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ANTIBACTERIAL POTENTIAL OF ETHYLACETATE EXTRACT FROM THE LEAVES OF *ELEPHANTOPUS SCABER* LINN.

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ABSTRACT

In the present investigation, evaluation of the antibacterial potential of ethylacetate leaf extract at a concentration of (5,10,15 and 20µg/ml) of the plant species, *Elephantopus scaber* (Asteraceae) was carried out against certain Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli, Proteus mirabilis and Vibrio cholerae*) by disc diffusion and minimum inhibitory concentration methods. The ethylacetate leaf extract posses significant antibacterial activity at 20µg/ml against the tested bacteria. Furthermore, gram negative bacterial species *Vibrio cholerae* was found to be more sensitive as compared to gram positive. Therefore, the results obtained suggest that the ethylacetate leaf extract exhibited effective antibacterial compounds.

KEYWORDS: ***Elephantopus scaber*, Asteraceae, ethylacetate leaf extract and Antibacterial activity.**

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INTRODUCTION

Plants are a goldmine of novel chemicals and an impressive number of modern drugs have been developed from them[1]. Plants have been used for the treatment of diseases all over the world before the emerging of modern medicines. Natural products have proven their potential to develop new lead for pharmaceutical, nutraceutical and agrochemical[2]. The importance of natural products in modern medicine has been discussed and the value of natural products in this regard can be assessed using 3 criteria

- The rate of introduction of new chemical entities of wide structural diversity, including serving as templates for semi synthetic and total synthetic modification,
- The number of diseases treated or prevented by these substances, and
- Their frequency of use in the treatment of diseases[3].

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern. There are a number of clinically efficient antibiotics becoming less effective due to the development of bacterial resistance[4]. The emergence of bacterial resistance to antibiotic is a major problem and therefore, it is a need to develop new antibiotics with novel mechanism of action. *Elephantopus scaber* Linn belongs to the family Asteraceae. It is an erect, perennial
plant growing up to 15-35 cm height. Rootstock are short, giving off many stout fibrous roots, stem are usually branched with white hairs. Leaves mostly radical oblong in shape and the base has tapering end[4]. The plant is widely distributed in Warmer parts of India (Figure 1).

Figure 1
Elephantopus scaber Linn (Garg 2008[5])

The major phytochemical constitutes of the plant are elephantopin, triterpenes, stigmasterol epifriedelinol and lupeol[6]. The different parts of the plant are used as astringent, antipyretic, diuretic, laxative, analgesic, anti-inflammatory, in bronchitis, small pox and cancer, in diseases of blood, skin and heart, anti-diarrhoel, as a hepatoprotective, antipoison, expectorant, in piles, dysuria, cough, swelling, snakebites, hemorrhoids and as a febrifuge[7].

MATERIALS AND METHODS

Plant material

Elephantopus scaber leaves were collected from Thiruvallur district, Tamilnadu, India. The plant was identified and authenticated by Dr. S.Sankaranarayanan, Head of the department,Department of Medicinal Botany, Sri Sairam Siddha Medical College, Tambaram, Chennai. The old, infected and fungus damaged leaves were removed. Fresh plant leaves were air-dried under shade at room temperature, ground with tissue blender into fine powder and stored in an airtight container for analysis.

Bacterial strains

The bacterial strains used for the assays were Gram positive:*Staphylococcus aureus* MTCC 29213; Gram negative: *Escherichia coli* MTCC 25922, *Proteus mirabilis* MTCC 13315 and *Vibrio cholerae* MTCC 12657. All bacterial

strains used for these studies were obtained from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology Sector 39-A, Chandigarh – 160036, India.

Preparation of ethylacetate leaf extract

The fresh shade dried leaf powder of *Elephantopus scaber* (500 g) was weighed and soaked in 1litre of ethylacetate at room temperature. After 24 hrs of soaking, the extracts were collected, filtered using Whattman filter paper (No.1), centrifuged at 5000 rpm for 15 minutes and the supernatant was collected, concentrated in a vacuum rotary evaporator and used for analysis.

Antibacterial activity of ethylacetate extract from the leaves of Elephantopus scaber tested against human pathogenic bacteria Agar disc diffusion assay

The preliminary testing of antibacterial potential of ethylacetate extract from the leaves of *Elephantopus scaber* was studied using disc-diffusion method[8].Bacteria obtained from the collection centre were grown on Muller Hinton (MH) agar plates. Four young colonies *(Staphylococcus aureus, Escherichia coli, Proteus mirabilis and Vibrio cholerae*) were suspended with 5ml of sterile saline (0.9%) and the density of the suspension adjusted to approximately 3×10^8 colony forming units (CFU). The sterile swab was used to inoculate the dried surface of MH agar plate by streaking four times over the surface of the agar, rotating the plate approximately by 90 ° to ensure an even distribution of the inoculums. The medium was allowed to dry for about 7 minutes before adding a sterile paper disc of 5 mm diameter. Each disc was tapped gently down onto the agar to provide uniform contact. 5, 10, 15 and 20 microlitres of the extract were introduced on each disc (five replicates) and without extract served as a negative control. The plates were incubated at 37 °C for 24 hrs and at the end of the incubation period, the antibacterial activities were evaluated by measuring inhibition zone diameters.

