Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG.: Research Article
Pharmacognostic Parameters for Evaluation of the Roots of *Echinops echinatus* marketed as *Brahmadandi*

Somashekar A. P and Mishra S. H*

**Pharmacy Department, Faculty of Technology & Engineering, Kalabhavan*
The M.S University of Baroda, Vadodara-390 001. Gujarat, INDIA
Phone: +91-265-2434187 ; E-mail: shmishra48@rediffmail.com

ABSTRACT

In ethnomedicinal practices the traditional healers use the roots of *E. echinatus* (*Brahmadandi*) in the treatment of various ailments related to reproductive disorders. Plants from other genus are also reported to be sold under the common trade name '*Brahmadandi*'. Scientific parameters are not yet available to identify the true plant material and to ensure its quality. Therefore the present work has been undertaken to establish the necessary pharmacognostic standards for evaluating the plant material. Various parameters like morphology, microscopy, physico chemical constants and phytochemical profiles of the roots were studied and the salient diagnostic features are documented. The characteristic HPTLC finger print profile of the major chemical constituents like terpenoids and phenolic compounds in the crude extract along with their *Rf* values and relative percentage in the extract were recorded.

KEY WORDS: *Brahmadandi*; *Echinops echinatus*; HPTLC finger print; pharmacognostic, microscopy

INTRODUCTION

Echinops echinatus Roxb, (Compositae), commonly known as *Brahmadandi*, is a pubescent annual herb of 1-3 ft height with branches widely spreading from the base. The species is found practically throughout India, Pakistan, Afghanistan, etc, (1). The Plant is bitter, increases the appetite and stimulates liver; used in diseases of the brain, pains in the joints, inflammations, etc. Roots and root bark of the plant are used in various indigenous systems of medicine for treating different ailments. The root is used as abortifacient and aphrodisiac (2), infusion of the root is given in seminal debility, impotence, hysteria, and its decoction is given in dyspepsia, scrofula, syphilis and fevers (3).

According to an ethnomedicinal survey carried out by Kakrani et al. (4), the rural population of Kutch region in Gujarat state, India, uses the suspension of root bark powder in milk (100g/ 250ml) for the treatment of diabetes. The traditional healers of Chhattisgarh in India use this herb in different ways both internally and externally for the treatment of sexual disorders. An aqueous paste of the root is applied in the lower abdominal region to hasten the process of delivery; also the patients are advised to take the paste internally for quick and safe delivery. In case of patients having poor sexual vitality, aqueous paste of the root bark powder is applied externally on the male genitals one hour before intercourse; pure honey can be used in place of water for better results. A paste prepared by mixing the root bark powder with the juice of *Datura stramonium* and *Blumea lacera* leaves is used to avoid premature ejaculation. The patients suffering from respiratory troubles, particularly asthma, are advised to inhale the fumes obtained by burning the leaves & roots of *E echinatus* in order to get quick and permanent relief (5).

Though the plant has been reported for many biological activities like anti-inflammatory (6), hypoglycemic and diuretic (7), antibacterial and antifungal (8), antispasmodic (9) etc, very little less has been reported on the chemical composition of the roots (10); however a recent study showed the presence of different flavonoids in the whole plant (11).

Traditionally roots are sold in different Indian markets under the trade name of '*Brahmadandi*'. According to the available literature, there exist plants from other genus which are also being sold under the same trade name '*Brahmadandi*' (12). But no scientific parameters are available to identify the true plant material and to ensure its quality. Therefore the present work has been undertaken to establish the various pharmacognostical and phytochemical parameters, which could serve as a measure of authentication and

quality control for commercial samples of the crude drug. In addition the detailed microscopy of the aerial parts of the plant (stem and leaf) has also been studied and documented.

MATERIALS AND METHODS

Plant material

The whole plant material of *E. echinatus* was collected in the month of August 2005 from the outfield of Gulbarga city, Karnataka, India and was authenticated in Botany Department of The M. S. University of Baroda, Vadodara, India. Voucher specimen (No: Pharmacy/EE/05-06/01/SP) has been deposited in the Pharmacy Department of The M. S. University of Baroda, Vadodara, India.

