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Evaluation of Anti-Microbial Activity of *Elaeocarpus tuberculatus* Roxb.

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Abstract: In the present study, the antimicrobial efficacy of acetone, methanol and water extracts of leaf, stem bark and fruit of *Elaeocarpus tuberculatus* Roxb. (Elaeocarpaceae) were 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. Results showed that the plant extracts exhibited a dose-dependent inhibition of microorganisms, especially, the acetone and methanol extracts of leaf and stem bark displayed maximum antibacterial activity against all the bacterial species studied. The plant extracts also displayed high antifungal activity against *Candida albicans* particularly, the acetone extract showed pronounced antifungal activity. Generally, the lower concentrations of the extracts were susceptible to the fungal pathogen.

Key words: *Elaeocarpus tuberculatus* · Phytochemical · Solvents · Anti-microbial assays · Agar well diffusion method

INTRODUCTION

Since the discovery of penicillin (1929) and its use in chemotherapy, a great number of important antibiotics have been found [1]. The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed 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 [2, 3].

Many studies indicated that in some medicinal plants there are many substances such as peptides, unsaturated long chain aldehydes, alkaloidal constituents, some essential oils, phenols and flavonoids which are soluble in water, ethanol, chloroform, methanol and butanol. These plants then emerged as compounds with potentially significant therapeutic application against human pathogens [4-7]. This has forced scientists to search for new antimicrobial substances from various sources like the medicinal plants [8-10].

Plants of the family Elaeocarpaceae have been reported to be used in traditional medicines particularly in India. A noteworthy chemical feature of Elaeocarpaceae is their ability to elaborate a series of oxygenated steroids or cucurbitacins and ellagic acid derivatives which abound in this family, hold some potential as a source of cytotoxic agents [11-13]. *Elaeocarpus,* a genus with about 360 species are reported from different parts of Asia, including Nepal, Bhutan, Sikkim, Tibet, Java, Indonesia, foothills of the Himalayas and various parts of India. About 25 species have been reported from India. *Elaeocarpus* species contain hard and highly ornamental stony endocarp of fruit (nut) commonly known as 'Rudraksh'. The stony endocarp is used as religious jewelry in the form of beads throughout India and Southeast Asia. Rudraksha beads users have repeatedly confirmed the medicinal properties such as dielectrical energy and permanent magnetic properties, controls heart beat and has a positive effect on blood pressure, stress, anxiety, depression, palpitations and lack of concentration [14].

Various species of *Elaeocarpus* have been known to possess antimicrobial [15], anti-arthritic [16], anti-diabetic activities [17, 18]. However, there is insufficient information regarding the antimicrobial activity of

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Elaeocarpus tuberculatus Roxb. In this paper, the antimicrobial property of crude extract of the leaf, stem bark and fruit 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 tuberculatus* Roxb. 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 has been deposited at the PG and Department of Botany, Vellalar College for Women, Erode (Tamil Nadu), 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 [19]. All the reagents used were of analytical grade.

Microbial Stains: 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 37°C 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 [20]. Bacteria were cultured overnight at 37°C 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 Analysis: 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 [21].

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 [22].

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 [23].

RESULTS AND DISCUSSION

The percentage yield and antioxidant phytoceuticals (phenols flavonoids and tannin) in the different solvent extracts (acetone, methanol and water) of *E. tuberculatus* leaf, stem bark and fruit are shown in Fig. 1 and Table 1, respectively. The maximum per cent yield was registered in the acetone extract of leaf (22.01%). The methanol

