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ANTIBACTERIAL ACTIVITY OF ELAEOCARPUS SPP: AN IN VITRO EVALUATION

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Conflicts of Interest: Nil

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ABSTRACT

The antibacterial action was tried against gram positive microorganisms (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative microbes (*Salmonella typhi, Escherichia coli, Klebsiella pneumonia*, *Pseudomonas fluorescens and Aeromonas hydrophylla*) by agar well diffusion methods. The concentration of (100µg/mL) of the extract was incorporated into the wells. An additional very much was bored into which the standard drugs Streptomycin and Ciprofloxacin was poured. The outcomes demonstrated the most elevated positive antibacterial movement with an inhibition diameter 4.7 mm in case of ethyl acetate extract, when compare to the other solvent extract of clinical pathogens. The purification and the determination of the active components responsible for the antibacterial activity from *Elaeocarpus spp*. seeds.

Keywords: *Elaeocarpus spp,* Streptomycin and Ciprofloxacin, Antibacterial activity

1. Introduction

Antibacterials are the agents which can successfully treat the contaminations brought about by the different strains of microscopic organisms (1). The recurrence of dangerous contaminations brought about by the pathogenic microorganisms has expanded worldwide and is turning into a significant reason for mortality and morbidity in creating nations (2). The antimicrobial properties have been examined by various investigations worldwide and a considerable lot of them have been utilized as helpful options due to their therapeutic properties (3). An assortment of antimicrobial compound is known and being used to control the microbial diseases. In any case, up to specific extents it failed to control the infectious diseases, particularly if there should be an occurrence of drug resistant pathogens (4). To counter this issue there is a persistent need of creating more current, more secure and increasingly powerful antimicrobial medicines (5). Most of the synthetic antimicrobial drugs are potentially toxic and possess many side effects on the host body; accordingly, there is a developing enthusiasm for the pharmacological assessment of different plants utilized in an alternate conventional arrangement of prescription. As for characteristic cause plants based antimicrobial compounds are less nontoxic, less expensive and ecofriendly (6).

Elaeocarpus ganitrus Roxb is an enormous evergreen wide leaved tree having a place with Elaeocarpaceae family. It is otherwise called *E. sphaericus, E.ganitrus* is a huge and evergreen tree normally known as an Utrasum Bead Tree. Leaves and seeds are known for different therapeutic properties and customarily used to pressure, hypertension, epilepsy, depression, lack of concentration, palpitation, asthma, nerve pain, migraine, anxiety, joint inflammation and liver diseases (7). Scientific advancement brought a positive methodology for the foundational investigation of *E. sphaericus* for its therapeutic properties and in most recent two decades *E. sphaericus* has been accounted for to display anti-inflammatory (8), antimicrobial (9), antihypertensive effect (10) and anxiolytic effects (11). However, most of these studies were wearied out on the leaves and very few efforts have been made of the seeds. The focus of this investigation was to decide the antibacterial action of the aqueous extract of *Elaeocarpus spp*. against an assortment of pathogenic microbes and analyzed the presence of amino acids.

2. Material and Methods

2.1. Chemical and reagents

Nutrient agar, Muller Hinton broth (MHB), Muller Hinton agar (MHA), Potato Dextrose Broth (PDB), Patato Dextrose Agar (PDA), Streptomycin and Ciprofloxacin Disc, were purchased from Himedia Pvt Ltd, Mumbai, India.

2.2. Sample Collection

Mature and healthy seeds of *Elaeocarpus spp*. were collected from local markets, Coimbatore, Tamil Nadu, India. The seeds were shade dried and pulverized to a coarse powder using manually operating pestle and mortar.

2.3. Preparation of solvent extract

The coarse powder of *Elaeocarpus spp*. Seeds (20gm) were extracted with 200mL of Petroleum ether, Ethyl acetate and Chloroform solvents were selected based on the polarity. This extraction was done in the soxhlet method. The extract was filtered using Whatman filter paper (No.1) and then concentrated using rotary evaporator and dried using lyophilizer. The final crude extract was stored at 4°C for further analysis. The extracted powder was dissolved in double distilled water as 100 µg/mL solution. This mixture was used to perform the antibacterial assay.

2.4. Test microorganisms

The following different clinical isolates of bacteria such as *Salmonella typhi, Escherichia coli, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas fluorescens*, *Aeromonas hydrophylla* and *Staphylococcus aureus* were used to study the antibacterial activity of *Elaeocarpus spp*.

