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PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *ELEPHANTOPUS SCABER* L. – A WILD MEDICINAL PLANT

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ABSTRACT

Elephantopus scaber L. is a popular plant that has been traditionally used as medicine to cure several diseases. It shows encouraging prospect in treatment of cancer, diabetics, rheumatism, fever, scabies and many other ailments. Phytochemical analysis revealed that the plant is a rich source of terpenoids and flavonoids. The present investigation deals with the ash and extractive values, fluorescence analysis and preliminary phytochemical screening of leaf powder of *Elephantopus scaber* L. Phytochemical analysis of the aqueous, methanol, Di ethyl ether and acetone fractionated portions of the leaf extracts revealed the presence of quinones, steroid, flavonoids, phenols, cardiac glycoside, cholesterol, tannins and terpenoids. Aqueous extract could indicate presence of saponins. Coumarins could be marked when methanol and water were used as solvent. Anthraquinones were found absent whereas alkaloids could be detected with all solvents except acetone.

KEYWORDS: Preliminary phytochemical screening, *Elephantopus scaber*, fluorescence analysis, pharmaceuticals.

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INTRODUCTION

Plants are known to contain organic substances synthesized by both primary and secondary metabolic processes. Secondary metabolites often play an important role in plant defense against herbivory, parasites, pathogens and in maintenance of its relationship with other species, thus affording us an opportunity since ancient times to use their secondary metabolites as medicines, flavoring agents and recreational drugs. Plants have been traditionally proving their excellence in medicinal use so that they can be used to prepare remedies for a variety of diseases utilizing their secondary metabolites selectively. As such scientists emphasize upon phytochemical screening of secondary metabolites of plants used in traditional systems for further pharmacological evaluation[1] and pharmacognostic characterization[2]. Accordingly *Elephantopus scaber* L., which has reputation of esteem in Indian ethnomedicine, was selected for phytochemical screening of its secondary metabolites.*Elephantopus scaber* L. belonging to the family Asteraceae is a terrestrial, scabrescent, aromatic, erect, stiff, perennial herb being about 60 cm in height. Leaves are mostly radical in basal rosette and a few cauline, finely dentate, obovate-oblong. Its capitula (heads) are numerous arranged in terminal dichotomous cyme with clusters of purple to dull pink flowers supported by a rigid cluster of ovate leaf like bracts wherein the inner bracts are leafy, distinct, pale green. It is one of such plants which has tremendous reputation in the indigenous traditional system of medicine in India by virtue of which it has drawn the attention and concern of scientists for validation of its medicinal properties through phytochemical screening and pharmacological evaluation [3, 4, 5, 6, 7.] Reports of investigations reveal the presence of trace elements such as Si, Ca, Cl, Mg, S, K and P in leaf and Al, Fe, Ti, Sr, V in roots and availability of Zn, Cu, As, Sr and Rb is less and equally present in roots as well as leaf [8]. The organic solvent extract like that of acetone of air-dried powdered seeds are known to contain terpenoids, flavonoids, steroids, glycosides, alkaloid, quinones, phenols[9].*Elephantopus scaber* possesses antimicrobial, hepatoprotective, antioxidant, antidiabetic, anti-inflammatory, analgesic, antiasthmatic, antiplatelet, anticancer and wound healing properties [10, 11] (Figs. 1 and 2).The objective of the present study is to set various pharmacognostic standards for use in detection of adulteration and quality control of the drug based on preliminary analysis of secondary metabolites in the leaves of *Elephantopus scaber* L. (Asteraceae) and other perspectives like ash and extractive values, fluorescence characteristics.

MATERIALS AND METHODS

Collection and authentication

Fresh leaves of *Elephantopus scaber* were collected randomly from their naturally occurring sites within Burdwan University Campus during the growing season i.e. pre-reproductive period (April to May, 2015). The plant was identified by one of the authors and a voucher specimen was kept in the department herbarium i.e. herbarium of Burdwan University (BURD) for the future references. The leaves were washed thoroughly under running tap water, blotted dry and then dried in the laboratory with the help of hot air oven at 50°C temperature.

Extract preparation

Four sets, each with eight grams of dried leaves, were prepared. The dried leaves after weighing out were crushed in a mortar with pestle to obtain coarse powder; to this powder was then added separately 80 ml each of solvents like water, methanol, di-ethyl-ether and acetone and boiled for 1-2minutes. These solutions were filtered using Whatman filter paper (number 40).

