>
<tr>
<td>2</td>
<td><em>E. serratus var serratus</em></td>
<td>55.63 ± 0.60⁰</td>
<td>65.28 ± 2.40⁰</td>
<td>-6.32 ± 21.24⁴</td>
<td>85.71</td>
</tr>
<tr>
<td>3</td>
<td><em>E. serratus var weibelii</em></td>
<td>42.58 ± 1.87ᵃ</td>
<td>55.56 ± 00³</td>
<td>25.54 ± 18.81ᵃ</td>
<td>85.71</td>
</tr>
<tr>
<td>4</td>
<td><em>E. tuberculatus</em></td>
<td>66.28 ± 1.36ᵇ</td>
<td>67.57 ± 8.78ᵃ</td>
<td>63.56 ± 6.77ᵇ</td>
<td>Nil</td>
</tr>
<tr>
<td>5</td>
<td><em>E. variabilis</em></td>
<td>72.78 ± 3.22ᶜ</td>
<td>59.72 ± 8.67ᵃ</td>
<td>75.94 ± 7.21ᶜ</td>
<td>Nil</td>
</tr>
<tr>
<td>6</td>
<td><em>Elaeocarpus</em> sp.</td>
<td>60.69 ± 1.68ᵇ</td>
<td>90.64 ± 1.30ᵇ</td>
<td>80.43 ± 2.13ᶜ</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Note: Mean values with different superscript are significantly different from each other as indicated by Scheffe’s post hoc test (Alpha=0.05). Data processed vertically for comparison.

Table No.2: Effect of aqueous extracts of *Elaeocarpus* spp. on Bacterial pathogens

<table>
<thead>
<tr>
<th>S.No</th>
<th><em>Elaeocarpus</em> spp.</th>
<th><em>Salmonella typhi</em></th>
<th><em>Escherichia coli</em></th>
<th><em>Shigella flexneri</em></th>
<th><em>Bacillus subtilis</em></th>
<th><em>Staphylococcus aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>59.90 ± 5.41ᵇ</td>
<td>21.84 ± 18.99</td>
<td>62.97 ± 6.65</td>
<td>61.54 ± 15.39</td>
<td>46.35 ± 9.36ᵃ</td>
</tr>
<tr>
<td>2</td>
<td><em>E. munronii</em></td>
<td>56.14 ± 7.14ᵇ</td>
<td>30.31 ± 2.88</td>
<td>62.10 ± 10.98</td>
<td>73.82 ± 13.71</td>
<td>79.49 ± 4.45ᵇ</td>
</tr>
<tr>
<td>3</td>
<td><em>E. serratus var serratus</em></td>
<td>62.86 ± 2.48ᶜ</td>
<td>37.39 ± 2.90</td>
<td>67.26 ± 11.04</td>
<td>75.00 ± 3.57</td>
<td>75.23 ± 11.43ᵇ</td>
</tr>
<tr>
<td>4</td>
<td><em>E. serratus var weibelii</em></td>
<td>65.20 ± 6.99ᶜ</td>
<td>31.87 ± 1.28</td>
<td>56.42 ± 6.27</td>
<td>60.64 ± 6.33</td>
<td>73.41 ± 6.11ᵇ</td>
</tr>
<tr>
<td>5</td>
<td><em>E. tuberculatus</em></td>
<td>42.67 ± 9.78ᵇ</td>
<td>23.84 ± 7.87</td>
<td>66.65 ± 5.57</td>
<td>59.08 ± 6.58</td>
<td>65.87 ± 6.80ᵇ</td>
</tr>
<tr>
<td>6</td>
<td><em>E. variabilis</em></td>
<td>36.63 ± 1.59ᵃ</td>
<td>32.31 ± 4.30</td>
<td>63.50 ± 3.08</td>
<td>69.68 ± 4.77</td>
<td>77.66 ± 6.26ᵇ</td>
</tr>
<tr>
<td>7</td>
<td><em>Elaeocarpus</em> sp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Mean values with different superscript are significantly different from each other as indicated by Scheffe’s post hoc test (Alpha=0.05). Data processed vertically for comparison.

Table No.3: Phytochemical screening of aqueous leaf extracts of six *Elaeocarpus* species

<table>
<thead>
<tr>
<th>S.No</th>
<th>Species</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Glycosides</th>
<th>Phlobatannins</th>
<th>Saponins</th>
<th>Steroids</th>
<th>Tannins</th>
<th>Terpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>E.munronii</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td><em>E.serratus var serratus</em></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td><em>Elaeocarpus sp.</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td><em>E.tuberculatus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td><em>E.variabilis</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td><em>Elaeocarpus sp.</em></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Two different unidentified species of *Elaeocarpus*
CONCLUSION

This work has revealed for the first time the antiphytotoxic effect of aqueous extracts of *Elaeocarpus* spp. It is a positive sign in the field of agriculture as the use of ecofriendly and cost effective biopesticide is replacing the harmful chemical pesticides which show environmental implications\(^\text{18}\). The antibacterial property of *Elaeocarpus* spp. against human pathogens may be useful in proving the therapeutic property of these plants. Though a great amount of work has been done on *E. ganitrus*, very less information is available about the above six species of *Elaeocarpus*. The present study has opened up the possibility of using these tree species in the field of agriculture also.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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