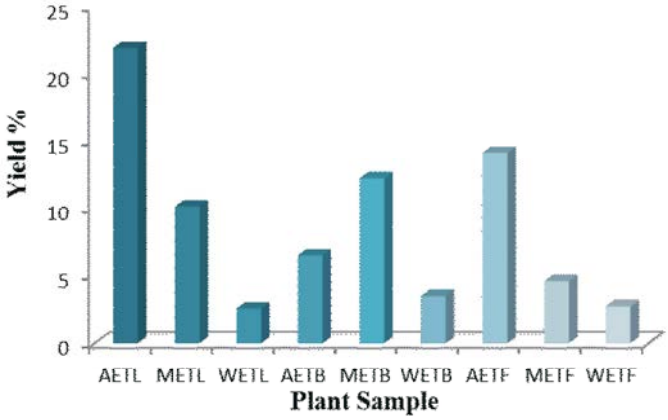

Fig. 1: The per cent yield of different solvent extracts of *Elaeocarpus tuberculatus*

AETL - Acetone extract of *E. tuberculatus* leaf
METL - Methanol extract of *E. tuberculatus* leaf
WETL - Water extract of *E. tuberculatus* leaf
AETB - Acetone extract of *E. tuberculatus* stem bark
METB - Methanol extract of *E. tuberculatus* stem bark
WETB - Water extract of *E. tuberculatus* stem bark
AETF - Acetone extract of *E. tuberculatus* fruit
METF - Methanol extract of *E. tuberculatus* fruit
WETF - Water extract of *E. tuberculatus* fruit

Table 1: Phytochemical screening of different solvent extracts of *Elaeocarpus tuberculatus*

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

extract of stem bark registered a yield percentage of 12.30%. Generally, the acetone and methanol extracts of plant parts contained more phytochemical 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 [24, 25]. Similarly, Singh *et al*. [26] 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%. Similarly, aqueous methanol was found to be more effective in recovering highest amount of phenolic compounds from *Moringa oleifera* leaves [22]. The differences in the extract yields from the tested plant materials in the previous analysis might be ascribed to the different availability of extractable components, resulting from the varied chemical composition of plants [27]. All the above reports were on par with the present investigation.

Table 2: Antimicrobial activity of different solvent extracts of leaf, stem bark and fruit of *Elaeocarpus tuberculatus*

			Zone of Inhibition (mm)				
Sample	Extraction medium	Concentration (µg/ml)	*Shigella sonnei*	*Salmonella typhi*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Candida albicans*
Leaf	Acetone	50	12±1.02	12±0.58	13±0.99	12±0.02	10±0.26
		100	14±1.01	12±0.87	13.5±1.13	16±0.48	12±1.02
		150	17±0.57	15±0.32	14.5±0.47	18±1.16	14±1.01
		200	20±0.62	16±0.25	17±0.95	19±1.01	17±1.23
	Methanol	50	12±0.82	11.5±0.78	11.5±0.14	10±0.05	9±0.58
		100	14±0.77	12±0.69	12±1.11	13±0.15	11±0.79
		150	16±0.98	14±0.99	13±1.05	14.5±0.38	14±0.64
		200	18±0.87	15±0.47	18.5±1.59	16±1.16	15±0.66
	Water	50	12±0.66	-	10±0.83	-	-
		100	13±0.12	11±1.05	11±0.74	10±1.02	-
		150	13.5±1.02	12±0.87	13±0.65	10.5±0.98	11±1.11
		200	14.5±1.01	16±0.22	14±0.77	12.5±0.51	13±0.86
Stem bark	Acetone	50	13±0.67	12±1.31	11.5±0.65	10.5±0.42	12±0.44
		100	14±0.76	14±1.08	13±0.88	12±0.17	13±0.31
		150	15±0.52	16±0.25	15.5±1.15	14±1.02	14.5±0.6
		200	16.5±0.33	17±0.15	17.5±1.62	18±1.16	16±0.91
	Methanol	50	11±0.25	11±0.34	12.5±1.58	9±0.39	-
		100	12±0.39	12±0.95	13±1.21	11±0.9	-
		150	13.5±0.32	13.5±0.75	14.5±0.52	14±0.65	10.5±0.52
		200	15.5±1.10	15±1.26	16±0.32	15±0.11	12±0.19
	Water	50	-	10.5±0.98	-	-	10.5±0.72
		100	10.5±1.21	11.5±0.75	12±0.74	-	12±0.21
		150	11.5±1.04	12±0.25	12.5±0.36	-	13±0.38
		200	13±0.45	14±0.85	16±0.98	11.5±0.77	15±0.27
Fruit	Acetone	50	10±0.78	9±0.61	-	9±0.36	9±0.49
		100	12.5±0.02	10±0.77	10±1.25	11±0.47	12±0.55
		150	13.5±0.18	11±0.12	11.5±1.03	11.5±0.82	14±0.71
		200	15±0.68	13.5±0.32	13.5±0.87	12±0.99	16.5±0.85
	Methanol	50	10±0.53	10±0.98	9±0.65	9±0.26	11.5±0.96
		100	10.5±0.49	11.5±0.42	10±0.98	10.5±1.07	13±0.22
		150	11±0.88	12.5±0.66	10.5±0.77	11.5±1.10	15.5±0.51
		200	12±0.79	14±0.87	12.5±0.54	13±0.09	16±0.49
	Water	50	-	-	-	-	9±0.37
		100	-	-	-	-	10±0.27
		150	10±0.91	-	-	-	11±0.23
		200	11.5±0.02	-	-	-	12±0.21
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)

