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Phytochemical Screening and Evaluation of *In-vitro* Anti-inflammatory Activity of Leaf Parts of *Elephantopus scaber*

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Abstract
Elephantopus scaber has tremendous reputation in indigenous traditional system of medicine in India by virtue of which it has drawn attention and concern of scientists for validation of its medicinal properties through phytochemical and pharmacological evaluation. The current was conducted with the main objectives of phytochemical screening and evaluation of *in-vitro* anti-inflammatory activities of leaf parts of *E. scaber*. Leaves of *E. scaber* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with methanol. Results delineated that the highest membrane stabilization *i.e.*, 98.93% and 95.84% was observed at 100 mg/ml concentration of methanolic leaf extract of *E. scaber* in HRBC membrane stabilization method and in heat induced hemolysis method respectively which was at par with membrane stabilization effects of standard diclofenac sodium. The major phytochemicals found in methanolic leaf extract of *E. scaber* were found to be alkaloids, flavonoids, saponins, phenolic compounds/tannins, and terpenoids. In conclusion, methanolic leaf extract of *E. scaber* has potential to exhibit anti-inflammatory properties, and hence could be explored for the development of natural anti-inflammatory agents.

Keywords: *Elephantopus scaber*, Leaf extracts, Anti-inflammatory agents, Flavonoids, Saponins

Introduction

Medicinal plant based traditional system of medicines are playing an important role in providing health care to large section of population, especially in developing countries. It is a well-known fact that the traditional system of medicines always played an important role in meeting the global health care needs. India has the unique distinction of having six recognized system of medicine in this category. They are Ayurveda, Siddha, Unani and Yoga, Naturopathy and Homeopathy.[1] Most of the traditional systems of India including Ayurveda have their roots in folk medicine. Traditional system of medicine in India functions through two major streams the local health tradition and the classical scientific system of tradition. The carriers of local health care system are millions of people who cure diseases at home as a birth attendant and practitioners of snake bite and jaundice treatments.[2]

Elephantopus scaber which is an erect herb up to 80 cm tall (Figure 1). The plant is a native to Tropical Africa, Eastern Asia, Indian Subcontinent, Southeast Asia and Northern Australia. Its natural habitat is subtropical or tropical moist montane forest. It is a perennial herb found as under growth in shady places. The whole plant of *E. scaber* is well known as herb of Chinese folk medicine which is widely used in the treatment of nephritis, edema, dampness, pain in the chest, fever and cough of pneumonia, scabies and arthralgia due to wounding.[3] The root decotion of *E. scaber* is widely used to treat diarrhoea, dysentery, stomach troubles and blood vomiting in tuberculosis in Nepal.[4,5] Sesequiterpenes lactones, triterpenoids, steroids, flavonoids and essential oil constituents have been reported from various part of the plant. The plant has been extensively screened for anticancer activity.[6]

Figure 1: Showing *Elephantopus scaber* plant

Thus, *E. scaber* has tremendous reputation in indigenous traditional system of medicine in India by virtue of which it has drawn attention and concern of scientists for validation of its medicinal properties through phytochemical and pharmacological evaluation. Most of the plants used in herbal medicine practices, used by plant healers of remote villages and primitive aborigines have not yet been completely investigated for their phytochemical constituents and pharmacological activities. With this background, the presented study was conducted with the main purpose of phytochemical screening and evaluation of *in-vitro* anti-inflammatory activities of leaf parts of *E. scaber*.

Materials and Methods

Collection Leaves of *E. scaber*

The leaves of *E. scaber* were purchased from local vegetable market in Chikkaballapur, Karnataka, India. The leaves were gently and thoroughly washed with running tap water to remove the dirt particles and wiped off, and sprayed with ethanol, and then shade dried. The dried leaves were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

Approximately 50 g of dried and coarsely powdered leaves of *E. scaber* were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 500 mL of methanol. The extract was concentrated by distilling the solvent in a rotary flash evaporator and dried at 40ºC. The extract was preserved in airtight containers and stored at room temperature until further use.

