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Pharmacognostical Evaluation of *Andrographis stenophylla* C. B. Clarke

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Abstract – *Andrographis stenophylla* C. B. Clarke., (Acanthaceae) is an erect glabrous undershrub with very narrow leaves and stems from a stout rootstock, the corolla pale with dark red stripes. The plant grows in hills of about 1200 meters height in South India. No scientific work reports are available with regard to this plant. The present study, thus deals with the detailed pharmacognostical evaluation of the plant *A. stenophylla* using light and confocal microscopy, WHO recommended physico-chemical determinations and authentic phytochemical procedures. The physico-chemical, morphological and histological parameters presented in this paper may be proposed as parameters to establish the authenticity of *A. stenophylla* and can possibly help to differentiate the drug from its other species.
Keywords – *Andrographis stenophylla,* Acanthaceae, pharmacognosy, HPTLC and HPLC

Introduction

Andrographis stenophylla C. B. Clarke., (Acanthaceae) is an erect glabrous undershrub with very narrow leaves and stems from a stout rootstock, the corolla pale with dark red stripes (Henry *et al*., 1982). The plant grows in hills of about 1200 meters height in South India. Literature survey reveals that there are no synonyms or common names available. The tribes living in and around Vadakkumalai hills, in Coimbatore district call this plant as Chiriyanangai, which is the vernacular name of *Andrographis paniculata.* The local tribes claim that the leaves of *A. stenophylla* are having more potential effect than *A. paniculata* in treatment of chronic fever, diabetes, wounds, ulcers, inflammations, cough, skin diseases and leprosy (Warrier *et al*., 1995). However, pharmacognosy information about this plant has not been published, particularly the necessary to define quality control procedures of the *A. stenophylla* as raw material. Hence, the present investigation deals with the pharmacognostical evaluation of the *A. stenophylla.* The study includes morphological and anatomical evaluation, determination of physico-chemical constants and the preliminary phytochemical screening of the different extracts of *A. stenophylla*.

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Experimental

Plant material – The plant *A. stenophylla* was collected from Vadakkumalai hills, in Coimbatore district, Tamil Nadu, India during the month of August. The botanical identity of the plant was confirmed at the Botanical Survey of India (BSI), Coimbatore, Tamil Nadu. A voucher specimen (P 101) has been deposited at the Museum of the Department of Pharmacognosy, C.L. Baid Metha College of Pharmacy, Chennai.

Chemicals and instruments – Compound microscope, glass slides, cover slips, watch glass and other common glass ware were the basic apparatus and instruments used for the study. Microphotographs were taken using a Leica DMLS microscope attached with Leitz MPS 32 camera. Solvents *viz.* ethanol (95%), petroleum ether, diethyl ether, chloroform, acetone, n-butanol and reagents *viz.* phloroglucinol, glycerin, HCl, chloral hydrate and sodium hydroxide were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India.

Macroscopic and microscopic analysis – The macroscopy and microscopy of the different parts like leaf, petiole, stem and root were studied according to the method of Brain and Turner (1975a). For the microscopical studies, cross sections were prepared and stained as per the procedure of Johansen (1940). The micropowder analysis was done according to the method of Brain and Turner (1975b) and Kokate (1986a).

Physico-chemical analysis – Physico-chemical analysis i.e. percentage of ash values and extractive values were performed according to the official methods prescribed (Indian Pharmacopoeia, 1966) and the WHO guidelines on quality control methods for medicinal plant materials (WHO/QCMMPM guidelines, 1992). Fluorescence analysis was carried out according to the method of Chase and Pratt (1949) and Kokoski *et al*. (1958).

Preliminary phytochemical screening – Preliminary phytochemical screening was carried out by using standard procedures described by Kokate, (1986b) and Harborne (1998).

HPTLC profile – Qualitative densitometric HPTLC analysis was performed for the development of characteristic finger print profile for successive chloroform ($CHCl_3$) and ethyl acetate (EtOAc) extracts of entire plant. Ten μL of the sample solutions were applied and the plates were developed in $CHCl_3$-MeOH (7 : 3) and (9 : 1) respectively for chloroform and ethyl acetate extracts. Developed plates were then scanned densitometrically at various wavelengths. R_f values, peak area and spectrum of each peak were determined for these extracts.