Microscopy

The plant materials were preserved in a mixture of solvents containing formalin, acetic acid and alcohol (70 %v/v) for histological studies; transverse sections (T.S) of the different organs of the plant material were taken using a microtome and stained with different stains (13); microphotographs of the sections were made using Olympus BX 40 microscope attached with Olympus DP12 digital camera.

Physico-hemical constants

Physico-chemical constants such as the percentage of total ash, acid-insoluble ash, water-soluble ash, water and alcohol soluble extractives and loss on drying (LOD) were calculated as per the Indian Pharmacopoeia (14). Total phenolic content in the crude drug was estimated according to the method given by Singleton & Rossi (15).

Phytochemical screening

For preliminary phytochemical studies 65 g of powdered material was extracted in a Soxhlet apparatus with petroleum ether (60-80), benzene, chloroform, ethyl acetate, methanol and water successively, obtained extracts were dried and weighed. The presence of various phytoconstituents viz. steroids and terpenoids (Leibermann Burchard test), alkaloids (Dragendroffs test), tannins and phenolics (Ferric chloride test), flavonoids (Shinoda test), Sugars (Fehling solution test), amino acids (Ninhydrin test), etc. was detected by usual methods prescribed in standard texts (16, 17).

Chromatographic finger printing

10g of powdered material was defatted with petroleum ether and then extracted with methanol (50ml x 3) on a water bath for 30 min, concentrated on a Rotary Evaporator (BUCHI Rotavapor R 200) and dried. A stock solution (10 mg/ml) was prepared in methanol. Suitably diluted stock solutions were spotted on precoated silica gel G60 F_{254} TLC plates (Merck) with the help of CAMAG Linomat V applicator. Plates were developed in solvent systems of different polarities to resolve polar and non polar components of the extract. The developed plates were scanned using TLC Scanner 3 (CAMAG). The photographs were made with the help of Reprostar 3 (CAMAG) digital camera.

The non polar components (steroids and terpenoids) in the extract were resolved using a solvent system of (Solvent 1) Toluene: chloroform: ethyl acetate (10:2:1) and the characteristic peaks of separated compounds were recorded under UV light at 254 nm and 366 nm. Subsequently the plate was derivatized using anisaldehyde sulphuric acid reagent (18) and the characteristic peaks of the detected compounds were recorded at 540 nm. Similarly the polar components (phenolic compounds) in the extract were separated using Toluene: ethyl acetate: formic acid: methanol (6:6:1.6:0.4) (Solvent 2), and the developed plate was derivatized using ferric chloride reagent (18), characteristic peaks of the detected compounds were recorded at 540 nm.

RESULTS

A brief taxonomic description of the plant

It is a much branched rigid annual herb of 0.3-0.9 m height; branches widely spreading from the base with cottony pubescence (Fig. 1). Leaves are sessile, 7.5-12.5 cm long, glabrous or minutely

scaberulous above, white with cottony wool beneath, oblong, deeply pinnatified, the lobes are triangular and oblong, sinuate and spinescent, the spines often 2.5 cm long. Balls of the heads white, normally 2.5-3.8 cm in diameter (excluding the spines (Fig. 1). Involucres surrounded by strong white bristles resembling pappus hairs; outer involucral bracts oblanceolates; intermediate bracts with 1 or 2 of the bracts often produced into sharp spines sometimes exceeding 2.5 cm long, causing the balls frequently to bristle with many spines. Pappus short, yellowish, forming a short cylindric brush above the achene. Achenes 4mm long, obconic, densely villous (1).

Macroscopic characters of the roots

The shade dried intact roots of *E. echinatus* (Fig. 2) are 30 - 50 cm long with a diameter of 0.5 – 1 cm. Outer surface is grayish brown in color with long longitudinal wrinkles and small rootlets in the lower region. The wood is smooth and yellowish white.