2.5. Antimicrobial activity

The agar well diffusion method was used to determine the inhibitory effects of the seed extracts against the microbes. The bacterial isolates obtained were first grown in nutrient broth for 24 hr at 37°C. The nutrient agar medium was prepared and it was poured into sterile petri dishes while hot and then they are allowed to set. After this stipulated period, 0.2 mL of the broth culture of the bacteria was aseptically inoculated on this plate using a sterile cotton swab and allowed to dry. Confluent growth is desirable for accurate results. Wells of 6 mm size was made in the agar plates with the help of the sterile stainless steel borer and these wells were loaded with concentration (100 µg/mL) of the *Elaeocarpus spp*. the plates were incubated at 37°C for 24 hr. Streptomycin and Ciprofloxacin disc were used as a standard for antibacterial assay respectively. The zone of inhibition was measured in mm diameter and recorded.

2.6. Amino acid analysis

Elaeocarpus spp. seeds were subjected to separate free and bound amino acids using an amino acid analyzer. Free amino acids were extracted from 1gm of *Elaeocarpus* spp. seeds in ethanol. This was followed by extraction of bound amino acids from acid hydrolysate of the residue by using butanol: formic acid: water (75: 13: 12 v/v/v) and buffer saturated phenol as solvent systems. Identification of the spots was carried out by comparing the Rf values of standard amino acids, run simultaneously in the Amino acid analyzer.

2.7. Statistical analysis

The values of antibacterial activity of *Elaeocarpus* spp. The extract is expressed as a mean ± standard deviation of the response of three replicate determination per sample. Results were analyzed statically by using Microsoft Excel 2007.

3. Results and discussion

Pathogenic microorganisms are one of the significant reasons for medical issues in people and animals and their infectious nature make it hard to control (12). In pre antibiotic era, microbial diseases were the significant reason for inopportune passing in people. Not long after the disclosure of antibiotics, the passing rate of microbial contamination has altogether diminished, even though, drug resistant microorganisms remain a noteworthy there at for individuals (13). Subsequently, fresher antimicrobial compounds, with low/no symptoms are attractive for pharmaceutical applications. Higher trees combined an assortment of phytochemicals mixes as optional metabolites to shield themselves from the microbial contaminations and ecological pressure conditions. These phytochemicals are the key mixes with numerous therapeutic properties and

can be abused for the improvement of new pharmaceutical molecule (14).

3.1. Antibacterial activity

In this study, aqueous extract of *Elaeocarpus* spp. seed was screened for antibacterial movement against Gram positive and Gram negative microscopic organisms detached from the clinical examples. The most noteworthy zone of restraint was appeared in gram negative microscopic organisms (Pseudomonas fluorescence) 4.2 mm, when contrasted with the other bacterial strains. Petroleum ether and Chloroform extracted sample was seen in gram positive and gram negative microscopic organisms of (*Staphylococcus aureus* and *Pseudomonas fluorescence*) 2.7mm, when validated with other bacterial strains. Ethyl acetate extracts showed a higher growth inhibition zone followed by chloroform extracts and comparatively other extract was found to be more active. The results further indicated that Gram's negative bacteria were more significantly inhibited the growth than Gram's positive bacteria.

Prior, chloroform, ethanol, benzene, petroleum ether and acetone concentrates of dried *E. sphaericus* fruits were accounted for to posses an expansive range of antimicrobial movement against an assortment of gram positive and gram negative microbes (15). Different organic solvent extracts of beads of *E. ganitrus* were screened for antifungal activity against five different fungal species. *C.albicans* and *A.niger*, whereas water extract inhibited on *A.niger* (16).

3.2. Amino acid analysis:

The results on amino acid composition of *Elaeocarpus* spp. seeds showed that sixteen free amino acids and eleven bound amino acids were detected. Many of these amino acids, excepting leucine/ Isoleucine, proline, serine and threonine, and which were present only in the bound form are common to both free and bound forms. Quantities of individual amino acids were variable with glutamic acid in free form and asparagine in bound form being most abundant. On the other hand, tyrosine was found to be traced.

Table 1: Antibacterial activity

Bacteria	Zone of inhibition in mm							
	Streptomycin	Ciprofloxacin	Petroleum ether		Ethylacetate		Chloroform	
			1%	2%	1%	2%	1%	2%
Salmonella typhi	10.5	12.5	0.7	1.3	2.5	3.7	0.7	1.7
Escherichia coli	12.5	13.5	0.7	1.3	2.7	3.7	1.3	2.5
Bacillus subtilis	10.7	12.3	1.3	1.2	1.3	2.5	0.7	1.5
Klebsiella pneumonia	10.3	10.0	1.3	2.5	1.5	2.0	1.3	2.0
Pseudomonas fluorescence	14.0	13.5	1.3	3.3	3.3	4.7	1.7	2.7
Aeromonas hydrophylla	15.5	15.7	0.7	1.7	2.5	4.5	1.1	2.5
Staphylococcus aureus	15.7	14.5	1.5	2.7	3.5	4.3	1.1	2.5

4. Conclusion

From the present screening, it could be inferred that the seeds of *Elaeocarpus spp.* was more powerful antibacterial against than seeds and could be contrasted with the known anti-infective agents. In addition, critical measures must be taken to save the customary information about therapeutic plants.

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