HPLC study – The presence of andrographolide was identified in ethanol (EtOH) extract by TLC with authentic sample. Hence, quantification of Andrographolide was carried out for the EtOH extract of the entire plant by HPLC. The linearity of the HPLC method was investigated for Andrographolide in the range of 15 - 240 μg/mL at five concentration levels. 5 gm of dried entire plant powder of *A. stenophylla* was extracted with EtOH (95%) by Soxhlet extractor for 5 hrs. Quantitative HPLC was performed on an isocratic high pressure liquid chromatograph (Shimadzu HPLC Class 10A Series) with two LC-10AT pumps, using a fixed wavelength guided by a programmable UV detector. The column used was Phenomenex RP-C18 (250 mm × 4.6 mm i. d., Particle size 5 μm). The HPLC system was equipped with the software, Class LC-10AT series, version 5. 03 (Shimadzu). The mobile phase, acetonitrile-water (35 : 65) was filtered before use through a 0.45 μm membrane filter. It was degassed with a helium spurge for 15 min and pumped from the respective solvent reservoirs to the column at a flow rate of 1.5 mL/min, which yielded a column back pressure of 160 - 180 kg/cm. The run time was set at 10 min the volume of injection loop was 20 μL. Prior to the injection of the drug solutions, the column was equilibrated for at least 45 min with the mobile phase flowing through the systems. The eluent was monitored at 230 nm and the data acquired was stored and analyzed with the software.

Results and Discussion

Macroscopic characters of the plant – The plant is an erect glabrous undershrub with very narrow leaves and stems from a stout rootstock, the corolla pale with dark red stripes. The branches often tetragonous, leaves are entire, often minutely linear up to 2.5 cm long. Flowers are in axillary and terminal, racemes, sometimes reduced to one flower, often unilateral; bracteoles minute, calyx five partite, lobes glabrous, lineolate. Pedicles are up to 0.4 cm long. Corrolla is small, usually pale but blotched and spotted with red and purple, tubular at base, prominently 2-lipped, the upper slightly bifid and the lower usually deflexed. Stamens are two in number with broad filaments, enlarged at base and sometimes in the middle. Anthers are 2-celled, usually bearded at base with a tuft of white hair. Ovary is 2-celled, ovules 2 - 6 in each cell, style slender and stigma minutely bifid. Fruits are an oblong or elliptic capsule compressed at right angles to the septum, 4 - 12 seeded. Seeds are hard and deeply pitted glabrous. Root is sub cylindrical, slightly tapering, branched and possesses longitudinal wrinkles. It has a fibrous fracture and is 3 - 7 cm long and 4 - 7 mm in thickness. The inner wood is yellowish brown in colour and bitter in taste.

Microscopic characters

Leaf – The leaf has thick lamina and prominent midrib. The lamina is uniformly smooth and even (Fig. 1A). The midrib has short conical adoxial hump and a wide hemispherical abaxial part. The midrib measures 500 μm both in vertical and horizontal planes. The adaxial hump has thin layer of epidermis and a mass of collenchyma cells. The abaxial midrib consists of 2 or 3 layers of collenchyma cells in the outer zone and thin walled compact parenchyma cells in the inner zone (Fig. 1B). The mesophyll tissue occurs as transcurrent band on the adaxial part of the midrib. The vascular tissues occur in a single, small arc-shaped bundle with adaxial vertical files of xylem elements and abaxial pad of phloem elements. An accessor strand may be seen on the lateral side of the main strand.

The lamina is 200 μm thick. It consists of broader adaxial epidermis with squarish cells; the cuticle is prominent; some of the epidermal cells are dilated into large circular lithocysts containing the calcium carbonate cystoliths. The abaxial epidermis is thin and consists of small rectangular or spindle shaped cells. The mesophyll is differentiated into a narrow zone of palisade cells and 5 or 6 layers of lobed, aerenchymatous spongy-parenchyma

cells. The leaf margin is slightly bent down; the structure of the margin is similar to the midlamina.

