ISSN:0975-5772

Pharmacognostic and phytochemical investigation of *Elephantopus scaber* L. (Asteraceae)

V.R. Mohan[1]*, P. Chenthurpandy[2] and C. Kalidass[1]

[1]Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tutuicorin-628 008, Tamil Nadu, India.

[2]Department of Botany, Kamaraj College, Tuticorin-1, Tamil Nadu.

Abstract :

Pharmacognostic investigation of anatomical sections of leaf, petiole, stem, peduncle and root of *Elephantopus scaber* was carried out to determine its anatomical features. The physico-chemical constant like ash and extractive values were determined. The total ash content of the *E. scaber* leaf is 6.32% and 10.53% for rhizome. The phytochemical analysis of the all the extracts revealed the presence of steroid compound. The results of the study could be useful for the identification and preparation of a monograph of the plant.

Key words: Elephantopus scaber; Pharmacognostic; phytochemical

Introduction:

Elephantopus scaber (Asteraceae) has been used to cause diuresis and antipyresis and to eliminate bladder stones. This genus has been reported to contain the hydroxylated germacranolides molephantin and molephantinin, which also possess cytotoxic and antitumor properties(1). Chemical study started from 1960's and showed that constituents of *E. scaber L.* include flavonoids, triterpenoids, flavonoid esters and sesquiterpene lactones. Sesquiterpene lactones are most important due to their anti-tumor activity (2,1). The major phytochemical constitutes of the plant are elephantopin, triterpenes, stigmasterol epifriedelinol and lupeol(3). Applied topically, they are employed as an antipyretic, for the treatment of erysipelas, skin infections, and measles. A preparation made from the roots is taken as a remedy for colic; the whole plant helps against diarrhea. It is one of the most popular cough remedies of Middle America(4).

In Chota Nagpur the root is given for fever. This plant is much used as a diuretic and febrifuge in Madagascar and as a vulnerary in Jamaica. In Taiwan the drug 'Teng-Khia-U' consists of the entire plant of *Elephantopus scaber, E. mollis* and *Pseudoelephantopus spicatus*(5). It has been proved that water extracts of these three plants possess a liver protective effect on carbon tetrachloride (CC14)-induced acute liver damage(6).

The literature survey revealed that the systemic evaluation including pharmacognostical study of this plant is still lacking. No scientific parameters are available to identify the true plant material and to ensure its quality. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies. The objective of the present study is to evaluate various pharmacognostic standards like microscopy of leaf, petiole, stem, peduncle and root; ash and extractive values, fluorescence analysis and preliminary phytochemical analysis of *Elephantopus scaber* leaf and rhizome.

Materials & Methods:

The leaf, petiole, stem, peduncle and root of *Elephantopus scaber* were collected from the well grown healthy plants inhabiting the natural forests of Kanyakumari district, Western Ghats, Tamil Nadu, India. For anatomical investigations standard microtome techniques(7) were followed. Take T.S. of 10 to 12 µm thickness were

prepared. These microtome sections were stained with 0.25% aqueous Toluidine blue (Metachromatic stain) adjusted to pH 4.7(8). Photomicrographs were taken with NIKON trinocular photo micrographic unit. The most accepted descriptive terms were being used to describe the root and stem anatomy(9,10).

a) Ph ysicochemical c onstant and fluorescence analyses:

These studies were carried out as per the standard procedures(11). In the present study, the leaf and rhizome powder was treated with 1N aqueous sodium hydroxide and 1N alcoholic sodium hydroxide, acids like 1N hydrochloric acid and 50% sulphuric acid. These extracts were subjected to fluorescence analysis in visible/daylight and UV light (254nm & 365nm). Various ash types and extractive values were determined by following standard method by African Pharmacopoeia(12,13).

b) Preliminary phytochemical analysis:

Shaded dried and powdered leaf and rhizome samples were successively extracted with benzene, chloroform and methanol. The extracts were filtered and concentrated using vaccum distillation. The different extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedure (11, 14).

