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Research Article

Pharmacognostical Studies and Phytochemical Investigation of *Barleria noctiflora* Linn (Acantheceae).

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ABSTRACT
Barleria noctiflora L. (Acantheceae), an important medicinal plant used in the Indian system of medicine. The aim was to investigate the pharmacognostical and phytochemical of *Barleria noctiflora L.* (Acantheceae) to find ethnomedicinally important in the current concepts and modern research to provide efficient and inexpensive medicine to the society, confidence in indigenous medicine. The *Barleria noctiflora L.* (*B. noctiflora*), were studied macroscopical, microscopical, physiochemical, phytochemical, fluorescence analysis and HPTLC methods for standardization as recommended by WHO. A bushy, much branched prickly shrub with 2-3 spines in axil of each leaf. An herb up to 0.6 – 1.5 m height with nodal spines, lanceolate leaves and pinky violet flowers. It occurs throughout the hotter parts of India. The physiochemical evaluation of total ash, water soluble ash, acid insoluble ash, alcohol soluble extractive value and water soluble extractive value and loss on drying were 13.5, 9, 2, 7.36, 8.24 and 2.67 percentage respectively. The microscopical character leaves shows upper epidermis and lower epidermis were along the midrib is narrow comprising of small slightly appellate thick walled cells with thick cuticle the cells. It has spongy parenchymatous ground tissue of vascular strand with dark bundle sheath. The stem showed phloem and a wider zone of Meta xylem element. Phloem has dilated rays and parallel lines of phloem elements. Preliminary phytochemical analysis revealed the presence of flavonoides, alkaloids, saponins, steroids, glycosides, tannins and phenols. The present study results were served as an important source of information to certain the identification for standards of this medicinal important plant for future applications.

Keywords: *Barleria noctiflora L.*, Microscopy, Pharmacognostic study, Phytochemical analysis.

INTRODUCTION

India has an ancient heritage of traditional medicine. Material media of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products. Indian traditional medicine is based on various systems including Ayurveda, siddha and unani[1]. Lot of efforts has been taken by the government and private sectors for the development of the traditional system based on these three methods. The world health organization (WHO) estimates that about 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs[2]. Herbal medicine are prepared from various plant part are contain many bioactive compounds are used primarily for treating. Due to the demand in the field of herbal medicines, it has become necessary of systematic knowledge about herbal drugs. The safety of herbals, it is better not to bury our phytotherapeutic heads in the sand like frightened ostrich in the hope that herbal health problems will dissolve by themselves. Thus we should accept that herbal medicines entail certain health risks and to look out actively for safety problems associated with herbal medicines several considerations [3]. Consistency in composition and biological activity are essential requirements for the safe and effective use of therapeutic agents. Quality is the critical determinant of safety and efficacy of botanical medicines[4]. Quality control of herbal medicines involves sensory inspection (macroscopic and microscopic examinations). *Barleria noctiflora L.* (*B. noctiflora*), belongs to the family Acanthaceae, which is being widely used as Folk and ayurvedic medicine. It is widely distributed throughout tropical region of India, Africa, Sri Lanka and other parts of Asia[5]. *B. noctiflora* is a shrub and it grows up to 90 cm height. Many of the members of the Acanthaceae family are used as medication for asthma[6]. The genus Barleria includes 28 taxa and 26 species. It has 3 unique characters calyx 4-partile with 2 large outer segments and 2 smaller inner ones, spheroidal, honey - combed pollen grains and the predominant with double cystoliths[7]**.** Most of the Barleria species are potent anti- inflammatory, analgesic, anti leukemic, antitumor, anti-hyperglycemic, anti-amoebic, virucidal & antibiotic[8]**.** Furthermore, literature on *B. noctiflora* revealed that there is no study till now on the pharmacognosy and biological activities. Thus the present study was carried out to investigate the pharmacognostical study of *B. noctiflora*.

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MATERIALS AND METHODS

Collection of plants

B. noctiflora was collected during winter season in and around erode District, Tamilnadu, India. It was identified and authenticated by Prof. P. Jayaraman, Director, National Institute of Herbal Science, Chennai-45, Tamilnadu, India (Ref no: PARC/2011/1015), and the voucher specimen was deposited at the same institute for future reference. Taxonomic description, vernacular names, habit and habitat of the plant, morphological characteristics were noted from the available literature[9-13].

