Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(6); 941-948

ISSN: 0975-4873

Research Article

Pharmacognostical Studies and Phytochemical Investigation of *Andrographis echioides* (L). Nees (Acanthaceae)

A Agnel Ruba, V.R. Mohan*

Ethnopharmacology Unit, PG & Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamilnadu, India.

Available Online: 9th June, 2016

ABSTRACT

Andrographis echioides (L). Nees (Acanthaceae) commonly known as 'Gopuram thangi' in tamil. The present investigation deals with the pharmacognostic studies of the leaf, stem and root of the above said plant. Pharmcognostic studies include microscopic, physico-chemical constant, fluorescent analysis and preliminary phytochemical evaluations. These findings should be suitable for inclusion in the proposal pharmacopoeia of Indian medicinal plants.

Keywords: *Andrographis echioides*, Pharmacognostic evaluation, pharmacopoeia.

INTRODUCTION

Now-a-days there is a renewed interest in drugs of natural origin simply because they are considered as green medicine and green medicine is always supposed to be safe. Another factor which emphasizes this attention is the incidences of harmful nature of synthetic drugs which are regarded as harmful to human beings and environment. The advantage of natural drugs is their easy availability, economic and less or no side effects but the disadvantage is that they are the victims of adulteration. The more effective the natural drug more is its demand and the chances of non-availability increases. To meet the growing demand, the natural drug is easily adulterated with low grade material. Adulteration or substitution is nothing but replacement of original plant with another plant material or intentionally adding any foreign substance to increase the weight or potency of the product or to decrease its cost. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification. Hence the need for evolving criteria for standard samples of crude drugs has become very important in pharmacognosy. 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 genus *Andrographis* is native of India contains 28 species of small annual shrubs essentially distributed in tropical Asia. *Andrographis* belongs to the family Acanthaceae. Some of them are medicinally important. The plants from genus *Andrographis* used in goiter, liver diseases[1], fever, fertility problems, bacterial[2], malarial, helmintic, fungal, diarrhea and larvicidal disorders[3,4]. Leaf juice boiled with coconut oil used to control falling and graying of hair[5]. *Andrographis echioides* (Acanthaceae) which is commonly known as false water willow is an herb commonly found throughout India. The literature survey revealed that the systemic evaluation including pharmacognostical study of this plant is still lacking. The present research work is concerned with the whole plant of the above mentioned Indian medicinal plant *Andrographis echioides*, which has reported folk-lore uses but yet not thoroughly explored so far for their exploitation in medicinal use. The first and foremost step is the characterization of different pharmacognostical parameters, botanical identification, photomicroscopic study, powder characteristics and fluorescence study has been included here. A preliminary phytochemical screening of whole plant has also been carried out.

MATERIALS AND METHODS

The whole plant of *Andrographis echioides* were collected from Surandai, Tirunelveli District, India. Identification and confirmation were done by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. Voucher specimens were deposited in the Ethnopharmacology unit, Botany Research Laboratory, V. O. Chidambaram College, Tuticorin, Tamil Nadu.

Macroscopical studies

The macroscopic characters like surface, shape, size, venation, phyllotaxy, length of the petiole, length of the leaf etc were noted.

Anatomical Studies

For anatomical studies, the required samples of leaf, stem and root were cut and removed from the plant and immediately fixed in FAA (formalin- 5 ml + acetic acid- 5 ml + 70% Ethyl alcohol- 90 ml). The specimens were left in the preservative for two days; then the materials were

*Author for Correspondence

PLATE -1
Andrographis echioides (L.) Nees
Anatomy of the leaf

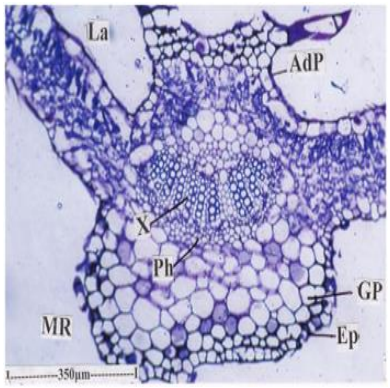

Figure a: T.S of midrib of the leaf.