Microscopic characters of the roots

Transverse section of *E. echinatus* root is circular in outline and shows the following regions (Fig. 3).

Periderm: In young root the epidermis is single layered with unicellular hairs where as in old roots the epidermis is replaced with periderm.

Periderm is 6-8 layers, thick and is not clearly distinguished into phellem, phellogen and phelloderm.

Cortex: Followed by periderm is a homogenous cortex comprising of 3-5 layers of thin walled large parenchymatous cells. The cortical cells are devoid of any cellular inclusions.

Endodermis: A distinct, single layer of endodermis separates the cortical region from vascular region and shows the presence of casparian thickening.

Vascular bundle: occupies more or less the central region and is separated into xylem and phloem by few layers of cambium. The xylem cylinder consists of patches of xylem included by parenchyma cells which are elongated thin walled and closely packed. Within the xylem patch medullary rays of 2-4 layers extend from the primary xylem to up to the outer phloem region. The xylem consists of few vessels and tracheids. Phloem cells are thin walled and are devoid of starch grains. Pericycle is made up of parenchyma cells.

Microscopic characters of the stem

The T.S of the stem is circular in outline and shows the following regions (Fig. 4).

Epidermis: It is single layered and shows the presence of thick cuticle. The epidermal cells are cuboidal, uniseriate multicellular trichomes emerge out from the epidermal cells.

Cortex: is heterogeneous having outer 4-5 layers of collenchyma cells and inner 3-4 layers of parenchyma cells. Parenchyma cells are compactly arranged.

Endodermis: separates the cortical region from the vascular region. Endodermis is single layered followed by a heterogeneous pericycle. Pericycle is made up of patches of sclerenchyma alternating with parenchyma cells. Parenchyma cells apparently show the presence of oil globules.

Vascular bundle: Phloem region shows the isolated sclerids, single leaf trace bundle separates out from the axial vasculature and traverse into the leaf base. Xylem forms a continuous cylinder it consists of vessels of large lumen, tracheids and xylem parenchyma.

Medullary rays: 1 - 2 layers of medullary rays traverse from the primary xylem to up to the outer phloem region. Primary vascular bundles are distinct and 19 - 20 in number.

Pith: comprises of small rounded compactly arranged parenchyma cells. Large number of pith cells shows the presence of spheraphides.

Microscopic characters of the leaf

Transverse section of the leaf shows dorsiventral nature of the leaf. Following are the important tissues in the lamina and the midrib regions (Fig. 5).

Lamina: Upper epidermis is single layered with rectangular cells having a thick cuticle on outer walls. Lower epidermis is identical to upper epidermis. A number of uniseriate, multicellular trichomes cover the entire lower region of the lamina. The uniseriate trichomes are very long with all the cells collapsed except the 4 - 6 cells at the base and close to the epidermis. Mesophyll is differentiated into upper palisade and lower spongy parenchyma.

Midrib: The midrib region is 3 - 5 ridged. Each ridge shows the presence of a vascular bundle. Below the upper epidermis and above the lower epidermis a patch of 4 - 5 layered collenchyma is seen. Rest of the midrib region is covered by rounded parenchyma cells having intracellular spaces. Vascular bundles are embedded in the parenchymatous tissue; each vascular bundle is caped on either side by a patch of sclerenchyma. Vascular bundles are endarch in nature.

Powder study of the roots

The various diagnostic characters of the root powder are depicted in Figure 6.

Cork: Thick rectangular cells placed in radial serration with thick suberin deposition.

Vessel elements: Large number of vessel elements either in entire or fragmented form showing various types of thickening like helical, simple pitted, bordered alternate pitting are found, though helical thickening predominates among all.

Tracheids: Occasionally few xylem parenchyma cells and tracheids creep in the powder.

Phytochemical studies

Various physico chemical constants, moisture content, total phenolic content of the roots were determined and the values are depicted in histogram (Fig. 7). The percentage of successive Soxhlet extractives was calculated and results are depicted in histogram (Fig. 8). Preliminary phytochemical studies showed the presence of steroids, terpenoids and phenolic compounds as the chief constituents in the extract (Table 1).

