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EVALUATION OF ANTIMICROBIAL POTENTIAL OF *ELAEOCARPUS SERRATUS* L.

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Elaeocarpus serratus, phytochemicals, antimicrobial activity, agar well diffusion method, zone of inhibition

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ABSTRACT: The *in vitro* antimicrobial activity of acetone, methanol and water extracts of leaf, stem bark and fruit of *Elaeocarpus serratus* L. (Elaeocarpaceae) was examined against four bacterial species (*Shigella sonnei*, *Salmonella typhi*, *Staphylococcus aureus and Klebsiella pneumoniae*) and a fungal species (*Candida albicans*) using the agar well diffusion method. Phytochemical screening was carried out for phenols, flavonoids and tannins. Results showed that the plant extracts exhibited a dose-dependent inhibition of microorganisms. The acetone and methanol extracts of leaf and stem bark of *E. serratus* displayed maximum antibacterial activity against all the bacterial species studied. The plant extracts also displayed high antifungal activity against *Candida albicans* especially, the acetone extract was found to be more active antifungal. Generally, the lower concentrations of the extracts were susceptible to the fungal pathogen. *E. serratus* extracts contained phenols, flavonoids and tannins at varying levels. The ability of the crude extracts of the test plant to inhibit the growth of bacteria and fungi is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infections.

INTRODUCTION: In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation, coupled with the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections are a serious medical problem [1, 2]. Therefore the scientists of the21st century are generally reviving our traditional knowledge and are screening various parts of plants scientifically used in the folklore medicine in search of newer lead compounds having antimicrobial efficacy [3, 4].

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Medicinal plants are known to produce certain bioactive molecules which react with organisms in the environment, inhibiting bacterial or fungal growth and protect the human body against pathogens [5, 6].

Elaeocarpus is a genus of tropical and subtropical evergreen trees and shrubs belonging to family Elaeocarpaceae. Studies indicate that various *Elaeocarpus* species contain chemical constituent such as triterpenes, tannins, indolizidine alkaloids, flavonoids, and ellagic acid derivatives [7, 8, 9].

Various species of *Elaeocarpus* have been known to possess anti-inflammatory [10], antimicrobial [11], analgesic [12] and antihypertensive [13] activities. However, there is insufficient information regarding the antimicrobial activity of *Elaeocarpus serratus* L. In this paper, the antimicrobial property of crude extract of the leaf, stem bark and fruit of *E. serratus* L. has been studied as part of the

exploration for new and novel bio-active compounds.

MATERIALS AND METHODS:

Plant materials and preparation of extract: The leaves, stem bark and fruits of *Elaeocarpus serratus* L. were collected from Upper Palani Hills of Western Ghats (Kodaikanal Forest Division), India and were authenticated at Botanical Survey of India (BSI), Southern Circle, Coimbatore, India and the herbarium of Voucher specimen number BSI/SRC/5/23/2011-12/Tech. 239 have been deposited at the Department of Botany, Vellalar College for Women, Erode (T.N), India. The plant materials were dried separately under shade and pulverized in a mechanical grinder and stored in a closed container for further use.

The air dried, powdered plant materials were extracted in the Soxhlet apparatus successively with different solvents in the increasing order of polarity [Acetone (56.5°C), Methanol (64.7°C) and Water (99.98°C)]. Each time before extracting with the next solvent, the powdered materials were dried in a hot air oven at 40°C. Finally, the materials were macerated using hot water with occasional stirring for 16 hours and the water extracts were filtered.

The different solvent extracts were concentrated, vacuum dried and weighed. The percentage yield was expressed in terms of air dried sample. The extracts were dried over anhydrous sodium sulphate, stored in sealed vials in refrigerator (5-8°C) until analysis[14]. All the reagents used were of analytical grade.

Test microorganisms: The test microorganisms used in this study (bacteria: *Shigella sonnei* MTCC 2957, *Salmonella typhi* MTCC 3216, *Staphylococcus aureus* MTCC 3381, and *Klebsiella pneumoniae* MTCC 3384; fungi: *Candida albicans* MTCC 183) were obtained from the culture collections of Manian Laboratories Pvt. Ltd., Coimbatore, India. Bacteria were cultured overnight at 37ºC in Mueller Hinton Broth (MHB) and fungi at 28°C for 72 hours in Potato Dextrose Broth (PDB) and used as inoculum.

