Research Article **CODEN: AJPAD7** **ISSN: 2321-0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

ANTIMICROBIAL ASSAY OF *ELAEOCARPUS* SPECIES OF WESTERN GHATS OF KARNATAKA

Sharvani K A[1], Jagadeesh D[2], Chandrakanth R[2], Sumana K[3], Seema M[3], Devaki N S*[2]

[1]Department of Botany, Yuvaraja's College, Mysore, Karnataka, India.
[2*]Department of Molecular Biology, Yuvaraja's College, Mysore, Karnataka, India.
[3]Department of Microbiology, J.S.S. College, Ooty Road, Mysore, Karnataka, India.

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. *weibelii* 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.

Author of correspondence

N.S. Devaki,
Department of Molecular Biology,
Yuvaraja's College, University of Mysore,
Mysore, India.

Email: devakins@yahoo.co.in

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* Zmarzty, *E. serratus* var. *wiebelii* Zmarzty, *E. tuberculatus* Roxb., *E. variabilis* Zmarzty and *Elaeocarpus* sp., are distributed in the districts which lie in the Western ghats[1]. 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, antioxidative, antiviral, antitumor, antihypertensive, antianxiety and antidepressant are studied using various species of *Elaeocarpus*[2-5].

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[6]. Plants are the important source of modern pharmaceutical drugs, nearly 25% of the pharmaceutically important drugs prescribed worldwide are derived from plants[7]. Plants showing antimicrobial activity possess healing property[8]. This property is attributed to the secondary metabolites such as alkaloids, flavonoids, steroids, tannins, terpenoids, saponins etc. present in the plants[9]. The antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world[10]. 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*. Phytofungal 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)[11].

Antifungal test (Poisoned food technique)

The antifungal activity of aqueous leaf extracts obtained as above was incorporated into PDA medium[12]. 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[13].

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,

phlobatannins, saponins, steroids, tannins and terpenoid according to standard methods[14].

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 phytofungal 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. *weibelii* aginst *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. *weibelii* 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 phytofungal and five bacterial pathogens.

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*.,[15] in *Elaeocarpous 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* [16] 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 weibelii* 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[2-4]. Similar results were reported by earlier researchers in the fruit extract of *E. sphaericus* against bacteria[14], aqueous leaf extract of *E.ganitrus* inhibited bacteria and fungi isolated from clinical samples[17,2]. The antimicrobial effects of *E. spaericus* fruit extract is correlated to the presence of phytochemicals viz., alkaloids and flavonoids[14].

Table No.1: Effect of aqueous extracts of *Elaeocarpus* spp. on fungal plant pathogens

S.No	*Elaeocarpus* spp.	Fungal plant pathogens			
		Fusarium oxysporum	***Magnaporthe grisea***	***Phytophthora arecae***	***Rhizoctonia solani***
		Percent inhibition in 50%			
1	*E.munronii*	58.81 ± 7.00^{b}	88.90 ± 00^{b}	11.16 ± 16.69^{a}	85.71
2	*E. serratus var serratus*	55.63 ± 0.60^{b}	65.28 ± 2.40^{a}	-6.32 ± 21.24^{a}	Nil
3	*E. serratus var weibelii*	42.58 ± 1.87^{a}	55.56 ± 00^{a}	25.54 ± 18.81^{ab}	Nil
4	*E. tuberculatus*	66.28 ± 1.36^{bc}	67.57 ± 8.78^{a}	63.56 ± 6.77^{bc}	Nil
5	*E. variabilis*	72.78 ± 3.22^{c}	$59.72 \pm 8..67^{a}$	75.94 ± 7.21^{c}	Nil
6	*Elaeocarpus* sp.	60.69 ± 1.68^{b}	90.64 ± 1.30^{b}	80.43 ± 2.13^{c}	Nil