Results and Discussion:

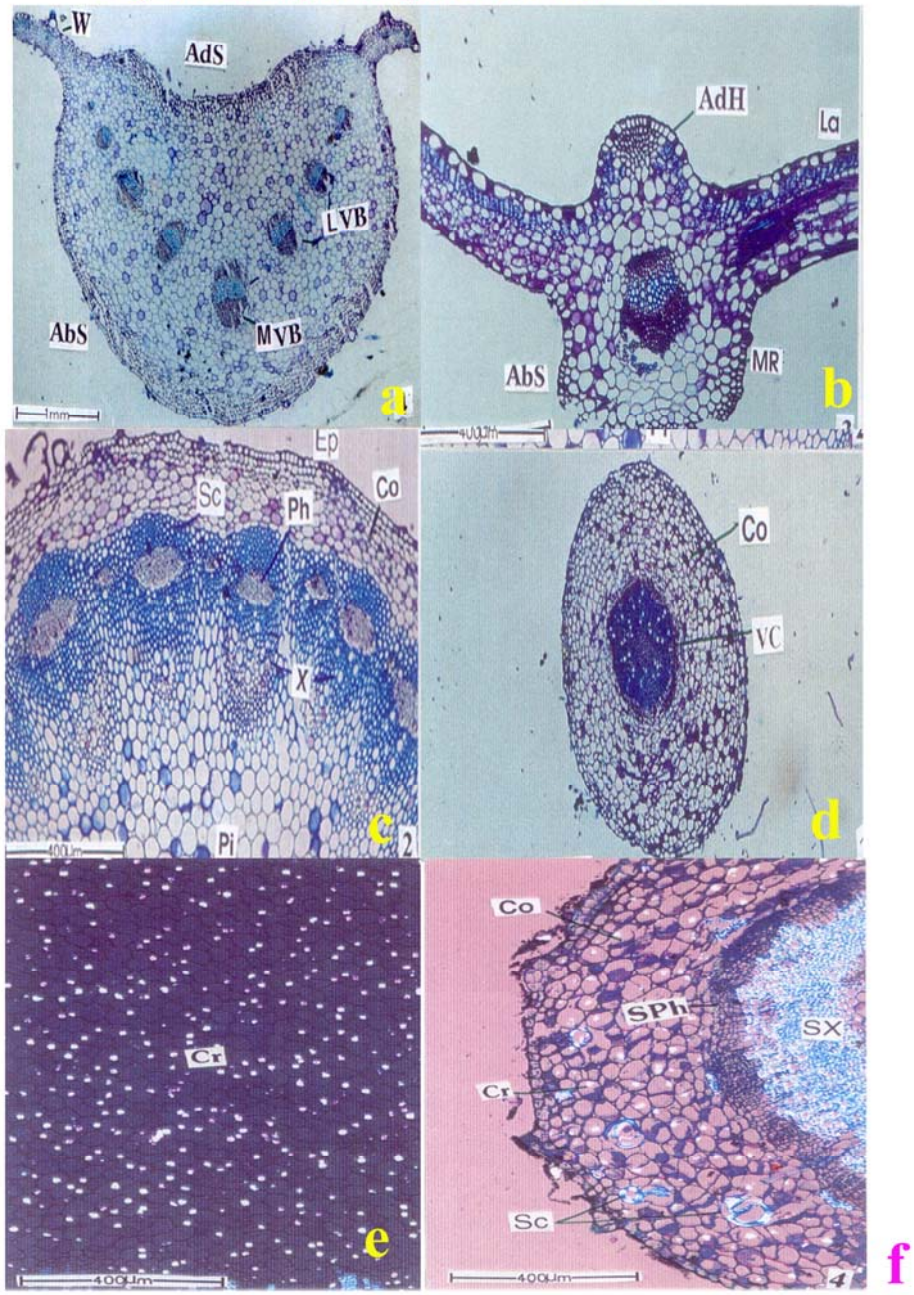
Elephantopus scaber showed the general characteristics are scapigerous, strigose herb growing up to 50 cm in height. The leaves are subradical, alternate, simple, oblanceolate, chartaceous. Capitula homogamous, disciflorous, sessile, aggregated in the dense stalked glomerules and subtended by floral leaves, receptacle flat; involucre tubular; florets purple, bisexual, achenes 10 ribbed; pollen spherical.