Macroscopical studies

The organoleptic studies were carried out by simple determination, technique like the shape, size, color, odour, margin and apex of the leaf and stem[14]. Morphological character of the plant leaf and stem were done[15-16].

Microscopical studies

T.S of Leaf and stem

To select healthy plant and normal organ. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin 5ml + Acetic acid 5ml + 70% of Ethanol 90ml) after 24hrs of fixing the specimen were dehydrated with graded series of Tertiary Butyl Alcohol (TBA) as per the schedule[17]. Infiltration of the specimen was carried by gradual addition of paraffin wax (melting point 58-60ºC) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the section was 10-12µm, de-waxing of the sections was by customary procedure[18]. The sections were stained with toluidine blue as per the methods[19]. The staining results were remarkable good and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies, etc. wherever necessary sections were also stained with safranin and fast green and IKI (for starch). Glycerin mounted temporary preparation were made from macerated / cleared materials. Powdered materials of different parts were cleared with sodium chloride and mounted in glycerin medium after staining. Different cell component were studied and measured[20-21].

Photomicrograph

Microscopic descriptions of tissue are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For the study of crystals, starch grains and lignified cells, polarized light was employed, since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy book[22-23].

Powder analysis

The dried powder of whole plant of *B. noctiflora* was passed through sieve no 60 and examined for its microscopic characters. The powder of drug was boiled with chloral hydrate to remove the coloring matters, mounted on the glass slides using glycerin and covered with a cover slip and viewed under microscope[24].

Quantitative Microscopy

The cleared materials were washed thoroughly and stained with safranin for quantitative microscopic studies of Stomatal Number, Stomatal Index, Vein-Islet and Vein termination number and Palisade Ratio.

Physicochemical Parameters

The various physiochemical parameters such as Total ash, water soluble ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value and loss on drying were determined.

Preliminary phytochemical analysis

The preliminary phytochemical analysis of the extract was carried out using standard methods. The presence and absence of the secondary phytoconstituents. Vitamins and inorganic elements were noted[25-26].

Fluorescence analysis

The dried Powder were treated with various chemical reagents and exposed to visible, day light and ultraviolet light to study their fluorescence behavior[27-28].

Chromatographic studies

HPTLC studies were carried out on alcoholic extract using camag HPTLC system equipped with Linomat IV sample applicator, Camag TLC scanner 3 and CATS 4 Software for interpretation of data. An aluminium plate (5X10 cm) precoated with slica gel 60 F_{254} (E Merk) was used as adsorbent. The plates were developed using Ethyl acetate: Methanol (6.5: 3.5) in a camag twin trough chamber to a distance of 8cm each after development using Dragendorff's reagent followed by 10% ethanolic sulphuric acid as post derivatasion reagent and scanned at 254nm[29].

Scientific classification[10-13]

Kingdom	:	Plantae
Phylum	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Lamiales
Family	:	Acanthaceae
Genus	:	Barleria
Species	:	noctiflora
Botanical name	:	*Barleria noctiflora*

Habit and habitat

A bushy, much branched prickly shrub with 2-3 spines in axil of each leaf. An herb up to 0.6 – 1.5 m height with nodal spines, lanceolate leaves and pinky violet flowers. It occurs throughout the hotter parts of India, Africa, Sri Lanka and other parts of Asia. A common under shrub occasionally found wild, but generally cultivated as a hedge plant or for its ornamental flowers. They have pungent odour and slightly sweet and sour taste. The plant has more important medicinal uses[14-16].

RESULTS

Macroscopical studies

In organoleptic evaluation, appropriate parameters like taste, odour, size, shape and color of the leaf stem were studied. The leaf is green and stems grey in color. The leaf is in oval like shape and stem cylindrical in shape. [Figure-

Table 1: Preliminary phytochemical screening of *B. noctiflora*.

Chemical constituents	Water extract	Alcohol extract	Chloroform extract	Petrolium ether extract
Flavonides	+	+	-	-
Alkaloids	+	+	-	-
Phenols	+	+	-	-
Saponins	+	+	-	-
Steroids	-	-	+	+
Glycosides	+	+	-	-
Tannins	+	+	-	-
Protein	+	+	-	-
Carbohydrate	+	+	-	-

[+] Denotes the presence of the respective class of compounds.

