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SCREENING OF PHYTOCHEMICALS, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *ELEPHANTOPUS SCABER* LEAVES

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ABSTRACT

Elephantopus scaber is native to India and is commonly used in a broad variety of medicinal applications. *Elephantopus scaber* Linn. (Asteraceae) is a scabrescent aromatic herb distributed in the moist deciduous forests of the central Western Ghats. Though the plant has been extensively used in different systems of medicine for the treatment of various types of diseases, yet, there are few reports in the literature of studies on its chemical composition and biological properties. In this study, the antioxidant and anti-bacterial activities of the plant were evaluated and the phytochemical analysis was performed. The antioxidant activity of the extracts was measured using DPPH method and antibacterial activity by disc diffusion assay. Our findings suggest that *E.scaber* leaves possess potential antioxidant and anti-bacterial activity, which could be considered as drug candidate against oxidative stress and bacterial infection related pathological processes in medicinal chemistry studies.

KEYWORDS: *Elephantopus scaber*, antioxidant, antibacterial.

INTRODUCTION

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Increase in drug resistance calls for alternate therapy. It is estimated that today, plant materials are present in, or have provided the models for 50% Western drugs.[1] Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic

treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency.[2-4] Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. *Elephantopus scaber* Linn belongs to the compositae family. It is an erect, perennial plant; grow up to 15-35 cm height. The plant has been extensively used in different systems of medicine, for the treatment of various types of diseases.[5,6] In the Indian system of medicine, the medicinal attribution of this species has been known for a long time, yet scientific validation is essential to establish the medicinal property. Hence, this study was undertaken to evaluate the antibacterial and antioxidant activity of ethanol extract of *Elephantopus scaber* leaves.

MATERIALS AND METHODS

Collection of plant materials

Elephantopus scaber leaves were collected from Kerala during the rainy season and the plant was authenticated by the botany department of our college and the voucher specimen was deposited. The leaves chosen for the study had been washed, macerated and lyophilised. About 500g of the leaves yielded 33g powder. The procedure was repeated to collect the needed quantity.

Preparation of plant extracts

100g of the powdered peels extracted in Soxhlet apparatus separately using 1 L of ethanol for 18h and then filtered. The filtrates were evaporated to dryness under reduced pressure and at a lower temperature in a rotary evaporator. The dried residues were stored in airtight containers for further use.

Culture

Bacterial cultures as *E.coli, Staphylococcus aureus, Proteus sps, Klebsiella pneumoniae* were obtained from the microbiology department of a local hospital, Tiruchirappalli, India. These clinical isolates were collected and stored in semi-solid agar and were plated out on nutrient agar plates and on MacConkey agar plates to check the viability of the bacteria.

Phytochemical screening

For qualitative phytochemical analysis, the ethanolic extract of leaves of *E.scaber* plant were tested by using standard protocols.

Antimicrobial Susceptibility Testing Disc Diffusion test (Kirby Bauer, 1966)[7]

All isolates were tested for susceptibility to the extracts and antimicrobial agents on Mueller Hinton agar (Hi-Media India) by the standard disc diffusion method recommended by the National Committee for Clinical Laboratory Standards.[8] The diameter of the zone of inhibition of growth was recorded, and interpreted by the criteria of CLSI.

Determination of Minimal Inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) can be determined by adopting the procedure outlined by CLSI using the microtitre plates and were examined for bacterial growth.[8] The Minimal Inhibitory Concentration (MIC) assay is performed to determine the concentration of the extract that is lethal to the target bacteria in vitro.

DPPH radical scavenging assay

The ability of *E.scaber* to scavenge 1, 1- diphenyl-2 picrylhydrazyl (DPPH) was measured by the reported method.[9] A mixture of absolute methanol and extract served as blank. Ascorbic acid was used as standard and different concentrations of the extract (100,200,300,400 and 500 µg/ml) were marked as tests. Finally DPPH reagent was added to all the test tubes including blank. Then, the absorbance of all samples was read at 515nm.

Calculation

% Antioxidant activity = {(absorbance at blank) – (absorbance at test) / (absorbance at blank)} X 100

RESULTS AND DISCUSSION

The ethanol extract obtained was subjected to preliminary phytochemical screening. The leaf extract of *E. scaber* shown the presence of various phytochemical constituent such as phenols, flavonoids, proteins and carbohydrates. The leaf extract was effective against all the tested bacterial isolates. The inhibition was dependent on the type of dissolution solvent used. Inhibition was most favoured when plant sample was dissolved in ethanol. The ethanol extract exhibited good antibacterial activity against the tested gram negative intestinal bacteria, where the zones ranged between 10mm and 18mm (Table 3). The antibacterial activity was dose dependent, where 50 µl gave the maximum effect. The DPPH radical scavenging ability of the ethanolic extract of *E.scaber* at different concentrations was compared with its reference standard ascorbic acid and the activity was highest at maximum dose of 500µg/mL with the inhibition percentage being 96% while that of ascorbic acid being

92.8% (Table 4).The effect seemed to be concentration dependent. The antioxidant property of DPPH radical scavenging is due to their hydrogen donating ability. Reactive oxygen species are essential for multiple normal physiological processes like cell differentiation, apoptosis, cell immunity and cellular defense against microorganisms at low concentration. Excess generation of these oxygen free radicals and oxidants generate a phenomenon called oxidative stress which cause oxidative damage to biomolecules resulting in lipid per oxidation, mutagenesis and carcinogenesis. It is also evident that overproduction of ROS plays an important role in the promotion and progression of human cancers including breast cancer. The human body is equipped with certain enzymatic and non- enzymatic antioxidant systems. Antioxidants are known to dispose, scavenge and suppress the formation of free radicals.[10]

Table 1: Phytochemical Screening of the ethanolic extract of *E.scaber* leaves.

S.No	Phytoconstituents	Presence/Absence
1.	Steroids	-
2.	Carbohydrates	-
3.	Proteins	+
4.	Aminoacids	+
5.	Anthacyanins	-
6.	Phenols	+
7.	Alkaloids	-
8.	Flavonoids	+
9.	Saponins	-

+ = presence - = absence

Table 2: Zones of inhibition of few antibiotics (ZOI in mm) on the chosen isolates.

S.No	Antibiotics	*E.coli*	*Klebsiella sp*	*Proteus sps*	*Staphyococcus sps*	*Streptococcus sps*
1.	Bacitracin (10 units/disc)	R	R	R	R	R
2.	Streptomycin (10 mcg/disc)	R	13mm	R	R	10mm
3.	Novobiacin (30 mcg/disc)	17mm	8mm	7mm	18mm	8mm
4.	Penicillin (10 units/disc)	R	R	R	R	R
5.	Ceftazidime (30 mcg/disc)	22mm	30mm	9mm	26mm	16mm

Table 3: Zones of inhibition of leaf extract *of E.scaber* (ZOI in mm) on the chosen isolates.

S.no	Chosen isolates	Zones of inhibition (in mm)		
		30µl	40µl	50µl
1.	*E.coli*	10 mm	12 mm	16 mm
2.	*Klebsiella sps*	R	14 mm	16 mm
3.	*Proteus sps*	11 mm	14 mm	18 mm
4.	*Staphylococcus sps*	R	14 mm	16 mm
5.	*Streptococcus sps*	R	R	15 mm

Table 4: Antioxidant activity of *E.scaber* leaves.

S.No	Concentration (in µg)	% of antioxidant activity of Std. (ascorbic acid)	% of antioxidant activity of extract
1.	100	65.5	34.3
2.	200	76.3	58.6
3.	300	79	85.6
4.	400	79.3	90.1
5.	500	92.8	96

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