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Research Article

STUDY OF PHYTOCHEMICAL AND ANTIMICROBIAL POTENTIAL OF METHANOL AND AQUEOUS EXTRACTS OF AERIAL PARTS OF *ELEPHANTOPUS SCABER* LINN.

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ABSTRACT

The present study was carried out to evaluate the phytochemical, antibacterial and antifungal activity of methanol and aqueous extract of *Elephantopus scaber* Linn. leaf on selected five bacterial pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Leuconostoc lactis* and *Salmonella typhi* and four fungal strains *Aspergillus niger, Aspergillus flavus, Rhizopus indicus* and *Mucor indicus*. For antimicrobial test, well diffusion technique was used and the zone of inhibition of microorganisms was measured in mm. The leaf extract of *E. scaber* showed not much potential antimicrobial activities against the selected strains and the maximum inhibition zone 28 mm was recorded from 200mg of methanol extract of *E. scaber* against *Streptococcus pyogenes* and minimum (18mm) by the above pathogen at 50 mg of methanol extract. The methanolic extract showed the maximum antifungal activity 32mm inhibition zone was recorded from 200mg of extract against *Mucor indicus* and minimum 14mm by 50 mg of extract against *Rhizopus indicus.* The phytochemical analysis revealed the presence of Alkaloids, Flavonoids, Saponins, Tannins, Phenols, Proteins and Carbohydrates in varying concentration.

Keywords: Phytochemical, Antimicrobial, *Elephantopus scaber*, Alkaloids, Flavonoids, Saponins

INTRODUCTION

The increasing failure and side effects of popularity used chemotherapeutic and appearance of multiple drug resistance phenotypes in pathogenic bacteria led to the search of new compounds with antimicrobial activity. Use of herbal products as antimicrobial agents may provide the best alternative to the wide and injudicious use of synthetic antibiotics. The demand on plant based therapeutics is increasing in both developing and developed counties due to growing recognition that they are natural products, non narcotic, easily biodegradable producing minimum environmental hazards, having no adverse side effects and easily available at affordable prices. Therefore researchers are progressively more turning their attention to natural products, looking for new leads to develop better drugs against microbial infections and screening of several medicinal plants for their potential antimicrobial activities (Ghosh *et al*., 2008).

For over several years medicinal plants have served as the models for many clinically proven drugs, and are now being reassessed as antimicrobial agents. Literally thousands of plant species have been tested against hundreds of bacterial strains in vitro and many medicinal plants are active against a wide range of gram-positive and gram-negative bacteria. However very few of these medicinal plant extracts have been tested against resistant bacteria. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Plants produce a wide array of organic compounds, usually secondary metabolites, which in addition to imparting characteristic odour, pigment and flavour properties, sometimes exhibit antimicrobial action. The extraction, and possible subsequent therapeutic application, of these biologically active phytochemicals is not a recent development, with many plant-derived antimicrobials undergoing clinical trials for human use (Cowan, 1999).

The investigation of traditional medicinal plants, function not only to scientifically validate purported properties of traditional medicinal plants, but also in the identification of possible sources of effective drugs. It follows, therefore, that as plant products become more widely exploited as a source of antimicrobial agents, those products in-turn require rigorous characterization, both in identification of active constituents, and in the study of mechanisms by which they exert their antimicrobial activity. However, few studies have included further investigations of the biological action of antimicrobial plant extracts (Ahmad and Beg, 2001).

The medicinal plant *Elephantopus scaber*, a member of the family Asteraceae known for its medicinal properties was also reported to posses antimicrobial activity (Avani and Neeta, 2005). A new terpenoid was isolated from the acetone extract of *E. scaber*. Biological testing of the compound demonstrated a significant anti-bacterial activity against a few multi drugs –resistant ESBL producing clinical isolates. But the mechanism of the anti-bacterial effect of the compound was not clearly understood to overcome this they tried in nonconventional methods of drug designing by the use of Bioinformatics approaches (Jasmine *et al*., 2007).

MATERIALS AND METHODS

Preparation of plant extracts

Fresh Plant leaf of *Elephantopus scaber* was collected from Nolambur village, Villupuram district, Tamil Nadu, India; they were identified with the help of Gamble's flora.