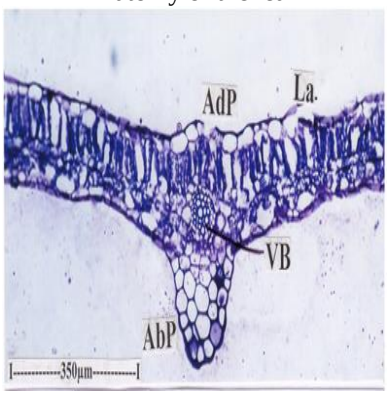

Figure b: T.S of leaf through lateral vein.

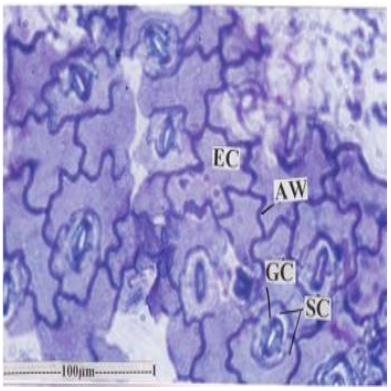

Figure c: Epidermal cells and stomata as seen in surface view.

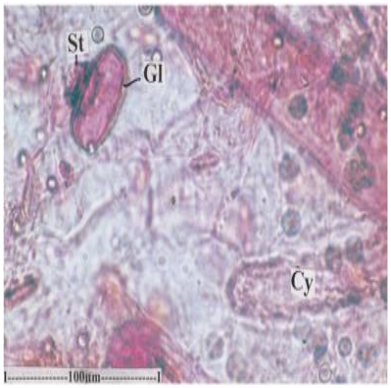

Figure d: Capitate type of glandular trichome.

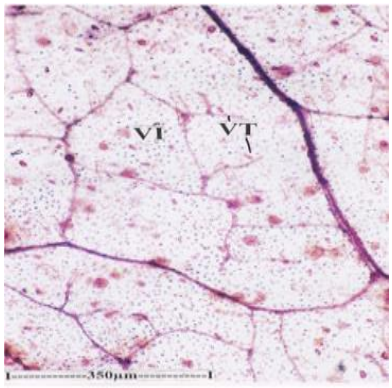

Figure e: Vein – islet – Enlarged.

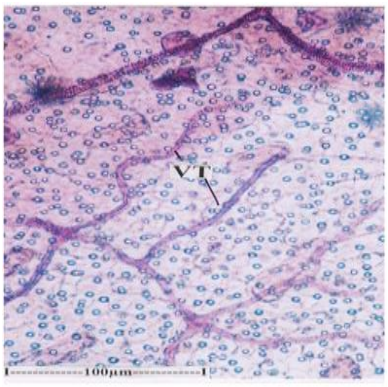

Figure f: Vein – terminations- Enlarged.

Adc- Adaxial cone, Adp- Adaxial part, Ep- Epidermis, GP- Ground parenchyma, La- Lamina, MR- Midrib, Ph- phloem, VB- Vascular Bundle, X- Xylem, Abp- Abaxial Part, La- Lamina, VB- Vascular bundle, AW- anticlinal Wall, GC- Guard Cells, SC- Subsidiary Cells, Cy- Cystolith, Gl- Gland, St- Stalk, VI- Vein Islet, VT- Vein Terminations.

washed in water and processed further. Standard microtome techniques were followed for anatomical investigation[6]. Transverse sections of the materials were made. The microtome sections were stained with 0.25% aqueous Toluidine blue (Metachromatic stain) adjusted to pH 4.7[7]. Photomicrographs were taken with NIKON trinocular photo micrographic unit.

Physicochemical and fluorescence analysis

These studies were carried out as per the standard procedures[8]. In the present study, the powered whole plant was treated with various chemical reagents like aqueous 1N sodium hydroxide, alcoholic 1N sodium hydroxide, 1N hydrochloric acid, 50% sulphuric acid, concentrated nitric acid, picric acid, acetic acid, ferric chloride and concentrated HNO_3+NH_3 These extracts were subjected to fluorescence analysis in day light and UV light (254nm and366nm). Various ash types and extractive values were determined by following standard methods[9].

Preliminary phytochemical analysis

Shade dried and powdered whole plant samples was successively extracted with Petroleum ether, benzene, ethyl acetate, methanol and ethanol. The extracts were filtered and concentrated using vacuum distillation. The different extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per the standard procedure[8,10].