Chromatographic finger print profile

HPTLC finger print profile of the extract for both polar and non polar compounds has been developed (Fig. 9 and Fig. 10). R_f values and the relative percentage of the separated compounds are recorded (Table 2).

Table 1: Phytochemical screening of the roots of E. echinatus

Class of compounds	Successive extracts					
	P	B	C	E	M	W
Alkaloids	-	-	-	-	-	-
Carbohydrates	-	-	-	-	+	+
Steroids/Terpenoids	+	+	+	+	-	-
Proteins & Amino acids	-	-	-	-	-	-
Saponins	-	-	-	-	-	-
Fixed oils/Fats	+	-	-	-	-	-
Flavonoids	-	-	-	-	-	-
Phenolics	-	-	-	+	+	+
Tannins	-	-	-	-	+	+

P: Petroleum ether; B: Benzene; C: chloroform; E: ethyl acetate; M: methanol;
W: water; '+': Present; '-': Absent

Table 2: R_f values and relative percentage of the separated compounds.

Peak	Solvent 1						Solvent 2					
	254 nm		366 nm		540 nm		254 nm		366 nm		540 nm	
	Max *Rf*	Relative %	Max *Rf*	Relative %	Max *Rf*	Relative %	Max *Rf*	Relative %	Max *Rf*	Relative %	Max *Rf*	Relative %
1	0.01	1.64	0.075	12.42	0.06	22.18	0.07	13.45	0.03	0.72	0.09	20.14
2	0.05	1.84	0.09	1.66	0.13	3.82	0.17	35.4	0.11	2.99	0.18	34.48
3	0.19	4.43	0.14	0.88	0.23	12.14	0.24	30.5	0.22	4.11	0.27	29.98
4	0.23	4.35	0.39	7.12	0.38	8.98	0.61	7.47	0.31	4.21	0.40	1.62
5	0.49	4.15	0.47	1.65	0.79	18.48	0.66	9.07	0.53	9.69	0.43	1.72
6	0.64	11.45	0.56	16.75	0.92	11.41	0.94	4.11	0.58	6.51	0.64	5.11
7	0.79	17.94	0.65	21.61	0.97	22.99			0.64	11.33	0.69	4.63
8	0.92	54.20	0.70	3.95					0.67	12.77	0.97	2.31
9			0.93	33.96					0.75	14.86		
10									0.86	10.91		
11									0.90	9.25		
12									0.95	12.64		

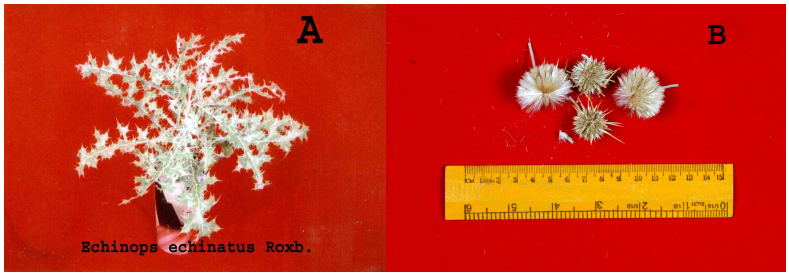

Figure 1. (A) Whole herb of E. echinatus; (B) Inflorescence

Figure 2. Crude drug (Roots of E. echinatus)