Generally, the results summarized that the different extracts showed a dose-dependent inhibition of microorganisms (Table 2). The diameter of growth inhibition ranged from 9 to 20mm. The highest zone of inhibition was observed against *Shigella sonnei* (20±0.62 mm) at 200 µg/ml. Normally, the water extracts possessed minimum activity. Moreover, 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 plant parts. The acetone and methanol extracts of leaf and stem bark at high concentrations showed zones of inhibition which was comparable to gentamycin.

In the present findings, the higher concentrations of acetone, methanol and water extracts (leaf, stem bark and fruit) were found to be active against the fungus with zones of inhibition ranging from 11 to 17mm. The acetone extract of leaf suppressed the growth of *Candida* to the maximum (ZI = 17±1.23 mm) at 200 µg/ml. Generally, the lower concentrations of the extracts were susceptible to the fungal pathogen. The various solvent extracts of the plant parts at higher concentrations 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.

Phenolics and polyphenols present in the plants were known to be toxic to the microorganisms [28]. Flavonoids have been reported to have both antibacterial and antifungal activities [29]. Tannins from *Dichrostachys cinerea* root bark possessed antibacterial activities [30]. In the present study, the phytochemical analysis of *E. tuberculatus* solvent extracts revealed the presence of phenols, flavonoids and tannins at varying intensity. The phytochemical characteristics possessed by *E. tuberculatus* may be attributed to its antimicrobial properties. The high inhibitory potential of

acetone and methanol extracts of leaf and stem bark might be due to the high solubility of the phytoconstituents in the organic solvents. Presence of these phytoconstituents in the plant pointed towards its pharmacological activities and supported the claim of the t raditional users. This findings are in agrement with those obtained by Sharker and Shahid [31] and Jayashree *et al.* [15].

In support of the present study, the results of Nair and Chanda [32] 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 [33]. It can be therefore inferred that the active principles of the plant may be more soluble in ethanol than in water. The present findings were also supported by Singh *et al*. [34] 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. Results of the present investigation agreed with the report of Jayashree *et al.* [15]. According to them, *Elaeocarpus serratus* acetone, methanol, water extracts generally produced a clear inhibitory effect on the bacteria and fungi.

CONCLUSION

In recent years, the indiscriminate use of synthetic drugs against microbial pathogens has resulted in mutation of strains making them insensitive to these chemical agents leading to the global hazard of drug resistance. It is high time the hidden wonders of plant molecules were revived with the modern tools of target-based screening to develop newer advanced generation of antimicrobials with novel modes of action. It is inferred from the current findings that the phytoconstituents along with some new microbicidal agents present in the plant extract reflects the high anti-microbial potential of *Elaeocarpus tuberculatus*. The implication of the broad spectrum action of these extracts is that they can be useful in antimicrobial formulation as well as in chemotherapy if the active principle can be isolated.

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