RESULTS

Macroscopic features

Erect herbs, 10-50 cm high. Whole plant villous. Leaves opposite decussate, 3-5 x 0.6-1.8 cm, elliptic oblong, obtuse to round at apex, attenuate at base. Inflorescence axillary, short unilateral racemes, simple or 1-2 branched, as long as or shorter than leaves. Calyx deeply 5-lobed; lobes 0.8-1 cm long, linear. Corolla tube 0.8-1 cm long, white, 2-lipped; lip with violet blotches; upper lip 2-lobed, 0.6-0.8 cm long, lower c. 0.8 cm long, 3-lobed. Stamens 2; filaments broad, hairy at base; anthers 2-celled. Style slender; stigma 2-fid. Capsules 0.8-1.2 cm long, 0.25-0.3 cm wide, compressed, broadened towards tip, attenuate at base, sparsely hairy towards tip. Seeds 0.15-0.2 cm across, black, pitted without; retinacula spiny.

Anatomy of the Leaf

The leaf is bisymmetrical with differences between the adaxial and abaxial sides. The midrib is thick with wide and slightly tall adaxial part and wide and thick abaxial

PLATE – ll
Andrographis echioides (L.) Nees
Anatomy of stem and the root

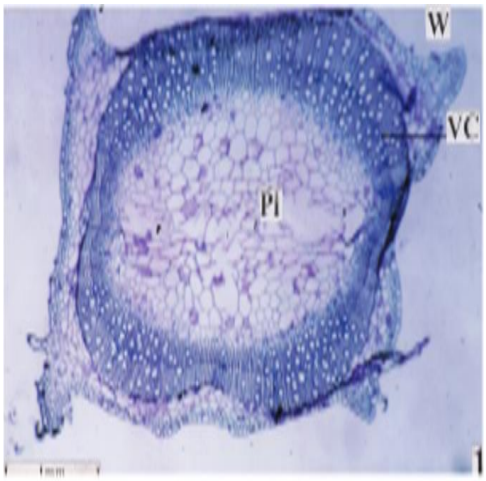

Figure a: T.S of stem- Entire view.

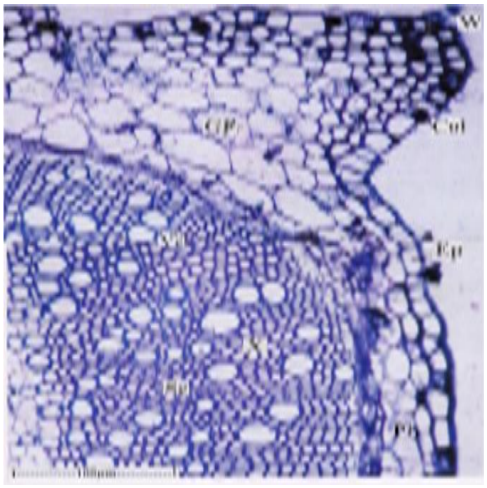

Figure b: T.S of stem- A sector enlarged.

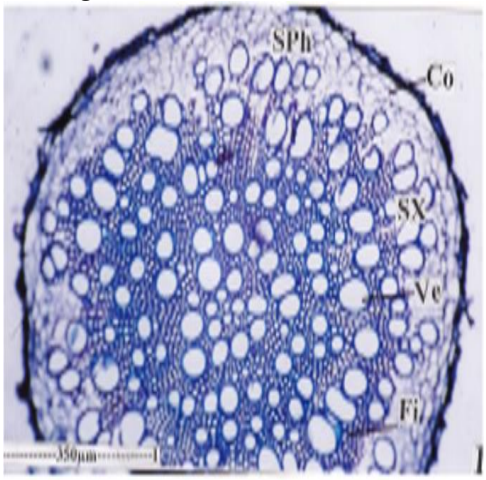

Figure c: T.S of root entire view.

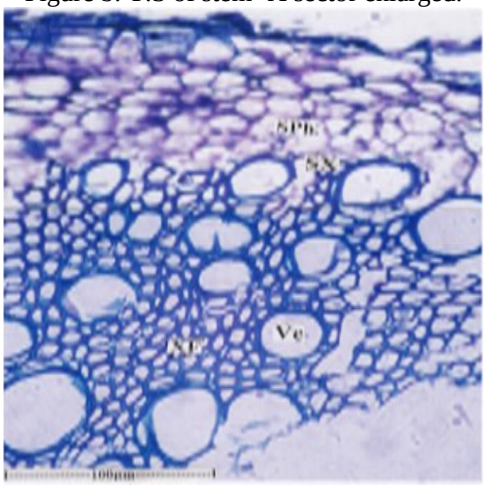

Figure d: T.S of Root- Vascular tissues enlarged.