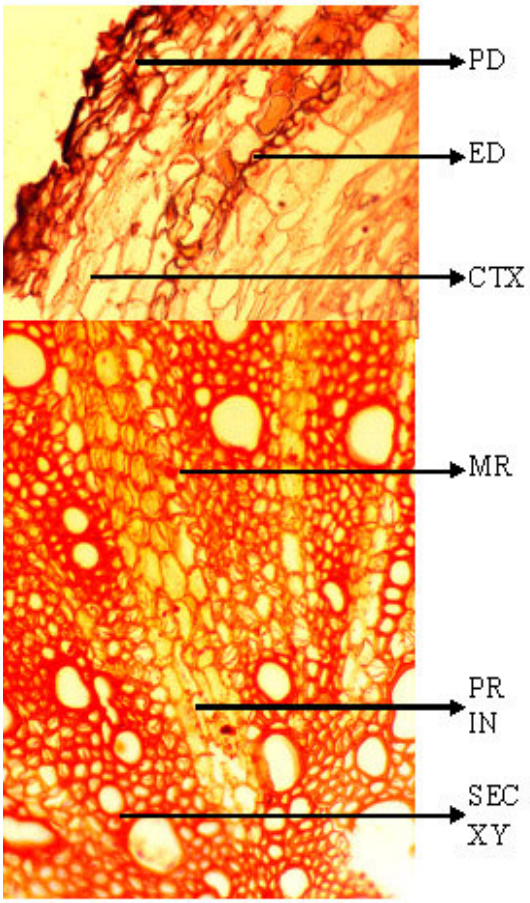

Figure 3 : Microscopy of the roots (X 10) (PD, Periderm; ED, endodermis; CTX, cortex; MR, bilayered medullary rays; PR IN, parenchyma inclusion; SEC XY, secondary xylem)

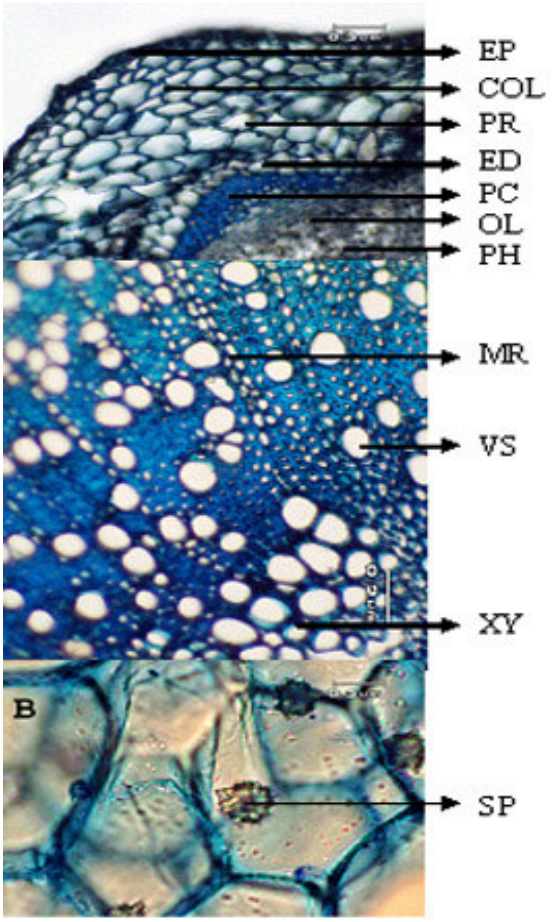

Figure 4. Microscopy of the Stem. [EP, epidermis; COL, collenchyma; PR, parenchyma; ED, endodermis; PC, pericycle; OL, oil globules; PH, phloem; MR, medullary rays; VS, vessels; XY, xylem (X 10), (B) SP, spheraphides (X 40)].

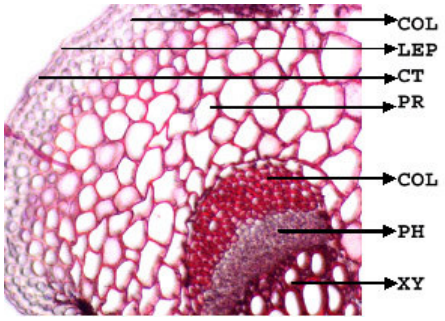

Figure 5. Microscopy of the Leaf. PH, phloem; COL, collenchyma; PR, parenchyma; LEP, lower epidermis; CT, cuticle; XY, xylem (X 10).presence of casparian thickening.