Preparation of powder (Harborne, 1973)

The leaves of plants were collected and dried under shade. These dried materials were mechanically powdered sheaved using 80 meshes and stored in an airtight container. These powdered materials were used for further physiochemical, phytochemical and fluorescent analysis

Extraction of plant material

Various extracts of the study plant was prepared according to the methodology of Indian Pharmacopoeia (Anonymous, 1966). The leaves were dried in shade and the dried leaves were subjected to pulverization to get coarse powder. The coarse powder material was subjected to Soxhlet extraction separately and successively with methanol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50^{0}C). Both the extracts were stored in a refrigerator in air tight containers. Both the extracts were analyzed for phytochemical screening of compounds, antimicrobial and pharmacological activity.

Phytochemical studies

Qualitative phytochemical analyses were done by using the procedures of Kokate *et al.* (1995). Alkaloids, carbohydrates, tannins, phenols, flavonoids, gums and mucilages, phytosterol, proteins and amino acids, fixed oils, fats, volatile oil and saponins were qualitatively analyzed.

Test organisms

The stored culture of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Leuconostoc lactis* and *Salmonella typhi* were collected from the Microbial Type Culture Collection (MTCC), The Institute of microbial Technology. Sector 39-4, Chandigarh, India.

The pathogenic fungal strains *Aspergillus niger, Aspergillus flavus*, *Rhizopus indicus* and *Mucor indicus* were collected from the Microbiological Lab, Christian Medical College, Vellore, Tamil Nadu, India.

Antibacterial Studies

Bacterial Media (Muller Hindon Media)

Thirty Six grams of Muller Hindon Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petridishes. The solidified plates were bored with 6mm dia cork porer. The plates with wells were used for the antibacterial studies.

Antifungal Studies

Fungal media (PDA)

Two Hundred gram of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20g of dextrose was mixed with potato infusion. 20g of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with 6mm dia cork porer.

Well diffusion method

Antibacterial and Antifungal activity of the plant extract was tested using well diffusion method (Bauer *et al*., 1996). The prepared culture plates were inoculated with different bacteria and fungus by using plate method. Wells were made on the agar surface with 6mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37±2°C for 24 hours for bacterial activity and 48 hours for fungal activity. The plates were observed for the zone formation around the wells.

The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

RESULTS AND DISCUSSION

Physico-chemical constants of leaf powder

The powder of the leaf was analyzed for various physicochemical constants and loss on drying (LOD).

Ash values

Total ash, water-soluble ash, acid-insoluble ash, acid soluble, alcohol solubility values of the leaf powder was done and the results are tabulated in Table 1.

Fluorescence analysis of leaf powder

The powder of root is examined in daylight, to detect the fluorescent compounds and the observations are given in Table 2. The fluorescence colour is specific for each compound. A non fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyze over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples (Pimenta *et al.,* 2006).

Preliminary phytochemical screening

The results of phytochemical examination of both the extracts were given in Table 3.

The phytochemical screening carried out on the *Elephantopus scaber* leaf extracts. Phytochemical compounds present were found to be reducing carbohydrates, saponins and proteins in both the extracts, flavonoids were found to be present in only the aqueous extract and alkaloids in methanolic extract only, while steroids, fixed oils & fats, gums & mucilage and volatile oils were not detected in both the extracts. The variation in type of phytochemicals present in different solvents as shown in the result of phytochemical screening might be attributed to the ability of the solvents to dissolve into solution specific type of phytochemicals as reported by Yusha'u *et al*. (2008).

Tannins bind to proline rich proteins and interfere with the protein synthesis (Shimada, 2006). Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Marjorie, 1999).

The results of antibacterial activity were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of bacterial growth and it as reported as positive and absence of zone as negative (Panthi and Chaudhury, 2006). The antibacterial activity of methanolic extract of *E. scaber* indicated that the crude solvent extracts possess antibacterial activities towards the Gram-positive bacterium *Streptococcus pyogenes* to more extent than Gram negative bacteria. Their was no marked activity against Gram positive and Gram negative bacteria when compared to both solvent extracts except methanol extract against *Streptococcus pyogenes* (Table 4).