Table 2: Florescence analysis of *B. noctiflora* powder

S.No	Reagents	Day light	Short UV	Long UV(365nm)
1.	Powdered drug	Pale yellows green	Yellowish Green	pale green
2.	Powder + 1 N NaOH	Pale Yellow	Pale Green	Dark green
3.	Powder + 1 N HCl	Pale Yellow	Pale Green	Pale Green
4.	Powder + 50% H2SO4	Dark brown	Brownish Green	Brownish Green
5.	Powder + H_2O	Pale green	Pale Greenish Yellow	Greenish Yellow
6.	Powder + Ammonia	Pale brown	Pale brown	Pale brown
7.	Powder +50%HNO3	Dark yellow	Pale yellow	Dark greenish yellow
8.	Powder + picric acid	Pale Yellow	greenish yellow	Dark green
9.	Powder + $Fecl_3$	Brownish Green	Brownish Green	Dark green
10.	Powder + Methanol	Pale green	Pale green	Light green

Table 3: Quantitative Microscopic data

S.No	Parameters	Value in 1 sq.mm (average of 10 fields)
1	Stomatal number	21.9
2	Stomatal index	9.9
3	Vein-islets number	4.3
4	Vein termination number	4.2
5	Palisade ratio	8.1

1]. The plants are pungent odour and slightly sweet and sour taste.

Morphological studies

The *B. noctiflora* leaf are dorsiventral variable in size , 1.5 – 2 cm long, 1- 1.5cm wide, simple, elliptic, acuminate, entire, acute reticulate, glabrous above, glabrous or pubescent beneath; petiole short. The stem 1- 6mm thick, terete, hard, glabrous, nodes swollen, branching at nodes, young stem grey, slightly four angled, usually with 2-3 divericate spines at axil of leaf mature stem cylindrical with longitudinally arranged or scattered, a few mature stem slightly hollow. The flower are sessile, often solitary in the axils, becoming spicate above, laciculate, acute bristle tipped early glabrous bracteoles, bristle tipped, calyx divided almost to the base[18-19].

Microscopical studies

T.S. of leaf

The T.S. of leaf through midrib shows the presences of unicellular hair are present in the epidermis. The upper epidermis and lower epidermis were along the midrib is narrow comprising of small slightly appellate thick walled cells with thick cuticle cells. The ground tissue of the midrib is homogenous and Parenchyma cells. The xylem part of the strand has several long parallel compact lines of xylem elements. The elements are narrow, elliptical and thin walled. Phloem occurs in broad and the continuous arc along the abaxial side of the xylem. The midrib were shows the trichome, and palisade tissue. [Figure-2A].

The lamina part shows the palisade spongy parenchyma tissues. The palisade cells are wide, cylindrical and compact. The epidermis is thin and has narrow rectangular thick cuticularised cells. The upper epidermis shows glandular hair, cuticle and trichome. It has spongy parenchymatous ground tissue of vascular strand with dark bundle sheath. The center of lamina part shows xylem vessels and vascular bundle. [Figure-2B]. A sector enlarged section shows the presence of phloem, xylem, and parenchyma glandular cells. [Figure-2C].

T.S. of Stem

The T.S. of stem shows the vascular cylinder consists of a thin, discontinuous layer of crushed phloem and a wider zone of meta xylem elements. Phloem has dilated rays and parallel lines of elements. Pith is wide and parenchymatous cells are circular to angular, compact and no intercellular spaces the presence of protoxylem and vessels. [Figure-3A]. A sector enlarged stem section were clearly shows

Figure 1: Plant of *Barleria noctiflora L.*

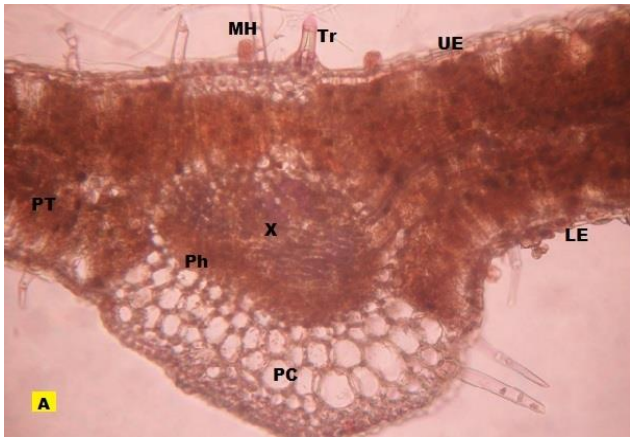
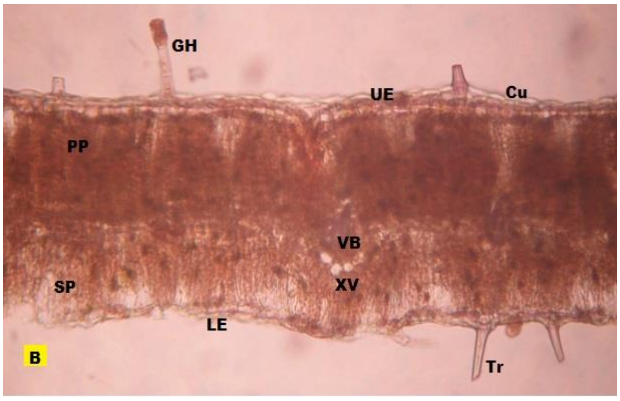