Pi- Pith, VC- Vascular Cylinder, W- Wing, Col- Collenchyma, Ep- Epidermis, GP- Ground Parenchyma, Ph- Phloem, Sc- Sclerenchyma (fibres), Ve- Vessels; X- Xylem, Co- Cortex, Fi- Fivres, Sph- Secondary

part (Plate I, a). The midrib is 600µm thick and 650µm wide. The adaxial part of the midrib consists of two or three layers of collenchyma cells. Beneath the collenchyma zone occur the palisade mesophyll cells of the lamina which is horizontally transcurrent (Plate I, b). The epidermis of the midrib consists of squarish, fairly wide, thick walled cells. The ground parenchyma cells are wide, polygonal, thin walled and compact. The vascular bundle is single horizontally elongated and slightly curved; it is collateral with adaxial xylem and abaxial phloem (Plate I, b). The xylem strand consists of small, angular and thick walled xylem elements arranged in thin vertical parallel lines. The phloem elements are small nests and are separate from each other.

Lateral Vein

(Fig.1.2) The lateral vein consists of a short and wide adaxial cone and widened abaxial part. It is 500µm thick. The palisade layer of the lamina is horizontally transcurrent in the adaxial conical part. The vascular bundle is single, circular and collateral. These are about six. Vertical lines of short xylem elements with thin layer of phloem. The ground tissue of the lateral vein is parenchymatous, wide, angular and thin walled (Plate I, b).

Smaller lateral vein

The thin lateral vein consists of flat adaxial side and thick conical abaxial part. It consists of parenchymatous ground tissue and transcurrent adaxial palisade zone. The vascular bundle is small, circular and collateral. The lamina is bilaterally symmetrical and it measures 140µm thick. The adaxial epidermis is fairly wide, rectangular and thin walled. The abaxial epidermis is narrow, square shaped and fairly thick walled. The palisade mesophyll is single layered and the cells are thin and vertically elongated. The cells are less compact. The spongy mesophyll tissue includes 4 or 5 layers of lobed loosely arranged cells (Plate I, 2).

Leaf Margin

The marginal part of the lamina is blunt and slightly conical. The epidermal cells are large, squarish and thick walled. There is no change in the structure of the mesophyll tissues of the leaf margin.

Epidermal tissue and stomata

PLATE – lll
Andrographis echioides (L.) Nees
Powder microscopy

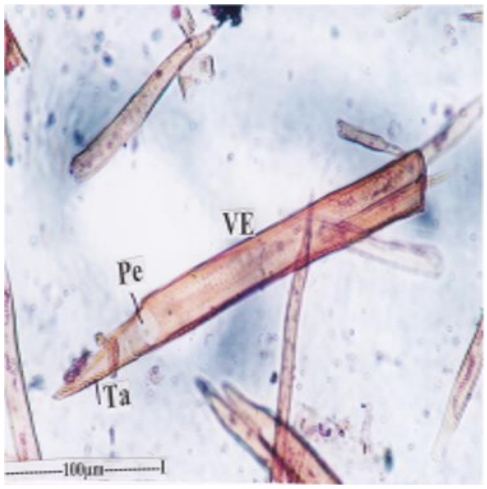

Figure a: Long narrow vessel element.

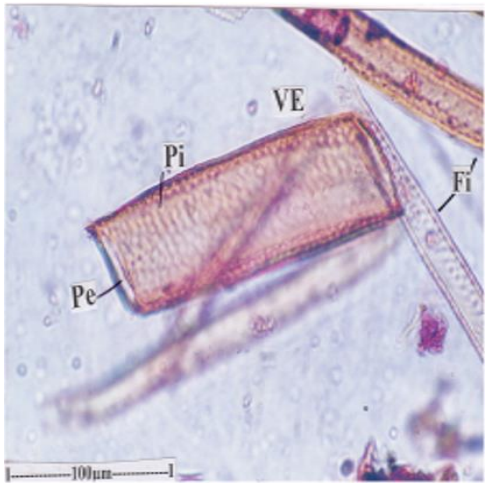

Figure b: Short wide vessel element.