The microscopic study of the lamina is 187 – 198 μm thick; dorsiventral, amphistomatic, epidermis rectangular to squarish; cuticle thin and even. Mesophyll palisade cells single layered, short and broadly cylindrical. Spongy mesophyll 3 or 4 layered; loosely arranged forming air spaces; Abaxial epidermal cells more frequently cubical; cuticle thick and even, anticlinal walls slightly wavy and thin. The midrib is 725 – 785 μm thick; project prominently both on the upper and lower sides of the midrib. On the abaxial side the midrib appears broadly hemispherical and on the adaxial side it is thick and flat; a few layers of collenchyma cells occur on the abaxial as well as adaxial hump; rest of the ground tissue is parenchymatous. Vascular bundle single, collateral with abaxial mass of sclerenchyma cells abetting the phloem; xylem elements disposed in 3 or 4 rows (Fig 1a).

The cross sectional outline varies significantly from the proximal to distal ends of the petiole. However, the vascular bundles are basically collateral throughout the length of the petiole, the distribution pattern of the bundle being variable. The petiole assumes U – shape and concavo-convex along middle part of the petiole (Fig 1b). The wings become gradually distinct towards the distal end merges with the lamina; the vascular bundles are distinct and range from 8 – 10. The median bundle is the largest, the lateral bundles are smaller and the wing bundles are the smallest. A narrow zone collenchyma occupies the subepidermal part of the adaxial and abaxial regions; rest of the ground tissue is parenchymatous.

The stem that arises from the root stock is circular, even and glabrous in cross section; it shows only primary state of growth. The cortex is narrow; the outer zone has two layers of collenchyma and inner zone is parenchymatous. The vascular cylinder consists of discrete collateral vascular bundles organized in a ring.

Figure 1. *Elephantopus scaber* Linn.



1a. T.S. of Petiole; 1b.T.S. of leaf through midrib with lamina; 1c. T.S. of stem half - portion enlarged; 1d. T.S of root; 1e. Crystal density in the pith cells of the root-stock; 1f. Rosette-type of crystal and annular sclereids in the cortical cells of the aerial stem.

AbS: Abaxial side; AdH: Adaxial epidermis; Co: Cortex; Cr: Crystals; Ep: Epidermis; La: Lamina; MR: Midrib; LVB: Lateral vascular bundles; MVB: Median vascular bundles; Ph: Phloem; Pi: Pith; Sc: Sclerenchyma; Sc: Sclereids; SPh: Secondary phloem; Sx: Secondary xylem; W: Wing; X: Xylem;

Table 1: Ash and Extractive values of the powdered leaf and rhizome of *Elephantophus scaber*

Ash Values			
Sl.No.	**Type of Ash**	**% of Ash in Leaf**	**% of Ash in Rhizome**
1.	Total ash	6.32 ± 0.25	10.53 ± 0.38
2.	Acid insoluble ash	3.42 ± 0.12	5.44 ± 0.26
3.	Water insoluble ash	1.76 ± 0.11	3.36 ± 0.21
4.	Sulphated ash	11.09 ± 0.14	15.39 ± 0.29
Extractive Values			
Sl.No. N	**ame of the Extract**	**Extractive Value (in %) of Leaf**	**Extractive Value (in %) of Rhizome**
1.	Ethanol Soluble Extract	9.18 ± 2.34	16.57 ± 1.89
2.	Water Soluble Extract	12.37 ± 1.48	21.65 ± 2.43

* Values are Mean ± S.E.