Phytochemical Screening

Phytochemical screening was carried out on the methanolic leaf extract of *E. scaber* by using standard procedure to detect phytoconstituents as described by sofora,[7] Trease and Evans[8] and Harborne.[9]

Test for alkaloids

Approximately 0.2g of methanolic leaf extract of *E. scaber* was warmed with 2% H_2SO_4 (2.0ml) for two minutes. The reaction mixture was filtered and few drops of Dragendrof's reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

Test for tannins and phenolic compounds

The methanolic leaf extract of *E. scaber* in **s**mall quantity was mixed with water and heated on water bath and filtered. To the filtrate, few drops of ferric chloride ($FeCl_3$) was added. A dark green colouration indicate the presence of tannins and phenolic compounds.

Test for glycosides

About 0.6g of methanolic leaf extract of *E. scaber* was hydrolyzed with HCl and neutralized with NaOH solution and few drops of Fehling's solution A and B were added. Formation of red precipitate indicates the presence of glycosides.

Test for saponins

About 0.2g of methanolic leaf extract of *E. scaber* was shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

Test for flavonoids

0.2g of methanolic leaf extract of *E. scaber* was dissolved in diluted 10%NaOH and few drops of 2M HCl was added. A yellow solution that turns into colorless indicate the presence of flavonoids.

Test for steroids

2 mL of acetic anhydride was added to 0.5g of methanolic leaf extract of *E. scaber* and then added 2 mL of H_2SO_4. The change of color from violet to blue or green or red showed the presence of steroids.

Test for terpenoids

0.3g of methanolic leaf extract of *E. scaber* was mixed with 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated 6M H_2SO_4 was carefully added to form a layer. Formation of reddish-brown coloration at the interface indicates positive results for the presence of terpenoids.

Evaluation of Anti-inflammatory Activities

HRBC membrane stabilization method

The anti-inflammatory activity of methanolic leaf extract of *E. scaber* was assayed by human red blood cell (HRBC) membrane stabilization method.[10] The blood was collected from a healthy human volunteer who had not taken any NSAIDs for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Different concentration of extracts was prepared (25, 50, 75, 100 mg/ml) using DMSO and to each concentration, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 37°C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated by spectrophotometer at 560nm. Diclofenac sodium (100 mg/ml) was used as a standard drug and a control was prepared by omitting the extracts.

Percentage protection (%) = 100 – [(optical density (OD) of sample/OD of control) X 100]

Heat-induced hemolysis method

The reaction mixture (2 ml) consisted of 1 ml of test sample solution i.e., different concentration of extracts was prepared (25, 50, 75, 100 mg/ml) using DMSO and 1 ml of 10% RBCs suspension, instead of test sample the only saline was added to the control test tube. Diclofenac sodium (100 mg/ml) was used as standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 minutes. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 minutes and the absorbance of the supernatants was taken at 560 nm.

Percentage of protection (%) = 100 – [(OD of sample/OD of control) X 100]

Results

Phytochemical screening

The major phytochemicals found in methanolic leaf extract of *E. scaber* were found to be alkaloids, flavonoids, saponins, phenolic compounds/tannins, and terpenoids. Whereas, phytochemicals *viz.* glycosides, and steroids were found to be absent (Table 1).

Table 1: Photochemical screening of methanolic leaf extract of *E. scaber*

Phytochemical Components	**Methanolic Leaf Extract of *E. scaber***
Alkaloids	+
Flavonoids	+
Glycosides	-
Saponins	+
Steroids	-

Phenolic compounds	+
Tannins	+
Terpenoids	+

The results of anti-inflammatory effects of methanolic leaf extract of *E. scaber* evaluated by HRBC membrane stabilization method was represented in Table 2. Results delineated that methanolic leaf extract of *E. scaber* provided membrane stabilization of 85.45%, 88.59%, 96.68%, and 98.93% at 25 mg/ml, 50 mg/ml, 75 mg/ml, and 100 mg/ml respectively. These findings revealed that highest membrane stabilization *i.e.*, 98.93% was observed at 100 mg/ml concentration of methanolic leaf extract of *E. scaber* which was at par with membrane stabilization effects of standard diclofenac sodium (99.89%).

Table 2: Anti-inflammatory effects of methanolic leaf extract of *E. scaber* in HRBC membrane stabilization method

Concentration of Methanolic Leaf Extract of *E. scaber*	**Protection (%)**
25 mg/ml	85.45
50 mg/ml	88.59
75 mg/ml	96.68
100 mg/ml	98.93
Diclofenac sodium	99.89

Values were expressed as Mean; n=3

The results of anti-inflammatory effects of methanolic leaf extract of *E. scaber* evaluated by heat induced hemolysis method was represented in Table 3. Results delineated that methanolic leaf extract of *E. scaber provided membrane stabilization of 46.49%*, 51.58%, 86.43%, and 95.84% at 25 mg/ml, 50 mg/ml, 75 mg/ml, and 100 mg/ml respectively. These findings revealed that highest membrane stabilization *i.e.*, 95.84% was observed at 100 mg/ml concentration of methanolic leaf extract of *E. scaber* in heat induced hemolysis method which was at par with membrane stabilization effects of standard diclofenac sodium (98.64%).