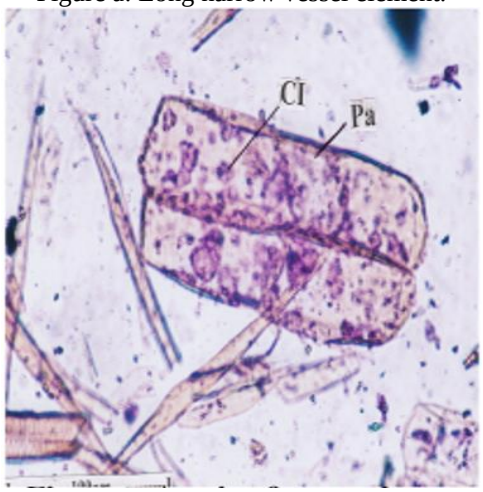

Figure c: A pair of parenchyma cells with dense cell inclusions.

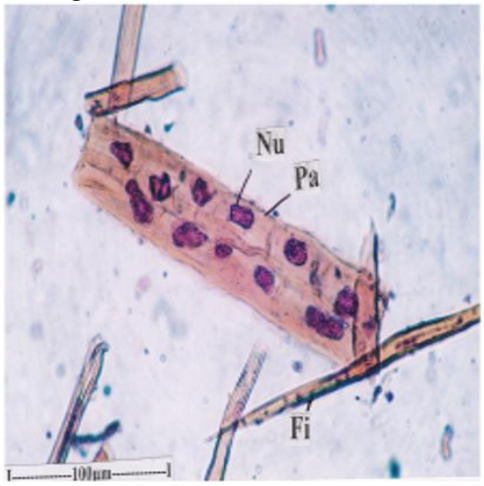

Figure d: Parenchyma strands with prominent nuclei.

Fi- Fibres, Pe- Perforation, Pi- Pits, VE- Vessel Element, CI- Cell Inclusious, Fi – Fibres, Nu- Nucleous, Pa- parenchyma cells.

The epidermal cells when viewed in surface view are fairly large with highly wavy anticlinal walls. The cells appear amoeboid in outline. (Plate I, c) The stomata are typically diachytic with one subsidiary cell at each end of the guard cells and the common walls of the subsidiary cells at right angles to the long axis of stomata. (Plate I, c) Narrow, elongated, rectangular cystoliths are fairly common in the mesophyll of the lamina. The cystoliths are 20×100µm in size (fig.4.1). These are also glandular epidermal trichomes seen in the powder (Plate I, d). The gland has short one-celled stalk and horizontally oriented circular gland. The gland is peltate type. The gland is 30µm in height and 50µm in breadth.

Venation Pattern

(Plate I, e, f**)** The vein lets are thin and less prominent. The vein islets are fairly distinct and polygonal in outline. The vein boundaries of the islets are thin. The vein terminatious is distinct. They are mostly unbranched or occasioually forked once. The terminations are long, thin and undulate.

Stem

The stem is four angled in transectional view with short, thick wings at each angle. (Plate II, a) The stem is 5.8µm in diameter. It consists of a thick epidermal layer of squarish, thin walled cells with prominant cuticle. The wings which are 200µm long and 150µm thick. The cells within the wings are collenchymatous. The cortical region is narrow comprising outer three layers of collenchyma and inner three layers of parenchyma. The vascular cylinder is hollow with wide central pith. The cylinder is thicker along the winged corners and thin in between the thick regions. The thick segments of vascular cylinder consist of dense vessels and fibers. The vessels are wide, circular, thick walled and solitary and measure 20-50µm in diameter (Plate II b). The xylem fibers are thick walled, polygonal in outline and radial in distribution. In the region in between the thicker segments of the xylem vessels are sparse only narrow thick walled fibres are seen. Phloem tissue occurs on the outer boundary of the xylem cylinder.