Petiole – Cross sections of the petiole from upper as well as lower regions were studied. The cross section of the upper part of the petiole has slightly convex adaxial side two thick and prominent lateral wings and broady hemispherical body (Fig. 1C). Thick and prominent epidermis is seen all around the petiole. The ground tissue consists 2 or 3 layers of outer collenchyma cells and remaining portions being compact parenchyma cells. The vascular system consists of a broad arc of main vascular bundle and small, less prominent lateral accessory

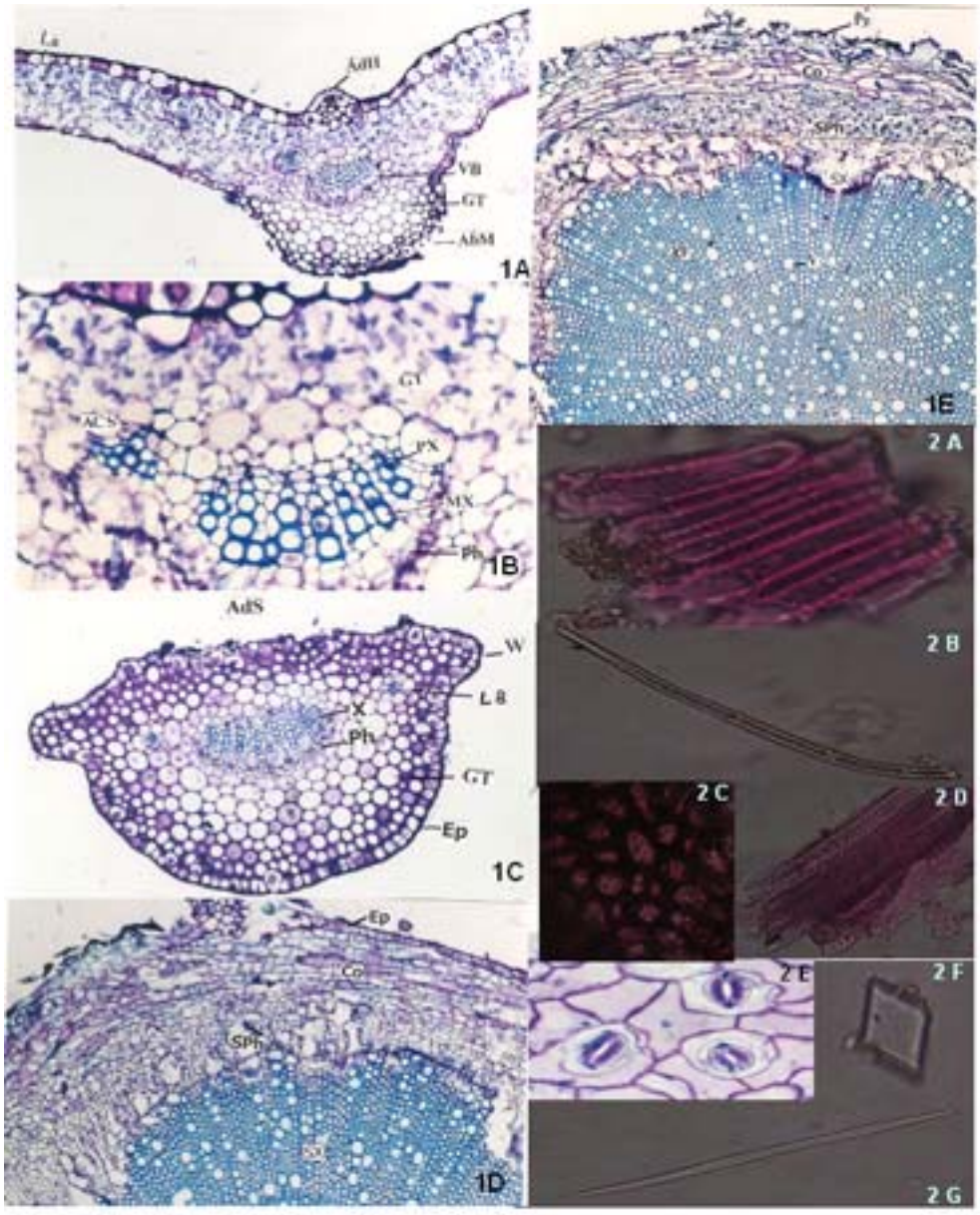

Fig. 1. A: T.S. of leaf through midrib with lamina, B: Midrib vascular bundle enlarged **(AbM-**Abaxial midrib; **ACS-**Accessory strand; **AdH-**Adaxial hump; **GT-**Ground tissue; **Mx-**Metaxylem**; Ph-**Phloem; **Px-**Proto xylem), C: T.S. of the petiole (**AdS**-Adaxial side; **Ep**-Epidermis; **GT**-Ground tissue; **Lc**-Lithocyst; **LB**-Lateral bundle; **Ph**-Phloem; **St**-Stem; **W**-Wing; **X**-Xylem), D: T.S. of the stem-a sector enlarged (**Co**-cortex; **Ep**-Epidermis; **Ph**-Phloem; **Pi**-Pith; **SPh**-Secondary phloem; **SX**-Secondary xylem; **W**-Wing; **X-**Xylem), E: Anatomy of the root-a sector enlarged (**Co**-Cortex; **Pe**-Periderm; **SPh**-Secondary phloem; **SX**-Secondary xylem; **V**-Vessel; **XF**-Xylem fibres).