Antimicrobial bioassay: The antimicrobial activity of the crude extracts was determined in accordance with the agar well diffusion method described by Sinclair and Dhingra,(1995)[15]. Bacteria were cultured overnight at 37ºC in Mueller Hinton Broth (MHB) and fungi at 28°C for 72 hours in Potato Dextrose Broth (PDB) and used as inoculum.

A final inoculum, using 100µl of suspension containing 108 CFU/ml of bacteria and 104 spore/ml of fungi were spread on Mueller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) medium, respectively. Subsequently, using a sterile borer, well of 9 mm diameter was made in the inoculated media. Addition of 50, 100, 150 and 200 µl of 20 mg/ml each extract was aseptically filled into the well. Negative control was prepared using the same solvent employed to dissolve the extracts. Gentamycin (50 µg/ml) and Amphotericin (100 units/disc) were used as positive control. The test plates were incubated at 37°C for 24 hours depending on the incubation time required for a visible growth. The diameter of zone of inhibition (mean of triplicates ± SD) as indicated by clear area which was devoid of growth of microbes was measured.

Statistical evaluation: Each assay in this experiment was repeated thrice and the values were expressed as mean of triplicate analysis of the samples (n = 3) ± Standard Deviation (SD).

Preliminary phytochemical analysis: The extracts of the plant were screened for phenols, flavonoids and tannins using the following procedure:

Phenols: The extract (50 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds [16].

Flavonoids: Four milliliters of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones [17].

Tannins: To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins [18].

RESULTS AND DISCUSSION: The percentage yield of extracts and the phytochemical constituents of the plant are shown in **Fig. 1 and Table 1**, respectively. The maximum per cent yield was registered in the acetone extract of leaf of *E.serratus* (27.67%).The methanol extract of stem bark of *E.serratus* registered an yield percentage of 16.0% . Generally, the acetone and methanol extracts of plant parts contained more constituents than the water extracts. This might be due to the fact that phenolics are often extracted in higher amounts in more polar solvents such as aqueous methanol/ethanol as compared with absolute methanol/ethanol [19]. This was in agreement with the findings of Shon *et al*. (2004)[20] who investigated that methanol and hot water were more efficient to extract antioxidant compounds from *Phellinus baumii*. Similarly, Singh *et al*. (2010)[21] showed that of all the solvents (pet ether, chloroform, ethanol and water) used, the ethanol extract of *Elaeocarpus ganitrus* had a maximum extractable value of 2.4% and chloroform had a minimum value of 0.5%.

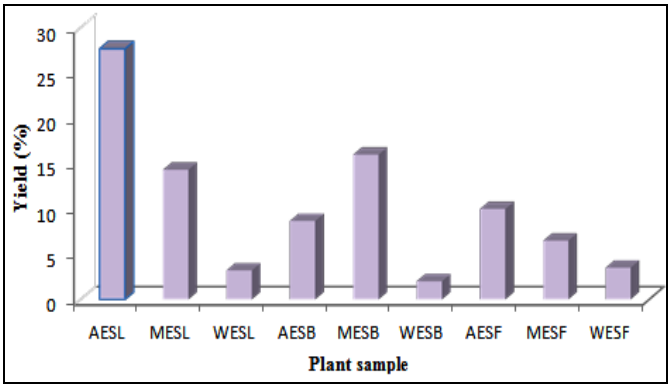

FIG. 1: THE PER CENT YIELD OF DIFFERENT SOLVENT EXTRACTS OF *ELAEOCARPUS SERRATUS*

Plant sample:

AESL - Acetone extract of *E. serratus* leaf MESL - Methanol extract of *E. serratus* leaf WESL - Water extract of *E. serratus* leaf

AESB - Acetone extract of *E. serratus* stem bark MESB - Methanol extract of *E. serratus* stem bark WESB - Water extract of *E. serratus* stem bark AESF - Acetone extract of *E. serratus* fruit