Table 2: Fluorescence analysis of the powdered leaf of *Elephantopus scaber*

Sl. No	Treatment	Day light	Short UV light (254 nm)	Long UV light (365 nm)
1	Powder as such	Yellowish green	Brownish yellow	Golden Yellow
2	Powder + 1N NaOH (aqueous)	Brownish green	Brownish green	Blackish green
3	Powder + 1N NaOH (alcoholic)	Green	Light green	Dark green
4	Powder + 1N HCL	Purple	Dark green	Purple
5	Powder + 50% H_2SO_4	Pale green	Bluish green	Bluish green

Size of the vascular bundles is not uniform. Each vascular bundle is thick, prominent and wedge shaped; phloem is embedded in a sclerenchymatous mass outside the xylem strands. Pith is wide and parenchymatous (Fig 1c & 1d).

The primary peduncle and the secondary peduncle are basically similar in the structural profile excepting total diameter and thickness of the vascular cylinder. The peduncles are generally circular and even in transactions. The epidermal layer is followed by 2 or 3 layers of collenchyma and less compact parenchymatous cortex. In the primary peduncle the vascular cylinder ensheaths wide parenchymatous pith; the xylem cylinder is thick and massive and comprises of fibers and scattered vessels.

Table 3: Fluorescence analysis of the powdered rhizome of *Elephantopus scaber*

Sl. No	Treatment Day	light	Short UV light (254 nm)	Long UV light (365 nm)
1	Powder as such	Yellowish green	Brownish yellow	Golden Yellow
2	Powder + 1N NaOH (aqueous)	Wine red	Brown	Dark brown
3	Powder + 1N NaOH (alcoholic)	Yellowish green	Yellow	Dark green
4	Powder + 1N HCL	Light brown	Light pink	Pink
5	Powder + 50% H_2SO_4	purple	Dark wine red	Dark wine red

Table 4: Preliminary phytochemical screening of *Elephantopus scaber* leaf and rhizome extract

Test Benz	ene		Chloroform		Methanol	
	leaf	rhiz ome	leaf	rhizome	leaf	rhizome
Alkaloid	-	-	-	-	-	-
Anthroquinone	-	-	-	-	-	-
Coumarin	-	-	-	-	-	-
Flavonoid	-	-	-	-	+	-
Phenol	-	-	-	-	+	+
Saponin	-	-	-	-	-	+
Steroid	+	-	+	+	+	+
Tannin	-	-	+	-	+	-
Terpene	-	-	+	+	+	+
Xanthoprotein		-		-	+	-
Sugar	+	+	+	+	+	+

Phloem occurs as thin sheath external to the xylem; discontinuous masses of pericyclic fibers are seen on the exterior of the phloem. The secondary peduncle has thinner vascular cylinder and lacks the pericyclic masses of fibres.

The adventitious roots arising from the root-stock were studied. The root exhibits a fairly good quantum of secondary growth. The epidermis is broken at several places, but no periderm is formed. The cortex is broad, styloid type of calcium oxalate crystals are fairly abundant in the cortical parenchyma (Fig 1e & 1f). The outline of the xylem cylinder is not perfectly circular; it has shallow ridges and furrows. The secondary xylem consists mostly of solitary vessels and more amount of fibres. The vascular cylinder is split into several blocks due to parenchymatous radial sector. Secondary xylem consists of radial files of vessels and fibres. Phloem occurs on the outer border of the secondary xylem, small patches fibres are seen outer to the phloem.

The physico-chemical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs by African Pharmacopoeia (12). Equally important in the evaluation of crude drugs, is the ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica (15). The total ash content of the *Elephantopus scaber* leaf is 6.32%

and 10.53% for rhizome. The water insoluble ash is less than that of acid insoluble ash at 1.76% and 3.42% respectively. The water extractive value of *E. scaber* is more than that of ethanol extractive value in the table 1. 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 (16). The fluorescence analysis of the powdered crude drug of *E. scaber* leaf and rhizome is presented in the table 2 and 3. The leaf powder emitted yellowish green under day light and brownish yellow under short light and long UV radiation respectively. When treated with aqueous and alcoholic NaOH it remained green in all conditions. Treatment with 1N HCL gave purple fluorescence in day light and long UV and dark green in short UV radiation. The powdered rhizome exhibited different fluorescence under different conditions.