Figure 2a: T.S. of *Barleria noctiflora L.* leaf



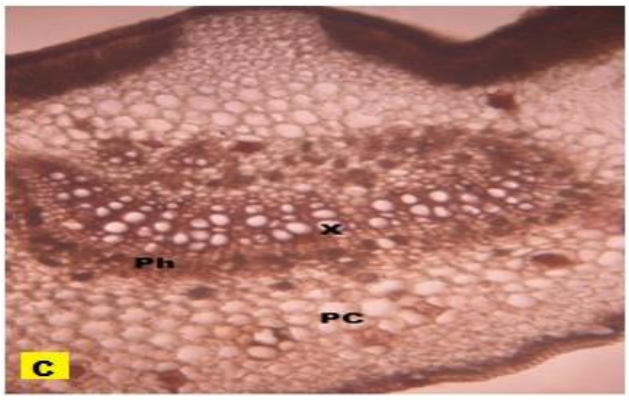
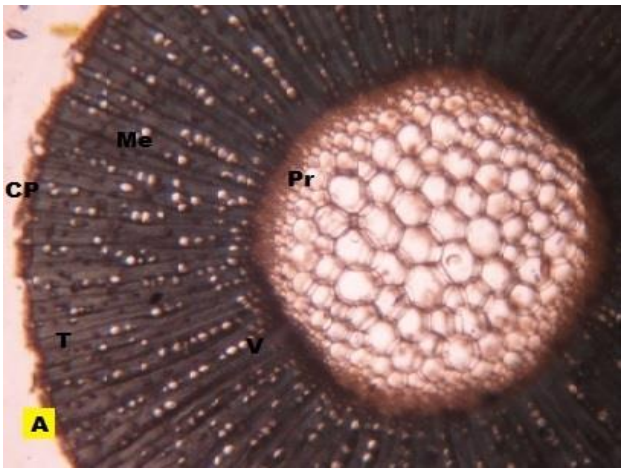


Figure 2b,c: T.S. of *Barleria noctiflora L.* leaf
A: T.S. of Mid rib, B: T.S. of Lamina, C: T.S. of Enlarge section
[Abbreviations: PT: Palisade tissue, Ph: Phloem, PC: Parenchyma cells, X: Xylem, MR: Midrib, LE: Lower epidermis, UE: Upper epidermis, Tr: Trichome, MH: Multicellular hair, PP: Palisade parenchyma, SP: Spongy parenchyma, VB: Vascular bundle, GH: Glandular hair, Cu: Cuticle.]



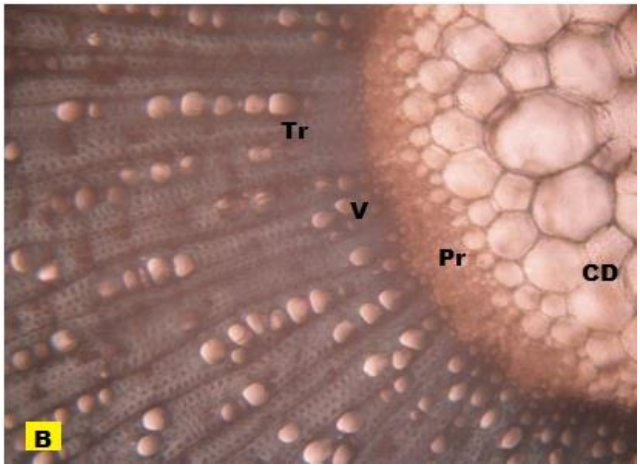


Figure 3: T.S. of *Barleria noctiflora L.* Stem
A: T.S. of stem, B: T.S. of Enlarge section
[Abbreviations: CB: Crushed phloem, T: Trachieds, Me: Metaxylem, Pr: protoxylem, V: Vessels, CD: Cell depositions.]

the presence of trachied, vessels, protoxylem, xylem and depositions cells. [Figure-3B].