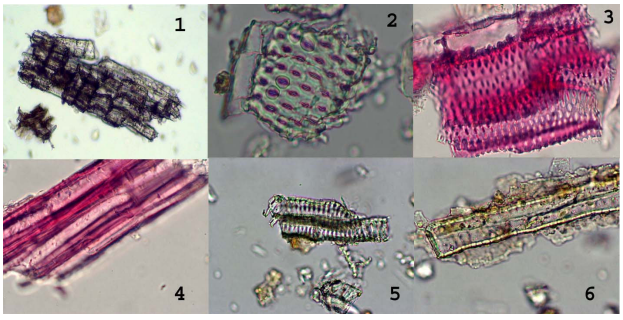

Figure 6. Diagnostic features for the powder microscopy of the roots of E. echinatus (X40). (1) cork cells; (2) vessels with alternate pitting; (3) vessels with simple pitting; (4) tracheids; (5) vessels with helical thickening; (6) xylem parenchyma.

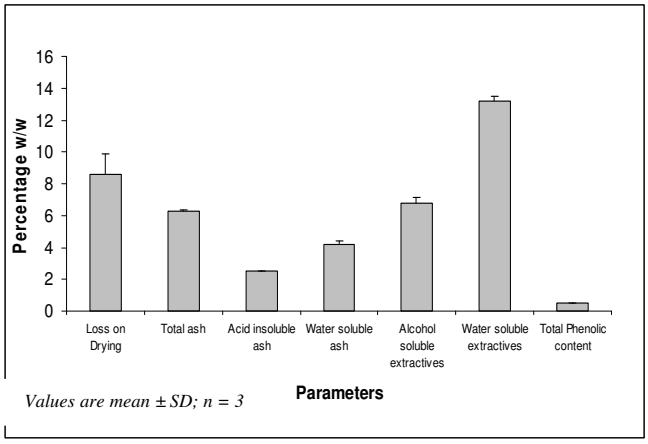

Figure 7: Physico chemical constants of E. echinatus root.

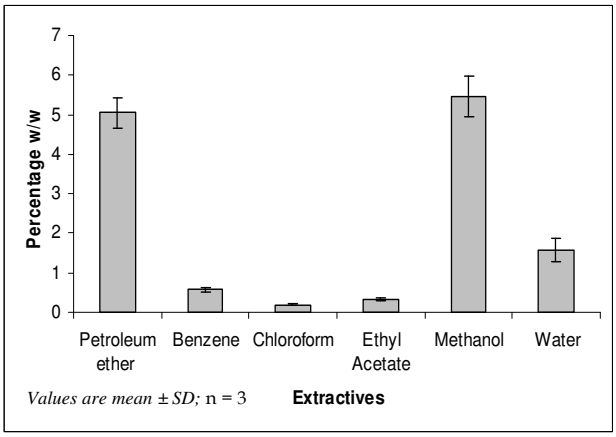

Figure 8: Successive extractive values of E. echinatus root.