Fig. 2. Powder microscopy.

A: Stone cells, **B**: Trichomes, **C**: Cork cells, **D**: Wood elements, **E**: Stomata, **F**: Prism ca. oxalate crystal, **G**: Less lignified fibre.

bundles. The vascular bundles are collateral with adaxial, parallel lines of xylem an abaxial pad of phloem. The basal part of the petiole is similar to the upper part. The adaxial side is flat with wavy surface. There are two prominent lateral wings; the abaxial part is broad and hemispherical. The ground tissue consists of outer zone of collenchyma and inner zone of parenchyma; the vascular bundle is single and broadly arc-shaped; the accessory lateral strands are absent.

Stem – The stem is four angled with thick, prominent wings along the angles. In a young stem, there is intact, thick epidermal layer with squarish cells and thick cuticle. Some of the epidermal cells are dilated into circular wide cavities enclosing the cystolith. The outer part of the cortex consists of three or four layers of collenchyma cells; the wings also consist of collenchyma cells. The wing cortex consists of chlorenchymatous tissue (Fig. 1D).

The inner cortex is chlorenchymatous in young stem and compact, compressed parenchymatous cells in the old stem. The vascular cylinders are thin and hollow the wide parenchymatous pith. The old stem has thick secondary xylem and broad secondary phloem. The secondary xylem exhibits indistinct growth wings with diffusely distributed vessels. The vessels are circular, thick walled and are in long or short radial multiples, the vessels are up to 25 μm in diameter. The xylem fibres are libriform-type with thick lignified walls narrow lumen. Secondary phloem is in narrow cylinder around the xylem. It consists of short, narrow rays and small nests of phloem.

Root – A thin root measuring 2.5 mm in diameter has thin, indistinct periderm, narrow cortex and thick, dense, solid vascular cylinder (Fig. 1E). The epidermis is disintegrated and the cortex consists of 4 or 5 layers of tangentially oblong and radially compressed parenchyma cells. Secondary phloem is in thin cylinder and consists of small groups of sieve elements. In the thin root, secondary xylem is in the form of solid, dense cylinder without growth rings. In fairly thick roots, the secondary xylem exhibits distinct growth-rings. The cortical zone and secondary phloem are broader and distinct. The root with a diameter of 2.5 mm, these are 3 growth rings; each growth-ring is demarcated by thick-walled fibres with narrow lumen; the vessels are semi-ring porous. The vessels are circular, mostly solitary, thick walled and range in diameter from 10 μm (late wood vessels) to 30 μm (early wood vessels) in diameter. The fibres are libriform type, thick walled and narrow lumened.

Powder characters – The leaf powder is dark green in colour with an unpleasant odour and intensely bitter in taste. On microscopical examination the powder showed numerous multi cellular (1 - 5 celled) covering trichomes which are, sometimes broken and rarely collapsed (Fig. 2B). Diacytic or caryophyllaceous type of stomata with irregularly shaped epidermal cells are seen (Fig. 2E). The stomata occur on the lower epidermis, the common wall of the subsidiary cells are at right angles to the long axis of the stomata. Some of the stomata have more than one set of subsidiary cells; the guard cells are either elliptic or circular.

The stem powder is light greenish brown in colour with a characteristic odour and bitter in taste. Thick walled brownish cork cells (Fig. 2C) and prism type calcium oxalate crystals are seen (Fig. 2F). Parenchymatous cells are rectangular to square in shape and thin walled. Lignified stone cells are rarely seen in groups (Fig. 2A) and concentric starch grains are also seen.

The root powder is slightly yellowish brown in colour with a characteristic odour and slightly bitter in taste. The wood elements are wide cylindrical cells with wide openings at the ends known as perforation plates and have lateral walls with reticulate thickenings. The wood elements are 120 - 230 μm in length and 30 - 60 μm in width (Fig. 2D). Fusi-form fibres are less lignified and appear in groups (Fig. 2G). The fibres have tapering ends with secondary wall thickening especially in the pits. The fibres measure 400 - 750 μm in length and 30 - 50 μm in thickness.