Protocol for analysis of phytochemicals in leaves of Elephantopus scaber

Test for Alkaloid: 2ml of 2N HCl was added to 2ml of plant extract, which was then shaken vigourously and kept aside for 5 minutes after which a few drops of Mayer's reagent ($Hgcl_2$ + KI in water) was added, shaken so that a creamy precipitation occurred[12, 13].Test for

Anthraquinone: 0.5 gms of crude powder was added to 20ml of each solvent and boiled and kept for 4 hours. 10 ml of the filtrate was mixed with 0.5 ml of ammonia solution to produce violet colour [13].Test for Cardiac glycoside: 2ml glacial acetic acid was taken along with 0.5 ml of 2% $Fecl_3$ and mixed with 5ml of plant extract after which 1ml conc. H_2SO_4 was added to form either green colour or brown ring[15].Test for Cholesterol: 2ml chloroform was mixed with 10ml of plant extract to which 10-12 drops of acetic acid were added and shaken vigorously. There after 2 drops of conc. $H_2SO_4$was added to change the colour from reddish brown to blue green[14].Test for Coumarin: 0.5 gms of crude powder was added to 5ml of each solvent and then the test tube mouth was covered with a filter paper treated with 1N NaOH. The mixture was boiled for a few minutes after which the filter paper was removed and examined under UV light to observe whether yellow fluorescence colour was formed or not[14].Test for Flavonoids: 0.2 gm of powdered sample was heated with 10 ml of each solvent in a steam bath for 3 minutes, after which 4ml of the filtrate was shaken with 1ml of 10% ammonia solution to observe yellow coloration[16].Test for Phenol: 4-5 drops of 2% $Fecl_3$ were added to 10 ml of each solvent extract for observing colour change of the solution[17].Test for Phlobatannins: 2ml of each solvent extract was boiled with 1ml of 1% HCl to form red or brown precipitation[16].Test for Quinones: 1ml of each solvent extract was mixed with 1ml of conc. H_2SO_4 to produce red or deep green coloration[18].Test for Saponins: Crude powder of sample (0.5 gm) was boiled with 15ml of solvent in a water bath. Intensive froth formation when present indicated the presence of saponins[19, 20].Test for Steroid: 5ml filtrate of each solvent extract was taken along with glacial acetic acid and cooled in an ice bath for 15 minutes. Then 0.5ml chloroform and 1ml conc. H_2SO_4 was added to form reddish brown ring between the two liquids[21].Test for Tannin: 0.5 gms of crude powder was added to 20ml of each solvent and boiled. A few drops of 2% $Fecl_3$ were added to the filtrate to form brownish green to blue black coloration[22].Test for Terpenoids: 2ml of chloroform was added to 5ml of plant extract to which 3ml of conc. H_2SO_4 was added to form a layer which finally led to green or reddish brown coloration[23].The fluorescence 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[24].

Fluorescence analysis

Leaf powder was subjected to fluorescence test subsequent to its treatments with freshly prepared chemical reagents. The treated samples were exposed to visible light and UV light[25, 26].

Extractive Values

(a) Water soluble Extractives

5gm of powdered drug was taken in conical flask. Then 100ml of water was added in the flask containing powdered drug and 5% solution in water was made. Then the flasks were closed with the help of the cotton plug. The mixture was shaken after regular interval of time without touching the solution on to the cotton plug. The mixture was kept for 24hrs (with regular shaking). After the period of 24 hours the solution was filtered out with the help of the Whatman filter paper. The upper solid content was discarded and the filtrate was collected. Empty dish was weighed and then filled with 25 ml of 5% solution of leaf extract, evaporated. Thereafter the evaporating dish was heated until a damp mass was formed. The evaporating dish was then cooled and weighed. Difference in weights of evaporating dish containing damp mass and empty evaporating dish was taken to directly calculate the water soluble extractive value.

(b) Alcohol soluble extractives

5gm of powdered drug was taken in each conical flask. Then 100ml of methanol was added to each of the flasks and 5% solution in methanol was made. Then the flasks were closed with the help of the cotton plug. The mixture was shaken after regular interval of time without touching the solution on to the cotton plug. The mixture was kept for 24hrs.

After the period of 24 hours the solution was filtered out with the help of the Whatman filter paper. Empty dish was weighed and then filled with 25 ml of 5% solution of leaf extract, evaporated. Thereafter the evaporating dish was heated until a damp mass was formed. The evaporating dish was then cooled and weighed. Difference in weights of evaporating dish containing damp mass and empty evaporating dish was taken for directly calculating the extractive value.