The resistance of Gram-negative bacteria to plant extracts was not unexpected as; in general, this class of bacteria is more resistant than Gram-positive bacteria. Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (Adwan and Abu-Hasan, 1998). The methanolic extract of *Elephantopus scaber* showed significant anti bacterial activity against the pathogenic bacteria (Suresh Kumar *et al.,* 2004).

The antifungal activity of both the extract of *E. scaber* leaf extracts were determined against four fungal strains and recorded in Table 5. The antifungal activity was also observed in dose dependent manner and highest activity was observed with methanolic extract against *Mucor indicus* (32mm) and minimum activity was recorded against *Rhizopus indicus* (14mm). There was no activity observed in aqueous extract against the tested fungus. Our findings are in accordance with the observations of Ravindra *et al*., who proved that highest antifungal activity was observed with methanolic extract of *Capparis pepiaria* against the tested fungal strains.

Table 1: Pharmacognostic characters of leaf of Elephantopus scaber

S. No	Parameters	Percentage yield (%)
1.	Total ash value	38
2.	Acid insoluble ash value	27
3.	Acid soluble value	73
4.	Solubility % in alcohol	45
5.	Solubility % in water	31

Table 2: Analysis of fluorescence characters of leaf powder of *Elephantopus scaber* in different chemical reagents

S. No	Chemical reagent	Appearance
1.	Powder colour	Green
2.	5% NaOH	Green
3.	10% NaOH	Light green
4.	Con. H_2SO_4	Brown
5.	Acetic Acid	Green
6.	1N NaOH in H_2O	Brown
7.	5% KOH	Brown
8.	50% HNO_3	Dark brown
9.	5% $FeCl_2$	Dark green
10.	1N HCl	Light brown
11.	Con.HNO_3	Light brown
12.	1N NaOH in Ethanol	Dark green
13.	50% H_2SO_4	Dark green
14.	50% HCl	Dark green
15.	Con. HCl	Dark green

Table 3: Results of phytochemical screening of aqueous leaf extracts of *Elephantopus scaber*

S. No.	Name of the compounds	Name of the test	Status of the substances	
			Aqueous extract	Methanolic extract
1.	Carbohydrates	Fehling's	++	+
		Benedict's	+	+
2.	Alkaloids	Mayer's	-	-
		Hager's	-	+
		Wagner's	-	-
		Dragen Dorfff's	-	+
3.	Steroids	Chloroform + Acetic acid + H_2SO_4	-	-
4.	Tannins & Phenols	10% Lead Acetate	+	-
		5% Ferric Chloride	-	+
		1% Gelatin	-	-
5.	Saponins	Foam test	+++	++
6.	Fixed oils & Fats	Spot test	-	-
7.	Gums & Mucilage	Alcoholic Precipitation	-	-
8.	Proteins	Biuret test	+++	++
9.	Flavonoids	NaOH / HCl	+	-
10.	Volatile oils	Hydro distillation method	-	-

++++ = High rich amount; +++ = Rich amount; ++ = Moderate amount; + = Minimum amount; - = absent

Table 4: Inhibition zone of Aqueous and Methanol extracts of *Elephantopus scaber* against bacterial pathogens

S. No.	Name of the organisms	Zone of inhibition					
		Aqueous extract			Methanol extract		
		50mg	100mg	200mg	50mg	100mg	200mg
1.	*Staphylococcus aureus*	-	-	-	-	-	-
2.	*Escherichia coli*	-	-	-	-	-	-
3.	*Leuconostoc lactis*	-	-	-	-	-	-
4.	*Salmonella typhi*	-	-	-	-	-	-
5.	*Pseudomonas aeruginosa*	-	-	-	-	-	-
6.	*Streptococcus pyogenes*	-	-	-	18±3.7	22±1.4	28±3.7

Table 5: Inhibition zone of Aqueous and Methanol extracts of *Elephantopus scaber* against fungal pathogens

S. No.	Name of the organisms	Zone of inhibition					
		Aqueous extract			Methanol extract		
		50mg	100mg	200mg	50mg	100mg	200mg
1.	*Aspergillus flavus*	-	-	-	-	-	-
2.	*Mucor indicus*	-	-	-	22±2.4	26±2.8	32±2.8
3.	*Aspergillus niger*	-	-	-	15±3.7	19±2.8	22±2.8
4.	*Rhizopus indicus*	-	-	-	14±2.8	18±3.7	23±2.8

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