Table 3: Anti-inflammatory effects of methanolic leaf extract of *E. scaber in heat induced hemolysis method*

Concentration of Methanolic Leaf Extract of *E. scaber*	Protection (%)
25 mg/ml	46.49
50 mg/ml	51.58
75 mg/ml	86.43
100 mg/ml	95.84
Diclofenac sodium	98.64

Values were expressed as Mean; n=3

Discussion

Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. *E. scaber* has been traditionally used as medicine to treat rheumatism, diarrhoea, gout, eczema, gum infections, toothaches, spider and snake bites. The extracts or compounds from *E. scaber* have been shown to have antibiosis, antivirus, and cytotoxicity actions in previous bioactivity studies. Particularly, the hepatoprotective and anti-inflammatory properties of sesquiterpene lactones have been studied. Hence, in the present study we aimed for phytochemical screening and evaluation of *in-vitro* anti-inflammatory activities of methanolic leaf extract of *E. scaber.*

Results of our study depicted that the major phytochemicals found in methanolic leaf extract of *E. scaber* were found to be alkaloids, flavonoids, saponins, phenolic compounds/tannins, and terpenoids. The highest membrane stabilization *i.e.*, 98.93% and 95.84% was observed at 100 mg/ml concentration of methanolic leaf extract of *E. scaber* in HRBC membrane stabilization method and heat induced hemolysis method respectively which was at par with membrane stabilization effects of standards diclofenac sodium. These findings inferred that the anti-inflammatory potential of methanolic leaf extract of *E. scaber* could be mainly due to presence of secondary metabolites such as alkaloids, flavonoids, saponins, phenolic compounds/tannins, and terpenoids.

It was evident from our research findings that methanolic leaf extract of *E. scaber* could inhibit lysis of the red blood cells membrane, and thereby provided the protection These activities are mainly accredited to flavonoids and saponins. In concurrence with our study findings literature findings revealed the content of flavonoids and saponins in plants could inhibit the phospholipase A2.[11] Phospholipase A2 is an enzyme that acts to break down the Sn-2 fatty acid of membrane phospholipid that encloses arachidonic acid which plays an important role in the inflammatory process.[12] The arachidonic acid is subsequently converted to prostaglandin by COX-1 and COX-2 enzymes. COX-2 is an enzyme responsible for the inflammatory process and its activity is induced by inflammatory, hormonal, and growth

factors. Flavonoids and saponins are also known to have an activity that can inhibit COX-2 which is a non-steroidal anti-inflammatory drug target.

Furthermore, *E. scaber* could protect the lysis of red blood cell membranes by exposure to harmful stimuli such as heat. Harmful stimuli could induce expression of TNF- α by activating immune response. TNF-α plays an important role in inflammation. TNF-α causes changes in vascular endothelium. In physiological conditions, leukocytes move freely along the vascular endothelium. TNF-α causes the vascular endothelium to become pro-inflammatory, subsequently increasing the adhesion of leukocytes to the vascular endothelium, transendothelial leukocyte migration, vascular leakage, and increased thrombosis.[13,14] Saponins contained in plants can inhibit TNF-α. Moreover, studies in the literature revealed that a substance that can inhibit inducible Nitric Oxide Synthase (iNOS) may act as an anti-inflammatory agent. Flavonoids, saponins, and tannins present in plants have been reported to inhibit iNOS in the literature by various research investigators.[15-18]

Conclusion

The results of this preliminary study clearly demonstrated that methanolic leaf extract of *E. scaber* has potential to exhibit anti-inflammatory properties. The anti-inflammatory properties of *E. scaber* could be accredited the presence of secondary metabolites mainly flavonoids and saponins. Hence, methanolic leaf extract of *E. scaber* could be explored for the development of natural anti-inflammatory agents. However, further *in-vivo* studies are recommended to ascertain the safety, efficacy and possible mechanism of action anti-inflammatory activities of *E. scaber*.

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