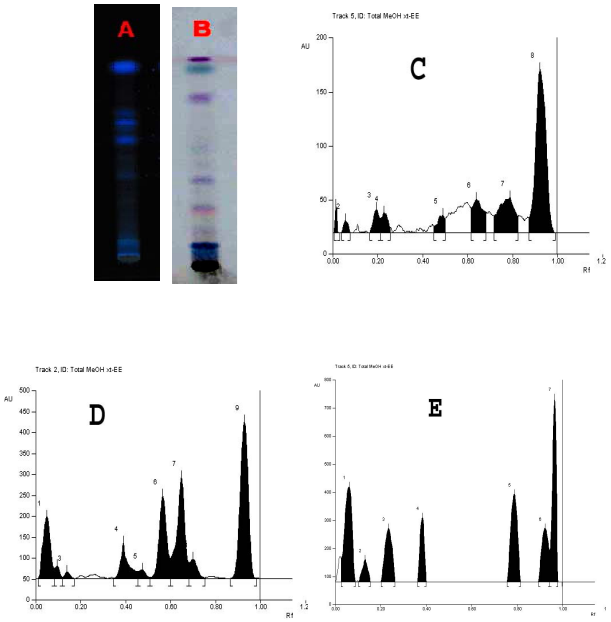

Figure 9. HPTLC Finger print profile of Methanol extract of E. echinatus root in solvent system 1. (A) fluorescent nature of the compounds; (B) after derivatization with anisaldehyde-H_2SO_4; (C) TLC chromatogram after densitometric scan under UV 254; (D) TLC chromatogram after densitometric scan under UV 366; (E) TLC chromatogram after densitometric scan at 540 nm.

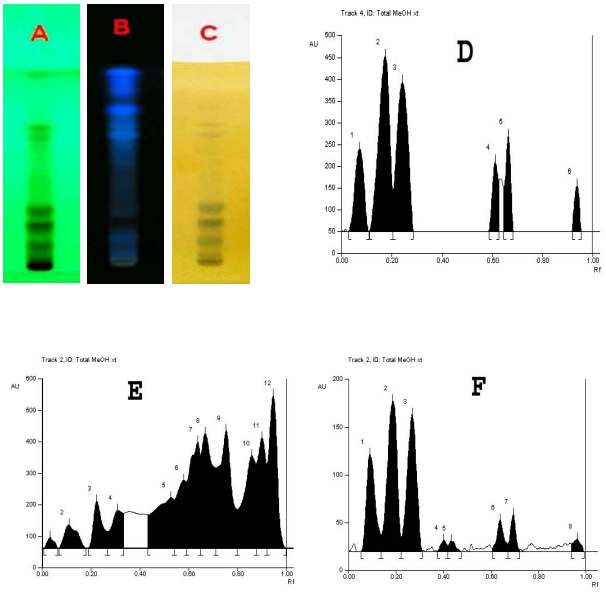

Figure 10. HPTLC Finger print profile of methanol extract of E. echinatus root in solvent system 2. (A) HPTLC profile under UV 254 nm; (B) fluorescent nature of the compounds under UV 366; (C) after derivatization with alcoholic $FeCl_3$ under white light; (D) TLC chromatogram after densitometric scan under UV 254; (E) TLC chromatogram after densitometric scan under UV 366; (F) TLC chromatogram after densitometric scan at 540 nm.

DISCUSSION

In ethnomedicinal practices the traditional healers use *E. echinatus* as '*Brahmadandi*' in treatment of various ailments especially in reproductive disorders. Plants from other genus are also reported to be sold under the common trade name '*Brahmadandi*' though the whole herbs can easily be differentiated by morphological and floral arrangements, but it becomes very difficult in case of roots, which are sold in the market as crude drugs in the form of dried and cut pieces. Therefore some diagnostic features have been evolved to identify and to differentiate the *E. echinatus* roots from the other crude drugs and

adulterants. Microscopic evaluation is an indispensable tool for identification of medicinal herbs and is one of the essential parameter in modern monograph. In this regard the important microscopic features of the various parts of the plant have been documented such as T.S of roots showed an endodermis having casparian thickening, presence of bilayered medullary rays and parenchyma inclusions in the xylem cylinder as the distinct features. Presence of heterogeneous cortex, heterogeneous pericycle, leaf trace bundles and large number of cluster crystals of calcium oxalate are the characteristic features observed in the microscopy of the stem. T.S of the leaf showed the presence of very long, uniseriate, collapsed trichomes. Ridged midribs showing the presence of patches of collenchyma, below upper & above the lower epidermis and patches of sclerenchyma, on either side of the vascular bundle are the characteristic features of the leaf microscopy.

However, the roots of *E. echinatus* are sold in the form of dried and cut pieces and are more likely to be substituted with roots of the other plants sold under the same trade name '*Brahmadandi*'. In such cases microscopy alone may be inadequate to identify the true plant material. Also microscopy does not reveal much about the deterioration of the crude drug, therefore many of the modern herbal pharmacopoeias and other regulatory agencies like WHO included TLC as a powerful and most economical tool for true identification of the plant material, especially in terms of its chemical constituents.