Table 1: Ash and extractive values of the powered whole plant of *A.echioides*

S. No	Types of ash	% of Ash
1	Total ash value of powder	10.84 ± 0.08
2	Water soluble	5.28 ±0.06
3	Acid insoluble ash	3.62 ± 0.04
4	Sulphated ash	11.36 ± 0.16
Extractive values		
S. No	Name of the extract	Extractive Value (%)
1	Petroleum ether	6.98 ± 0.03
2	Benzene	5.48 ± 0.04
3	Chloroform	6.76 ± 0.02
4	Acetone	6.48 ± 0.11
5	Methanol	8.79 ± 0.07
6	Ethanol	9.10 ± 0.14
7	Water	9.48 ± 0.13

* All values are mean of triplicate determination

Phloem consists of a few layers of small, darkly stained sieve elements and parenchyma cells.

Root

The root is circular in cross sectional view. It exhibits advanced stage of secondary growth. It is nearly 1mm in diameter. The root consists of thick dark layer of crushed epidermal layer. The cortex is much reduced to two or three layers of parenchyma cells. Secondary phloem is very thin and less prominant. The secondary xylem is a thick solid cylinder, occupying major portion of the root (Plate II, c, d). Secondary xylem includes both wide and narrow vessels which are solitary. The vessels are circular and thick walled. They are 20-50µm in diameter. The vessels are embedded in xylem fibres. The fibres are narrow, thick walled and lignified (Plate II, c, d).

Powder Microscopic observation

Powder preparation of the plant was examined under the microscope and the following inclusions were recorded.

Vessel elements (Plate III a)

Long narrow cylindrical vessel elements with thick and long tails were often seen in the powder. The perforations at the end walls are wide and circular. These types of vessel elements are 450µm long. These are wider and cylindrical vessel elements. These vessel elements are 320µm long. The vessel elements have dense, multiseriate bordered pits on the lateral walls. The end wall perforations are wide and circular.

Fibres

Long, narrow, highly thick walled lignified fibres are very common in the powder. They have narrow lumen and prominant, slit - like simple pits.

Parenchyma cells (Plate III, c, d)

Wide, rectangular, thin walled parenchyma cells fairly abundant in the powder. Some of the parenchyma cells have prominant spherical inclusions. Other parenchyma cells have only large cylindrical nuclei. The cells are uninucleate. The former type of parenchyma cells are 90µm wide and 320µm long. The second types of cells are short and occur in vertical rows.

Powder microscopy

The powder includes - narrow long cylindrical as well as wide short vessel elements with simple perforations; fibres with thick lignified walls and some cell inclusious and rectangular parenchyma cells and squarish cells in strands with prominant nuclei and cytoplasmic contents.

Pharmacochemical characterization

The physicochemical parameters like ash and extractive values, fluorescence analysis of leaf of *A. echioides* were determined. Preliminary phytochemical screening was also performed and results are presented below

Ash value and extractive value

The results of the ash and extractive values of whole plant powder of *A. echioides* are depicted in Table 1. The total ash content of the powdered whole plant of *A. echioides* is 10.84% respectively. The amount of acid insoluble ash presents in whole plant of *A. echioides* was 3.62% respectively. The water soluble ashes of whole plant of *A. echioides* were 5.28% respectively. The amount of sulphated ash present in whole plant of *A. echioides* were 11.36% respectively.

Percentage of the extractive values of various extracts is given in Table 1. The results showed that various extracts of whole plant contain greater proportion by mass of the extractive values. Petroleum ether, benzene, chloroform, ethyl acetate, methanol, ethanol and aqueous soluble extractive values of whole plant were 6.98%, 5.48%, 6.76%, 6.48%, 8.79%, 9.10% and 9.48% respectively Table 1. In whole plant extracts, water soluble extractive values were higher followed by alcohol and chloroform soluble extractive values while the least amount of extractive value was observed in petroleum ether extract.

Fluorescence analysis

Fluorescence analysis of whole plant powder were studied at day light and UV light (245 nm and 365 nm) and the observations are presented in Table 2. Fluorescence studies of whole plant powder revealed the presence of fluorescent green with 1N HCl, Conc. HCl, Conc. H_2SO_4, 50% HNO_3, petroleum ether and acetone under UV light of shorter wavelength.

Preliminary phytochemical screening

The results of preliminary phytochemical screening of whole plant extracts of *A. echioides* are presented in Table 3. The methanol and ethanol extracts of the whole plant powder shows the presence of alkaloid anthraquinone, catechin, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotein.