RESULTS AND DISCUSSION

While identifying various secondary metabolites present in leaf extracts of *Elephantopus scaber* through routine chemical tests using different solvents, anthraquinones were found to remain totally absent. Alkaloids could be detected with all solvents except acetone. Presence of quinones, steroid, flavonoids, phenols, cardiac glycoside, cholesterol, tannins and terpenoids is indicated in extracts obtained using all the solvents. Aqueous extract could indicate presence of saponins. Coumarins could be marked when methanol and water were used as solvents. The preliminary analysis of secondary metabolites in the leaves of *Elephantopus scaber* (Asteraceae) using standard procedures reveals a wide diversity in form of alkaloid, cardiac glycoside, cholesterol, coumarin, flavonoids, phenols, phlobatannins, quinones, saponins, steroid, tannins and terpenoids and thus unveiling a high therapeutic potential (Table I).When powder samples of leaves were passed through different treatments for observation under both ordinary and ultra violet lights, colour transformation to various extents against specific treatments was noted (Table II). Ash value is useful in determining authenticity and purity of sample and also these values are important qualitative standards (Fig 3). The Water-soluble extractive value plays an important role in evaluation of crude drugs. Extractive value less than expected indicates addition of exhausted material, adulteration or incorrect processing during drying or storage. The alcohol-soluble extractive value was also indicated in a similar way as the water-soluble extractive value. The water soluble extractive value proved to be higher than alcohol soluble extractive value. This shows that the constituents of the drug are more extractable and soluble in water as compared to alcohol (Table III).

Table I
Phytochemical constituents in leaves of Elephantopus Scaber in different solvents

Sl no.	Compound	Methanol	Water	Di ethyl ether	Acetone
1	Alkaloid	+	+	+	-
2	Anthraquinone	-	-	-	-
3	Cardiac glycoside	+	+	+	+
4	Cholesterol	+	+	+	+
5	Coumarin	+	+	-	-
6	Flavonoids	+	+	+	+
7	Phenol	+	+	+	+
8	Phlobatannins	-	-	+	+
9	Quinones	+	+	+	+
10	Saponins	-	+	-	-
11	Steroid	+	+	+	+
12	Tannin	+	+	+	+
13	Terpenoids	+	+	+	+

+ = Present, - = Absent

Table II
Fluorescence analysis of the powdered samples of leaf of Elephantopus scaber

Sl no.	Treatment	Ordinary Light	Ultra-violet Light
1	Powder as such	Brownish green	Greenish black
2	P+ Distilled water	Brownish green	Yellowish green
3	P+ 1(N) NaOH in methanol	Black	Brownish black
4	P+ 1(N) NaOH in ethanol	Brownish green	Yellowish brown
5	P+ 1(N) NaOH in water	Blackish green	Brownish green
6	P+ Iodine	Deep brown	Blackish green
7	P+ HCL(conc.)	Brownish green	Yellowish green
8	P+ HNO_3(conc.)	Yellowish brown	Brown
9	P+ H_2SO_4(conc.)	Brownish green	Yellowish brown
10	P+ 50% HCL	Brownish green	Pale green
11	P+ 50% H_2SO_4	Pale green	Yellowish brown
12	P+ Acetic acid	Brownish green	Yellowish black
13	P+ CCl_4	Black	Brown
14	P+ Methanol	Blackish green	Blackish brown
15	P+ Ethanol	Greenish black	Yellowish brown
16	P+ Acetone	Brownish green	Greenish black
17	P+ 40% NaOH+ Lead acetate	Blackish green	Greenish black

P = Powder

Table III
Extractive Values of the leaf and rhizome Powders of Elephantopus scaber

Sample	Water soluble Extractives	Alcohol soluble extractives
Leaf Powder	12.07 ± 1.30	8.78 ± 2.04
Rhizome Powder	21.05 ± 2.13	15.77 ±1.49

Fig 1: Plant in vegetative condition

Fig 2: Plant in reproductive stage

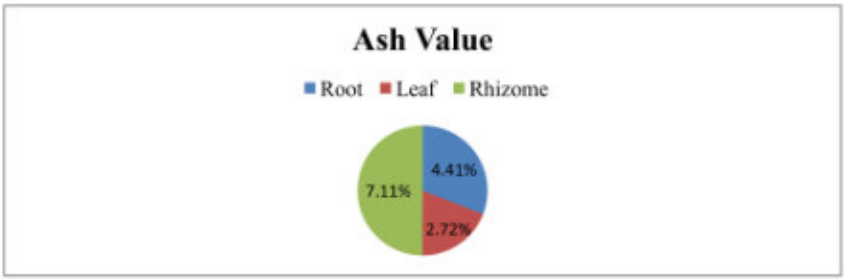

Fig 1: Graphical representation of Ash value of different parts of the plant.

CONCLUSION

The secondary metabolites are the micromolecules produced by a plant precisely in response to its environmental experience mainly as chemical weapons against parasites, predators and pathogens or as anti-stress substances. Thus each type of secondary metabolite is a strategic product of metabolism for putting up resilience against environmental resistance and ensuring adaptation and evolution. The diversity of secondary metabolites as seen in *Elephantopus scaber* is the reflection of its evolutionary advancement and its potential for therapeutic efficacy against diverse pathogenic organisms. Thus the present study on the preliminary phytochemical evaluation of the leaf of *Elephantopus scaber* can provide useful information to authenticate and to substantiate the genuine crude drug along with its phyto-constituents and phytochemical nature, thus proving helpful to set up a standard necessary for drug-cognizance and prevention of its adulteration.

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CONFLICT OF INTEREST

Conflict of Interest declared none.

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