In the present investigation, methanol extract of *E. scaber* leaf was accounted for the presence of flavonoids, phenols, steroids, tannins, terpenes, xanthoproteins and sugar in the table 4. The chloroform extract has tested positively for steroids, tannin, terpene and sugar. Benzene extract has only steroid and sugar compounds. All the three extracts have steroid compounds. Similar report was obtained for the rhizome of *E. scaber* (Table 4). Therapeutically terpenoids exert wide spectrum of activities such as antiseptic, stimulant, diuretic, anthelmintic, analgesic and counter-irritant (17). They are also medicinally used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and as antidote (18). Our study suggests that *E. scaber* may be a potential resource of steroid compound and useful for the identification and preparation of a monograph of the plant.

References:

[1] Lee K H, Cowherd C M and Wolo M T. Antitumor agents. XV: deoxyelephantopin, an antitumor principle from *Elephantopus carolinianus* Willd. *J Pharm Sci* 1975, 64, 1572–3.

[2] Fuchino H, Koide T, Takahashi M, Sekita S and Satake M. New sesquiterpene lactones from *Elephantopus mollis* and their leishmanicidal activities. *Plant Med* 2001, 67, 647–53.

[3] Rajkapoor B, Jayakar B and Anandan R. Antitumor activity of *Elephantos scaber* Linn. Against Dalton's Ascitic Lymphoma. *Indian J Pharm Sci.*, 2002, 64, 71 – 73.

[4] Morton J, *Atlas of Medicinal Plants of Middle America, Bahamas to Yucatan*. Springfield, IL: Thomas, 1981, p420.

[5] Sasaki S, *The Medicinal Plant of Formosa.* Published by South Villa Books Centre, Taipei, Taiwan, ROC, 1982, 853 pp.

[6] Lin J K – Tsai S H – Lin-Shiau S Y .Anti inflammatory and anti tumor effects of flavonoids and flavanoids. *Drugs Fututre,* 2001, 26, 145 – 157.

[7] Johansen D A, *Plant Microtechnique*. McGraw – Hill Book Company, Inc. New York and London, 1940, 1-523.

[8] O' Brien T P, Feder N and Mc Cull M E, *Polychromatic staining of plant cell walls by toluidine blue* – Eds. O. Protoplasma, 1964, 59: 364-373.

[9] Junikka L, *Survey of English Macroscopic bark terminology*. IAWA Bulletin, 1994, 15: 3-45.

[10] Trockenbrodt M, Survey and discussion of the terminology used in bark anatomy. *IAWA Bulletin*, 1960, 11, 141-166.

[11] Lala P K, *Lab Manuals of Pharmacognosy*. CSI Publishers and Distributors, Calcutta, 1993, 5th Edition.

[12] African pharmacopoeia, Determination of Ash, Sulfated ash and Acid insoluble ash. OAU/STRU. Nigeria, 1986, 11, 40.

[13] Anonymous, *Indian Pharmacopoeia* vol. I & II. Government of India. Ministry of Health and family welfare. The controller of publication. Civil lines. Delhi-110054, 1996.

[14] Brinda P, Sasikala B and Purushothaman K K, Pharmacognostic studies on *Merugan kilanzhu*. *BMEBR*, 1981, 8, 1-13.

[15] Musa K Y, Katsayal A U, Ahmed A, Mohammed Z and Danmalam U H, Pharmacognostic investigation of the leaves of *Gisekia pharmacioides*. *African Journal of Biotechnology*, 5, 2006, 956-957.

[16] Pimenta A M, Montenegro M C, Ara Ujo A N, Mart'inez J C, Application of sequential

injections analysis to pharmaceutical analysis. *Journal of Pharm. Biomed. Annuls*, 2006, 40, 16-34.

[17] Gokhale S G, Kokate C K and Purohit A P, A text Book of *Pharmacognosy*, Nirali Prakashan, Pune, 2003.

[18] Ali M, *Text book of Pharmacognosy*. CBS Publishers and Distributors, New Delhi, 1994, p. 405.