Preliminary phytochemical screening – Preliminary

Table 1. Preliminary phytochemical screening of the entire plant powder of *A. stenophylla*

Test	Hexane	Benzene	Chloroform	Acetone	Ethanol	Water
Carbohydrates	–	–	–	–	+	+
Phytosterols	+	–	–	–	–	–
Terpenes	+	+	–	–	–	–
Lipids and fats	+	+	–	–	–	–
Saponins	–	–	–	–	–	–
Phenolic compounds and tannins	–	–	–	+	+	+
Flavonoids	–	–	–	+	+	–
Gums and Mucilage	–	–	–	–	–	+

+ Denotes the presence of the respective class of compounds

Table 2. Ash values of the entire plant powder of *A. stenophylla*

Parameters	Values % (w/w)
Total ash	12.38
Acid insoluble ash	3.90
Water soluble ash	6.94
Sulphated ash	13.38

Table 3. Extractive values of the entire plant powder of *A. stenophylla*

Parameters	Values % (w/w)
Water soluble extractive	2.58
Ethanol soluble extractive	1.02
Ether soluble extractive	0.91

Table 4. Fluorescence analysis of the entire plant powder of *A. stenophylla*

Treatment	Day light	UV light (254 nm)
Powder as such	Dark green	Light brown
Powder + 1N NaOH (Aqueous)	Pale green	Light green
Powder + 1N NaOH (Alcoholic)	Pale green	Light yellowish green
Powder + 1N HCl	Brownish green	Fluorescent green
Powder + Ammonia	Dark brownish green	Fluorescent greenish brown
Powder + Iodine	Brownish green	Brownish green
Powder + $FeCl_3$	Slightly greenish brown	Dark greenish brown
Powder + 1N H_2SO_4	Brownish green	Dark brown
Powder + Acetic acid	Light brownish green	Dark brownish green
Powder + 1N HNO_3	Slightly reddish green	Reddish green

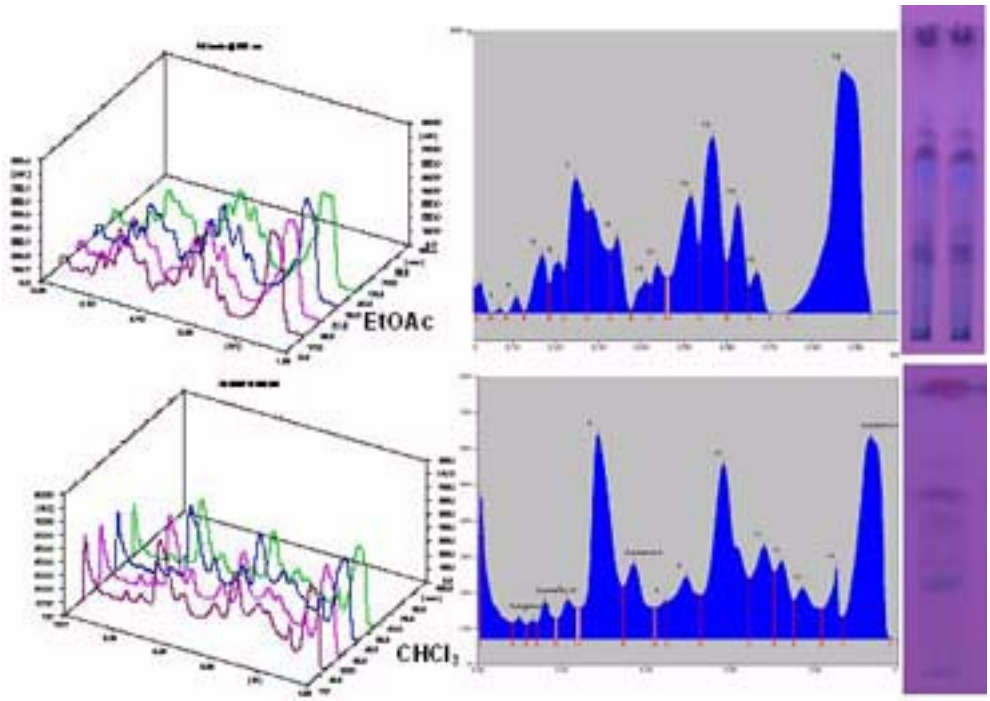

Fig. 3. HPTLC chromatogram of ethyl acetate and chloroform extracts of *A. stenophylla* (**EtOAc**-Ethyl acetrate extract; **$CHCl_3$**-Chloroform extract).

phytochemical screening revealed the presence of terpenes, phytosterols, phenolic compounds, carbohydrates and flavonoids (Table 1).