MESF - Methanol extract of *E. serratus* fruit

WESF - Water extract of *E. serratus* fruit

TABLE 1: PHYTOCHEMICAL SCREENING OF DIFFERENT SOLVENT EXTRACTS OF *ELAEOCARPUS SERRATUS*

Sample	Extraction medium	Phenols	Flavonoids	Tannins
Leaf	Acetone	+++	+++	+++
	Methanol	+++	++	+++
	Water	++	+	++
Stem Bark	Acetone	+++	++	+++
	Methanol	+++	+++	++
	Water	+	+	++
Fruit	Acetone	++	++	++
	Methanol	++	+++	+++
	Water	+	+	+

\+ = Present in small amount (concentration); ++ = Moderately present; +++ = Present in large amount

The antimicrobial activity of different extracts of *E. serratus* is shown in **Table 2**. The plant extracts showed a dose-dependent inhibition of micro-organisms.

Among the extraction medium, acetone and methanol extracts of leaf and stem bark of *E. serratus* displayed maximum antibacterial activity against all the bacterial species studied. The water extracts of leaf, stem bark and fruit were found to be more susceptible to the bacterial species and showed no zone of inhibition except *Shigella sonnei*. The standard antibiotic gentamycin inhibited the growth of all the bacterial species effectively at a lower concentration of 50 µg/ml.

Generally, the inhibition of all the bacterial species (except *Klebsiella pneumoniae*) by the standard antibiotic gentamycin (50 µg/ml) was higher than the various solvent extracts of the test plant.

In the present findings, *E. serratus* extracts displayed high antifungal activity against *Candida albicans*. The water extract of leaf and acetone extract of fruit of *E. serratus* produced the maximum inhibition zone of 18±1.05 and 18±0.92 mm at 200 µg/ml. On the whole, the various solvent extracts of the plant parts at higher concentrations (150 and 200 µg/ml) inhibited the fungal growth more effectively than the standard amphotericin which showed an inhibition zone of 11±0.02 mm at a concentration of 100 units per disc. Generally, the lower concentrations of the extracts were susceptible to the fungal pathogen.

TABLE 2: ANTIMICROBIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF LEAF, STEM BARK AND FRUIT OF *ELAEOCARPUS SERRATUS*

Sample	Extraction medium	Concentration (µg/ml)	Zone of Inhibition (mm)				
			Shigella sonnei	***Salmonella typhi***	***Staphylococcus aureus***	***Klebsiella pneumoniae***	***Candida albicans***
Leaf	**Acetone**	50	13±0.27	11±1.22	11±1.01	11±0.98	10±0.52
		100	15±0.85	12±0.77	13±0.12	13±0.46	12±1.02
		150	16±0.15	15±0.46	15±0.01	15±0.61	13±0.96
		200	18±0.77	17±1.49	16±0.27	17±0.84	15±0.25
	Methanol	50	11±0.28	10±1.68	12±0.78	10±0.97	-
		100	14±1.05	13±0.91	13±0.66	13±1.12	9±0.47
		150	15±0.83	16±0.45	16±0.45	14±1.16	12±0.98
		200	16.5±1.87	18±0.41	17±0.99	16±1.87	15±0.21
	Water	50	-	-	-	-	-
		100	-	-	-	-	11±0.62
		150	9±0.11	-	-	-	13±0.77
		200	10±1.65	-	-	-	18±1.05
Stem bark	**Acetone**	50	11.5±1.53	11.5±1.21	13±0.62	1.2±0.84	-
		100	13±0.66	14±1.88	15±0.31	1.3±0.25	8.5±1.03
		150	14.5±0.95	15±0.69	16±0.48	1.4±0.37	11±0.54
		200	16±0.32	17±0.65	16±1.02	1.6±0.68	12±0.44
	Methanol	50	11±0.54	12±1.62	10±0.98	11±1.25	10.5±0.89
		100	13±1.15	14±0.50	12±0.61	14±1.85	11±0.69
		150	14±0.65	15±1.65	14±0.77	15±0.97	11.5±0.47
		200	15±0.88	17±1.66	16±0.24	15±0.82	14±0.25
	Water	50	-	-	-	-	-
		100	-	-	-	-	-
		150	9±0.41	-	-	-	-
		200	10±0.35	-	-	-	10±0.81
Fruit	**Acetone**	50	9±0.97	9±0.19	9±1.11	9±1.06	-
		100	11±0.17	10±1.51	11±1.08	10±1.55	11±0.94
		150	11.5±0.54	10.5±1.65	11.5±0.55	12±0.17	15±0.44
		200	13±1.23	13±0.98	12.5±0.78	13±0.28	18±0.92
	Methanol	50	9±1.54	9.5±1.17	10±0.25	-	11±0.87
		100	9.5±1.88	10±0.79	11±0.29	10±0.51	14±1.12
		150	10.5±0.89	11±0.88	11.5±0.02	11±0.49	16±1.04
		200	12±0.73	11.5±1.12	12.5±0.16	12±0.66	17±1.62
	Water	50	-	-	-	-	-
		100	-	-	-	-	-
		150	8±0.32	-	-	-	-
		200	9±0.14	-	-	-	12±0.11
Gentamycin		50	23±0.66	20±0.03	21±0.09	12±0.02	-
Amphotericin		100 units/disc	-	-	-	-	11±0.02