Powder microscopy

The powder is pale green in color with pungent odour and slightly sweet and sour taste. The microscopic study of powder revealed the presence of bunch of vessels, bundle of fiber, transgenital parenchyma cells. The lengths of fibre are nearly 2mm. The unicellular covering trichome which are slightly curved and diacytic stomata. The seen in piece of epidermal tissue with trichome, parenchymatous tissue, trachied stone cells and vessels elements. [Figure-4].

Physicochemical Parameters

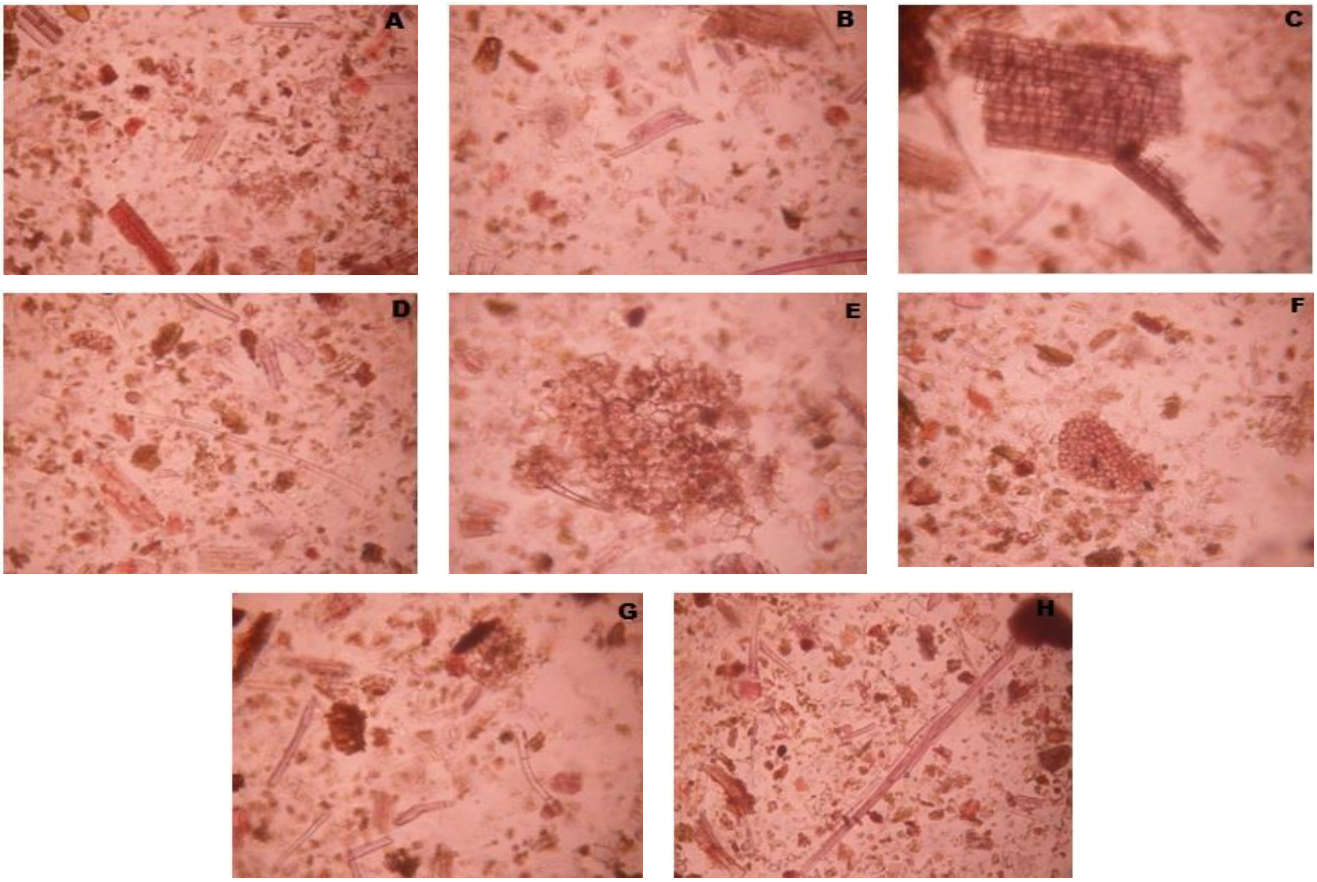


Figure 4: Powder microscopy of *Barleria noctiflora L.*
A: Bunch of vessels, B: Bundle of fiber, C: Transgenital parenchyma cells, D: length of fiber, E: Epidermal tissue with trichome, F: Parenchymatous tissue, G: Stomata, H: Trachied stone cells.