Physico-chemical studies – Ash value of a drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values (Table 2) of the powdered *A. stenophylla* root revealed a high concentration of sulphated ash. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The water soluble extractive (Table 3) was high in *A. stenophylla.* The results of fluorescence analysis of the drug powder are presented in Table 4.

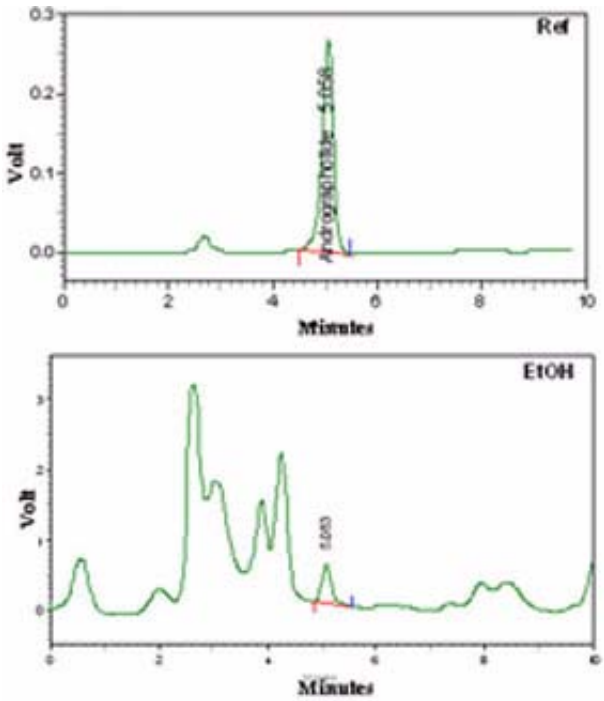

Fig. 4. HPLC profile of ethanol extract of entire plant *A. stenophylla* and Andrographolide reference (**EtOH**-Chromatogram of ethanol extract; **Ref**-Chromatogram of andrographolide reference).

HPTLC profile – A densitometric HPTLC analysis was performed for the development of characteristic finger print profile for EtOAc and $CHCl_3$ extracts of entire plant of *A. stenphylla* (Fig. 3), which may be used as markers for quality evaluation and standardization of the drug. Most of the compounds have shown maximum absorbance at 265 nm for EtOAc and $CHCl_3$ extracts. The bands in the sample were obtained at R_fs, 0.11, 0.17, 0.21, 0.25, 0.28, 0.34, 0.41, 0.44, 0.52, 0.57, 0.63, 0.67 and 0.87 (EtOAc extract) and 0.13, 0.16, 0.21, 0.29, 0.37, 0.44, 0.50, 0.58, 0.68, 0.72, 0.77, 0.85, 0.93 ($CHCl_3$ extract), which can be used as identifying markers.

HPLC study – The development of HPLC methods for the determination of drugs has received considerable attention in recent years because of its importance in the quality control of drugs and drug products. The aim of HPLC study was to quantify the amount of andrographolide present in the EtOH extract and entire plant of *A. stenophylla.* The retention time of andrographolide was found to be 5.0 - 5.1 min (Fig. 4). The calibration curve was constructed by plotting the area of the andrographolide against concentration. It was found to be linear with a correlation coefficient of 0.9965, the representative linear regression equation being $Y = 34489X - 125604$, $r^2 = 0.9965$. The amount of andrographolide present in the EtOH extract was found to be 3.022% and the entire plant of *A. stenophylla* contains 0.6045% of andrographolide.

Conclusion

As there is no pharmacognostic/anatomical work on record of this much valued traditional drug, the present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. While discussing the purpose of systematic anatomy, Metcalfe and Chalk (1981) pointed out that any exercise that involves the identification of plant material when it is in a fragmentary or partly decomposed condition can be achieved by the method of comparative histology. As regards, the subterranean plant parts, the correct identification of roots can often be achieved only by microscopical investigation.

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