Minimum inhibitory concentration (MIC)

The MIC of ethylacetate extract from the leaves of *Elephantopus scaber* was determined by dilution method[9]. The strains were grown in MH broth to exponential phase representing 3×10^8 CFU/ml. Different dilutions of ethylacetate extract from the leaves of Elephantopus scaber were prepared to give concentrations at 5, 10, 15 and 20 µg/ml respectively. 0.5 ml of each concentration was added into separate test tubes containing 4ml of MH broth inoculated with 0.5 ml bacterial suspension at a final concentration of 10^8 CFU/ml. Each MIC was determined from five independent experiments performed in duplicates. The tubes containing 4.5 ml of bacterial inoculates and 0.5 ml of ethylacetate were used as bacterial controls. 4.5 ml of uninoculated MH broth and 0.5 ml uninoculated broth solution served as a blank. The tubes were incubated at 37 °C for 18 h; inhibition of bacterial growth was determined by measuring the absorbance at 560 nm.

RESULTS AND DISCUSSION

Anowara Jenny et al[10] used petroleum ether, chloroform and methanol as a solvent for extraction in the aerial part of *Elephantopus scaber*. S.Suresh kumar et al[11] used methanol as a solvent for extraction. In the present study we used ethylacetate as a solvent for extraction. The antibacterial activity of ethylacetate extract from the leaves of *Elephantopus scaber* was significant in inhibiting of human pathogenic bacteria. The plant extract showed a broad spectrum of antibacterial activity. The maximum inhibitory activity is noted for one of the gram negative species *vibrio chlorea*. The zone of inhibition for *vibrio chlorea* is found to be 12.6±0.55 in the concentration range of 20µg/ml in the table1. The least inhibitory activity is seen in *proteus mirabilis* with values of 6.4±0.61 at 20µg/ml in the table 1. The disc diffusion assay of this extract showed the gradient value against the concentration used to inhibit the bacteria.

Table 1
Antibacterial activity of ethylacetate extract of Elephantopus scaber against bacterial species[Diameter of the inhibition zone (mm)*]

MICROORGANISM	ETHYLACETATE EXTRACT FROM THE LEAVES OF *ELEPHANTOPUS SCABER*			
	5µg/ml	10µg/ml	15µg/ml	20µg/ml
Staphylococcus aureus	5.7±0.64	6.6±0.79	7.4±0.40	8.05±0.45
Vibrio cholera	9±0	10.33±0.58	11.03±0.37	12.6±0.55
Escherichia coli	4.3±0.81	5±0	6.9±0.28	7.5±0.5
Proteus mirabilis	3±0.5	4.1±.0.5	5.33±0.58	6.4±0.61

The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates ± SD of three replicates.

The minimal inhibitory concentration of ethylacetate extract of *Elephantopus scaber* by two-fold micro broth dilution method against the human pathogenic bacteria was determined. Table-2 indicates that the extract was found to be most significant in the inhibition of bacteria. MIC of ethyl acetate extract showed gradient value against the concentration used to inhibit the bacteria. Furthermore, one of the gram negative bacterial species *Vibrio cholerae* was found to be more sensitive as compared to gram positive and other negative species.

Table 2
Minimal Inhibitory Concentration (MIC) of different phytochemical extracts against bacteria [Optical density values at (560nm)*]

MICROORGANISM	ETHYLACETATE EXTRACT FROM THE LEAVES OF *ELEPHANTOPUS SCABER*			
	5µg/ml	10µg/ml	15µg/ml	20µg/ml
Staphylococcus aureus	0.678±0.05	0.462±0.07	0.396±0.01	0.233±0.04
Vibrio cholera	0.326±0.05	0.225±0.01	0.142±0.01	0.106±0.03
Escherichia coli	0.660±0.04	0.569±0.09	0.469±0.04	0.309±0.06
Proteus mirabilis	0.647±0.03	0.557±0.02	0.464±0.01	0.332±0.01

The Minimal Inhibitory Concentration was determined by measuring the turbidity of the bacterial culture that is the mean of triplicates ± SD of three replicates.

The Minimal Inhibitory Concentration values further support that the ethylacetate extract from the leaves of *Elephantopus scaber* is found to be most efficient in one of the gram negative bacterial species *vibrio chlorea*.

CONCLUSION

The current investigation supports the traditional *Elephantopus scaber* knowledge of local users and encourages the use of plants as an alternative or supplementary medicine to reduce the burden of high cost, risk of side effects and progressively increasing drug resistance of pathogens.

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