The physicochemical evaluation of Total ash, water soluble ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value and loss on drying were calculated and recorded. The total ash value, water soluble ash and acid insoluble ash were 13.5, 9 and 2% respectively. Extractive values of alcohol soluble and water soluble extractive value were 7.36 and 8.24%. The moisture content of the drug is not too high as the general requirement for moisture content in crude is not more than 14%w/w[30]. The moisture content (loss on drying) was 2.67%.

Preliminary phytochemical analysis

Preliminary phytochemical analysis revealed the presence of flavonoides, alkaloids, saponins, steroids, glycosides, tannins, phenols, protein and carbohydrates in various extract. The crude drug of powder presence of vitamin C, vitamin D and inorganic elements like sodium, iodine, phosphate, and chloride. [Table-1].

Fluorescence analysis

Powdered drug under Ultra violet and ordinary light when treated with different reagent emitted various color radiation which help in identifying the drug in powder form. [Table- 2].

Quantitative Microscopy

The observed values for stomatal number, stomatal index, veinislets, vein termination number and palisade ratio. [Table-3].

HPTLC finger print profile

HPTLC finger print of alcoholic extract of plant revealed 5 phytoconstituents having Rf values 0.01, 0.05, 0.54, 0.62 and 0.67, with a most pronounced spot of maximum area of Rf is 0.62. [Figure-5].

DISCUSSION

Pharmacognostical standardization was carried out on the basis of detailed botanical evaluation of the leaves and stem of *B. noctiflora* which includes morphology and microscopy as well as WHO recommended, physicochemical studies. The results of the standardization may throw an immense light on the botanical identity of the leaves and stem of *B. noctiflora*. This may furnish a basis of judging the authenticity of the plant and also to differentiate the drug from its adulterants and other species. The macroscopic characters were examined to identify the right crude drug.

Transverse section of the leaves showed a fairly prominent midrib, lateral veins and dorsiventral lamina. The vascular strand is omega shaped with an abaxial arc and two laterals out curved wings. The lamina has smooth even surface with two layers of palisade cells, the marginal part of the lamina is slightly curved down and bluntly conical. The stem has thin continuous epidermal layer, a wide cortex with homogenous layers of parenchyma cells, vascular cylinder consists of a thin, discontinuous layer of sclerenchyma abutting in phloem and a wider zone of xylem elements.