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

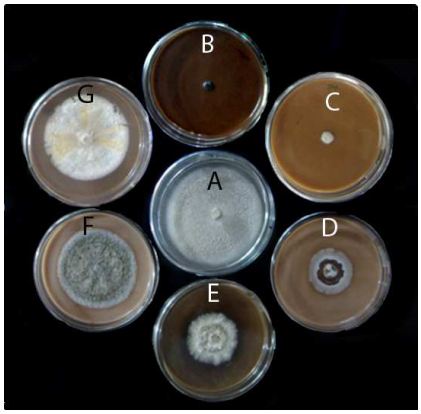
S.No	*Elaeocarpus* spp.	Bacterial species				
		Salmonella typhi	***Escherichia coli***	***Shigella flexneri***	***Bacillus subtilis***	***Staphylococcus aureus***
1		Percentage of Relative Inhibition Zone Diameter (% RIZD)				
2	*E. munronii*	59.90 ± 5.41^{bc}	21.84 ± 18.99	62.97 ± 6.65	61.54 ± 15.39	46.35 ± 9.36^{a}
3	*E. serratus* var *serratus*	57.14 ± 7.14^{bc}	30.31 ± 2.88	62.10 ± 10.98	73.82 ± 13.71	79.49 ± 4.45^{b}
4	*E. serratus* var *weibelii*	62.86 ± 2.48^{c}	37.39 ± 2.90	67.26 ± 11.04	75.00 ± 3.57	75.23 ± 11.43^{b}
5	*E. tuberculatus*	65.20 ± 6.99^{c}	31.87 ± 1.28	56.42 ± 6.27	60.64 ± 6.33	73.41 ± 6.11^{b}
6	*E. variabilis*	42.67 ± 9.78^{ab}	23.84 ± 7.87	66.65 ± 5.57	59.08 ± 6.58	65.87 ± 6.80^{ab}
7	*Elaeocarpus* sp.	36.63 ± 1.59^{a}	32.31 ± 4.30	63.50 ± 3.08	69.68 ± 4.77	77.66 ± 6.26^{b}

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

S.No	Species	Alkaloids	Flavonoids	Glycosides	Phloba tannins	Saponins	Steroids	Tannins	Terpenoids
1	*E.munronii*	-	+	+	+	-	+	+	+
2	*E.serratus var serratus*	-	+	-	-	+	-	+	-
3	*Elaeocarpus* sp.*	+	+	+	-	+	+	+	-
4	*E.tuberculatus*	+	+	+	+	+	+	+	+
5	*E.variabilis*	-	+	+	-	-	+	+	+
6	*Elaeocarpus* sp.*	-	+	+	-	+	-	+	+

*Two different unidentified species of *Elaeocarpus*



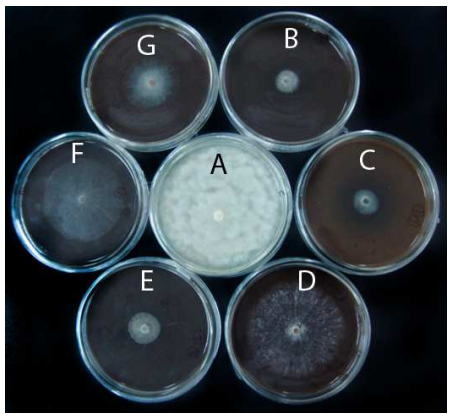


Figure No.1 **Figure No.2**

Figure No.1: Fourteen day old culture of *Magnaporthe grisea* grown on Potato Dextrose Agar medium supplemented with leaf extracts of different species of *Elaeocarpus*: A. Control B. *Elaeocarpus* sp. C. *E. munronii* D. *E. tuberculatus* E. *E. serratus* var. *serratus* F. *E. variabilis* G. *E. serratus* var. *weibelii*
Figure No.2: Seven day old culture of *Phytophthora arecae* grown on Potato Dextrose Agar medium supplemented with leaf extracts of different species of *Elaeocarpus*: A. Control B. *E. variabilis* C. *Elaeocarpus* sp. D. *E. serratus* var. *serratus* E. *E. tuberculatus* F. *E. munronii* G. *E. serratus* var. *weibelii*

CONCLUSION

This work has revealed for the first time the antiphytofungal 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[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