Values are mean ± SD (n=3)

Phenolics and polyphenols present in the plants were known to be toxic to the microorganisms [22]. Flavonoids have been reported to have both antibacterial and antifungal activities [23]. Tannins from *Dichrostachys cinerea* root bark possessed antibacterial activities [24]. In the present study, the phytochemical analysis of *E. serratus* solvent extracts revealed the presence of phenols, flavonoids and tannins at varying intensity. The phytochemical characteristics possessed by *E. serratus* may be attributed to its antimicrobial properties.

The high inhibitory potential of acetone and methanol extracts of leaf and stem bark of *E. serratus* might be due to the high solubility of the phytoconstituents in the organic solvents. The phytoconstituents might be present in higher concentrations in the leaf and stem bark along with some new microbicidal agents reflecting its higher bactericidal and fungicidal potential. Presence of these phytoconstituents in the leaf and stem bark pointed towards the pharmacological activities of this plant and supported the claim of the traditional users.

In support of the present study, the results of Nair and Chanda (2007) [25] revealed that the ethanol extracts were more potent than aqueous extracts of all the plants studied. Similar trend was also noted by Ekwenye and Edeha (2010) [26]. According to them, the ethanol extracts of *Citrus sinensis* exhibited inhibitory activities that were found to be a little higher than aqueous extract on the bacterial species investigated. It can be therefore inferred that the active principles of the plant may be more soluble in ethanol than in water. Results of the present investigation agreed with the report of Arokiyaraj *et al* (2009) [27].

According to them, *Vitex doniana* and *Cajanus cajan* acetone, methanol, ethanol extracts generally produced a clear inhibitory effect on the bacteria. The present findings were also supported by Singh *et al* (2010) [21] who evaluated the petroleum ether, chloroform, ethanol and water extracts of dried fruits of *Elaeocarpus ganitrus* for antifungal activity on different fungal strains. The chloroform and ethanol extracts were found to be more active antifungals.

CONCLUSION: It is inferred from the current findings that the phytoconstituents might be present in high concentrations in the leaf of *Elaeocarpus serratus* along with some new microbicidal agents reflecting its higher bactericidal potential. However, further studies are necessary to find out the active principles responsible for these activities which can be used as natural antimicrobial agents for human consumption and cure of infectious diseases.

REFERENCES:

1. Marchese A and Shito GC: Resistance patterns of lower respiratory tract pathogens in Europe. Int. J. Antimicrobial Agents 2001; 16: 25–29.
2. Poole K : Overcoming antimicrobial resistance by targeting resistance mechanisms. J.Pharmacy Pharmacol. 2001; 53: 283-284.
3. Afolayan AJ : Extracts from the shoots of *Arctotis artotoides* inhibit the growth of bacteria and fungi. Pharm. Biol. 2003; 41: 22-2
4. Karaman I, Sahin F, Güllüce M, Ogütçü H, Sengül M and Adigüzel A: Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J. Ethnopharmacol.2003; 85(2-3): 231-235.
5. Yano Y, Satomi M and Oikawa H: Antimicrobial effect of spices and herbson *Vibrio parahaemolyticus*. Int. J. of Food Microbiol. 2006;111: 6-11.
6. Wojdylo, A., Oszmianski, J. and Czemerys, R. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, 105: 940-949.
7. Fang X, Phoebe Jr CH, Pezzuto JM, Fong HH, Farnsworth NR, Yellin B, and Hecht S.M: Plant anticancer agents, XXXIV. Cucurbitacins from *Elaeocarpus dolichostylus.* J.Nat.Prod., 1984; 47 (6): 988 - 993.
8. [8] Ito A, Chai HB, Lee D, Kardono L B S, Riswan S, Farnsworth N R, Cordell G A, Pezzuto J M and Kinghorn A D: Ellagic acid derivatives and cytotoxic cucurbitacins from *Elaeocarpus mastersii*.Phytochemistry 2002; 61 (2):171-174.
9. Rodriguez N, Vasquez Y, Hussein A A, Coley P D, Solis P N, Gupta M P: Cytotoxic cucurbitacin constituents from *Sloanea zuliaensis.* J. Nat. Prod. 2003; 66: 1515.
10. Singh R K, Bhattacharya S K Acharya SB: Studies on extracts of *Elaeocarpus sphaericus* fruits on *in vitro* rat mast cells. Phytomedicine 2000; 7(3): 205-7.
11. Singh R and Nath G: Antimicrobial Activity of *Elaeocarpus sphaericus.*Phytother. Res. 1999; 13: 448–450.
12. Bhattacharya S K, Debnath PK and Pandey VB: Pharmacological investigations on *Elaeocarpus ganitrus.* Planta Medica 1975; 28: 174-177.
13. Pandey VB and Bhattacharya SK: Scientific appraisal of rudraksha (*Elaeocarpus ganitrus*): chemical and pharmacological studies. J. Res. Edu. Ind. Med. 1985; 4: 47-50.
14. Anonymous: Pharmacopoeia of India, Ministry of Health, Govt. of India Publication, New Delhi 1985.
15. Sinclair J B, Dhingra OD: Basic Plant Pathology Methods, 1995: 287–305.
16. Harborne J B: Phytochemical methods. A guide to modern techniques of plant analysis, Ed.2 Chapman and Hall, London, 1973:4 and 140

17. Siddiqui A A and Ali M: Practical Pharmaceutical chemistry. CBS Publishers and Distributors, New Delhi, First Edition, 1997: 126-131.
18. Iyengar MA: Study of Crude Drugs. Manipal Power Press, Manipal, India 8th ed., 1995: 2.
19. Sultana B, Anwar F and Przybylski R: Antioxidant activity of phenolic components present in barks of barks of *Azadirachta indica, Terminalia arjuna, Acacia nilotica, and Eugenia jambolana* Lam. trees**.** Food Chem. 2007; 104: 1106- 1114.
20. Shon M Y, Choi S D, Kohng G G, Nam S H and Sung N J: Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onion. Food Chem. Toxicol. 2004; 42: 659-666.
21. Singh B, Chopra A, Ishar M P S, Sharma A and Raj T: Pharmacognostic and antifungal investigations of *Elaeocarpus ganitrus* (Rudrakasha). Indian J. Pharm. Sci. 2010; 72:261-265.
22. Mason T L and Wasseman B P: Inactivation of red beet betaglucan synthase by native and oxidized phenolic compounds. Phytochemistry 1987; 26: 2197-02.
23. Tsuchiya H, Sato M, Miuazaki T, Fujiwara S and Tanigaki S: Comparative study on the antibacterial activity of phytochemical flavonones against methicillin resisitant *Staphylococcus aureus.* J. Ethnopharmacol. 1996; 50: 27-34.
24. Banso A. and Adeyama SO: Evaluation of antibacterial properties of tannins isolated from *Dichrostachys cinerea*. African J. of Biotech. 2007; 6(15): 1785-87.
25. Nair R and Chanda S V: Antibacterial activities of some medicinal plants of the Western Region of India. Turk. J. Biol. 2007; 31: 231-236.
26. Ekwenye U N and Edeha OV: The antibacterial activity of crude leaf extract of *Citrus sinensis* (Sweet Orange). International Journal of Pharma and Bio. Sciences 2010;1(4): 742 - 750.
27. Arokiyaraj K, Perinbam P, Agastian R and Mohan K: Phytochemical analysis and antibacterial activity of *Vitex agnus-castus*. International Journal of Green Pharmacy 2009; 34:162-164.

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