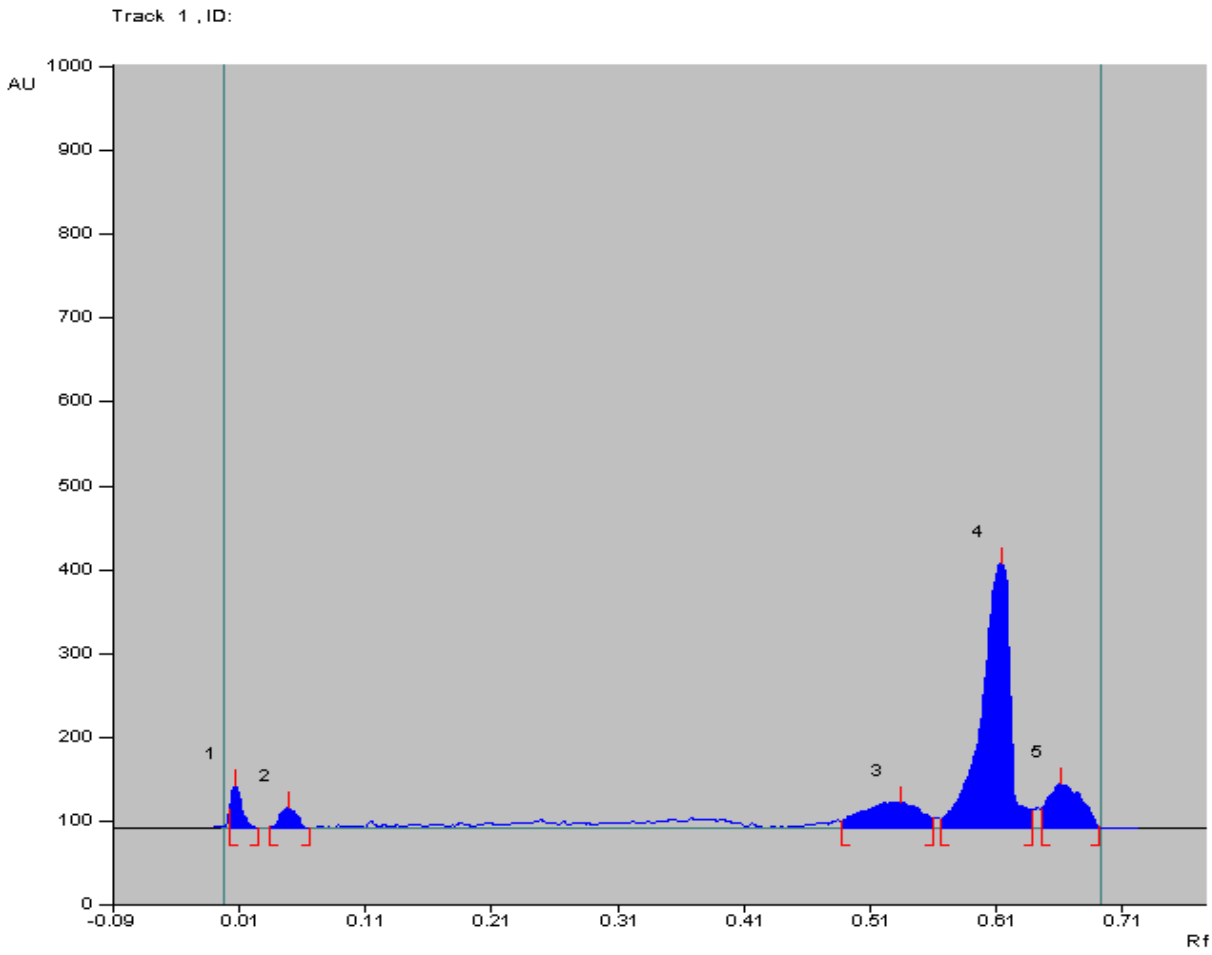


Figure 5: HPTLC profile of alcoholic extract of *Barleria noctiflora L.*

The powder characters of a drug are mainly used in the identification of the drug in the powder form. The *B. noctiflora* powder is dark greenish grey in color with pungent odour and slightly sweet and sour taste. On microscopical examination the powder showed numerous unicellular covering trichomes, which are slightly curved. Diacytic stomata made up of rectangular or polygonal epidermal cells with thin anticlinal walls are seen. Quantitative microscopic data are especially useful for identifying the different species of genus and also helpful in the determination of the authenticity of the plant. The physicochemical parameters are mainly used in judging the purity and quality of the drug. An ash value of a drug gives an idea of the earthy matter or inorganic composition or other impurities present along with the drug.

The ash values are important since ash may be derived from the plant itself (physiological or natural ash) as well as from the extraneous matter, especially sand and soil adhering to the surface of the drug (non physiological ash). The determination of physiological and non physiological ash together is called as total ash. The total ash may vary within wide limits for specimen of genuine drug due to variable natural or physiological ash, in such cases the ash obtained is treated with acid in which most of the natural ash is soluble leaving the silica as acid- insoluble ash which represents most of the ash from the contaminating soil. Extractive values give an idea about the chemical constituents present in the drug as well as useful in the determination of exhausted or adulterated drugs. The results suggest that the powdered have high water soluble extractive value. The loss on drying reveals the percentage of moisture present in the drug, since moisture facilitates the enzyme hydrolysis or growth of microbes which leads to deterioration. The crude fiber content which was studied can be implied to determine the nutritive value. Fluorescence analysis of powdered leaves was studied in both UV and day light. The powder showed green fluorescence in UV light, which indicates the presence of chormophore in the drug. Preliminary phytochemical analysis revealed the secondary metabolites are known to support bioactivity in this plant.

The present investigation of *B. noctiflora* can be concluded that this pharmacognostic study yielded a set of parameters which could serve as an important source of information to ascertain the identity and determination of quality and purity of plant material for future studies. This simple but reliable standardization will be useful to a lay person in using the drug as home remedy and also in the pharmaceutical industry for testing the raw material.

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