We are thankful to University Grants Commission, New Delhi for the financial support, University of Mysore and Yuvaraja's college, Mysore for the facilities. We are grateful to Dr. Lancy D'Souza for helping with the statistical analysis.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBILIOGRAPHY

1. Saldanha C J. Flora of Karnataka, *Oxford and IBH Publishing Co., New Delhi*, Vol.I, 1984, 535.
2. Joshi S C, Jain P K. A review on ethnomedicinal and traditional uses of *Elaeocarpus ganitrus* Roxb. (Rudraksha), *International Journal of Pharma and Bio sciences*, 5(1), 2014, 495-511.
3. Shah G, Singh P S, Mann A S, Shri R. Scientific basis for the chemical constituent and therapeutic use of *Elaeocarpus* species: A review, *International Journal of Institutional Pharmacy and Life Sciences*, 1(1), 2011, 267-278.
4. Singh R K, Acharya S B, Bhattacharya S K. Pharmacological activity of *Elaeocarpus sphaericus*, *Phytotherapy Research*, 14(1), 2000, 36-39.

5. Pullaiah T. Encyclopedia of World Medicinal Plants, *Regency Publication, New Delhi*, Vol-2, 2006, 2442.
6. Bodeker G, Ong C K, Grundy C, Burford G, Shein K. WHO Global Atlas of Traditional, Complementary and Alternative Medicine, *World Health Organization, Kobe*, 2005, 347.
7. Rates S M K. Plants as source of drugs, *Toxicon*, 39, 2001, 603-613.
8. Selvamohan T, Ramadas V, Kishore S S S. Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria, *Advances in Applied Science Research*, 3(5), 2012, 3374-3381.
9. Singh B, Chopra A, Ishar M P S, Sharma A, Raj T. Pharmacognostic and antifungal investigations of *Elaeocarpus ganitrus* (Rudrakasha), *Indian Journal of Pharmaceutical Sciences*, 72(2), 2010, 261-265.
10. Bhalodia N R, Nariya P B, Acharya R N, Shukla V J. Evaluation of in vitro antioxidant activity of flowers of Cassia fistula Linn, *International Journal of PharmTech Research*, 3(1), 2011, 589-599.
11. Rojas J, Ochoa J V, Ocampo S A, Muñoz J F. Screening for antimicrobial activity of ten medicinal plants used in *Colombian folkloric* medicine: a possible alternative in the treatment of non-nosocomial infections, *BMC Complementary and Alternative Medicine*, 6, 2006, 1-6.
12. Nene Y L, Thapliyal B W. Fungicides in Plant Disease Control, *Oxford and* IBH *Publisher house New Delhi*, 1979, 425.
13. Mohana D C, Raveesha K A. Anti-fungal evaluation of some plant extracts against some plant pathogenic field and storage fungi, *Journal of Agricultural Technology*, 4, 2007, 119-137.
14. Harborne J B. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, *Chapman and Hall, New York*, 1973, 279.
15. Kumar G, Karthik L, Rao K V B. Antimicrobial activity of *Elaeocarpus ganitrus* Roxb (Elaeocarpaceae): An *in vitro* study, *Elixir BioTechnology*, 40, 2011, 5384-5387.
16. Rongai D, Milano F, Scio E. Inhibitory effect of plant extracts on conidial germination of the phytopathogenic fungus *Fusarium oxysporum*, *American Journal of Plant Sciences*, 3(12), 2012, 1693-1698.
17. Singh R K, Nath G. Antimicrobial activity of *Elaeocarpus sphaericus*, *Phytotherapy Research*, 13(15), 1999, 448-450.
18. Mazid S, Kalita J C, Rajkhowa R C. A review on the use of biopesticides in insect pest management, *International Journal of Science and Advanced Technology*, 1(7), 2011, 169-177.

Please cite this article in press as: Devaki N S *et al*. Antimicrobial Assay of *Elaeocarpus* Species of Western Ghats of Karnataka, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*, 3(1), 2015, 14 - 19.