Thus the HPTLC finger print profiles of the major chemical constituents in the crude extract along with their *Rf* values and percentage proportions were recorded which would serve as a reference standard for the scientist engaged in research on the medicinal properties of this plant.

Studies on physicochemical constants and phytochemical screening can serve as a valuable source of information and provide suitable standards to determine the quality of this plant material in future investigations or applications.

ACKNOWLEDGEMENT

One of the authors Mr. Somashekar Padashetty would like to thank All India Council for Technical Education (AICTE), New Delhi, for providing the financial assistance, in the form of National Doctoral Fellowship, to carryout this work and to Dr. (Mrs.) Susy Albert for providing technical assistance during the studies.

REFERENCES

1. Anonymous, *The wealth of India*, Raw Materials, vol. 3. (CSIR, New Delhi, 1952) p.127.
2. K.R. Kirtikar, B.D. Basu, *Indian Medicinal Plants* vol. 2. (Periodical experts, Delhi, 1975) p. 1415.
3. A.K. Nadkarni, *Indian Materia Medica* vol. 1. (Bombay Popular Prakashan Pvt. Ltd, Bombay, 1976) p. 468.
4. H.N. Kakrani, B.H. Kakrani, A.K. Saluja. Traditional treatment of diabetes through herbs in kutch district, Gujarat state. *Planta indica* **1:** 16-21 (2005).
5. P. Oudhia. Medicinal Herbs of Chhattisgarh, India having less known uses of Brahmadandi.http://botanical.com/site/column_poudhia/250_brahmadandi.html. (2003)
6. B. Singh, S.S. Gambhir, V.B. Pandey, V.K. Joshi. Anti-inflammatory activity of *Echinops echinatus*. *J. Ethnopharmacol* **25:** 189-199 (1989).
7. Z. Abraham, S.D. Bhakuni, H.S Garg, A.K. Goel, B.N Mehrotra, G.K. Patnaik. Screening of Indian plants for biological activity Part XII. *Indian J. Exp. Biol.* **24:** 48-68 (1986).
8. K.M Savitasharma, B.K Metha. Antimicrobial activity of *Echinops echinatus* root extracts. *Fitoterapia* **60:** 82-83 (1989).
9. O.S. Bhakuni, M.L. Dhar, M.M Dhar, B.N Dhawan, B.N Mehrotra. Screening of Indian Plants for biological activity Part II. *Indian J. Exp. Biol.* **7:** 250-262 (1969).
10. S. Sharma, M. Porwal, B.K. Mehta. Chemical investigation of *Echinops echinatus*. *J. Ind. Chem. Soc* 65: 69-70 (1988).
11. S. Singh, R.K. Upadhyay, M.B. Pandey, J.P. Singh, V.B. Pandey. Flavonoids from *Echinops echinatus*. *J. Asian Nat Prod Res*, **8:** 197–200 (2006).
12. R K Issar. The botanical identification of market sample of *Brahmadandi*. *Jour. Res. Ind. Med.* **9(1):** 92-93 (1974).
13. D.A. Johansen. *Plant Microtechnique*. New York, McGraw-Hill, pp. 126-154 (1940).
14. Anonymous. *Indian Pharmacopoeia* vol 2. (Government of India, New Delhi, 1996).
15. V.L. Singleton, J.A.Rossi. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **16:** 144-153 (1965).
16. P.J. Houghton, A. Raman. *Laboratory Handbook for the Fractionation of Natural Extracts*. (Chapman and Hall, London, 1998) pp. 154-162.
17. K. Peach, M.V. Tracy *Modern Methods of Plant Analysis* vols. 3-4. (Springer-Verlag, Heidelberg, 1955).
18. E. Stahl. *Thin Layer Chromatography*; spray reagents, 2nd ed. (Springer-Verlag, New York, 1969) pp. 854-90.