DISCUSSION

Pharmacognostical studies

According to world health organization (WHO) more than 80% of the world's population relies on herbal medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Herbal formulation involves use of fresh or dried plant part. Therefore, proper and correct identification of the plant material is very much essential. Correct identification of the starting material is an essential prerequisite to ensure reproducible quality and will contribute immensely to it's safely and efficacy[11].

Table 2: Fluorescent analysis of powdered whole plant of *A. echioides*

S. No	Experiments	Visible/ day light	UV –light	
			254 nm (short wave length)	365 nm (long wavelength)
1	Powder as such	Green	Dark green	Dark green
2	Powder+1NNaOH(Aqueous)	Yellowish green	Greenish yellow	Dark green
3	Powder+1N NaOH(Alcohol)	Yellowish green	Greenish yellow	Dark green
4	Powder +1N HCL	Green	Fluorescent green	Brown
5	Powder +Conc. H_2SO_4	Light green	Fluorescent green	Dark blue
6	Powder +50% H_2SO_4	Green	Light green	Dark green
7	Powder +Conc.HNO_3	Green	Green	Dark green
8	Powder +Conc.HCL	Light green	Fluorescent green	Bluish green
9	Powder +50%HNO3	Yellowish green	Fluorescent green	Pink
10	Powder +40%NaOH + 10% Lead acetate	Pale green	Dark green	Violet
11	Powder +Acetic acid	Green	Light green	Dark green
12	Powder +Ferric chloride	Dark green	Dark green	Dark green
13	Powder +HNO_3 +NH_3	Yellowish green	Yellowish green	Dark blue
14	Powder +HNO_3	Green	Yellowish green	Blue
15	Powder +Benzene	Yellowish green	Pale yellow	Dark brown
16	Powder +Petroleum ether	Yellowish green	Fluorescent green	Dark green
17	Powder +Acetone	Dark green	Fluorescent green	Dark green
18	Powder +Chloroform	Yellowish green	Pale yellow	Brown
19	Powder +Methanol	Yellowish brown	Pale yellow	Dark brown
20	Powder +Ethanol	Yellowish green	Green	Dark brown

Pharmacognostic studies are pivotal in herbal technology as it ensures plant identify, lays down standardization parameters which will help and prevent adulterations. Such studies will help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products. As literature survey and scientific data revealed that a large number of indigenous drugs have already been investigated as regards their botany and chemistry is concerned, however a systematic standardization including pharmacognostical and phytochemical study is still lacking. The present study attempts a modest comprehensive investigation of the whole plant of *A. echioides*. Since the whole plant of *A. echioides* as the folklore claims has therapeutic qualities the present investigation has laid down a set of anatomical features of the leaf which can be employed for its botanical diagnosis. The salient features of identification of the fragmentary sample are as follows.

Salient anatomical features of A. echioides

- The leaf consists of thick midrib and main lateral vein which possess adaxial cone, abaxial thick and wide midrib proper.
- The vascular strand is single and collateral.
- The palisade layer is horizontally transcurrent along the adaxial part of the midrib.
- The lamina is dorsiventral with single vertical row of palisade cells.
- The epidermal cells of the lamina are fairly wide with thick, highly wavy anticlinal walls. The stomata are diacytic type.
- Long, scale- shaped calcium carbonate cystoliths and short stalked, thick circular glandular trichomes are seen in the leaf.
- The venation is loosely reticulate with wide vein- islets and branched vein terminations.
- Stem is 4- angled with short thick wings at the angle. The vascular cylinder is also angular with four thick segments of secondary xylem alternating with thin segments of xylem fibres. The vessels are fairly wide, circular, thick walled and solitary.
- The root has very thick, dense circular cylinder secondary xylem, occupying the major portion of the root.
- The vessels are circular wide, thick walled and solitary. They are narrow and wide cells.

Microscopical evaluation is the simplest and reliable tool for correct identification of herbs as well as small fragment of crude drugs or powdered drugs and detection of adulterants and substituents[12,13]. The external features coupled with the microscopic features are specific for *A. echioides*. These features are discussed and proposed as microscopic diagnostic protocol for the taxon studied.

Physicochemical parameters

The physicochemical parameters help in judging the purity and quality of the drug. The drug powder was evaluated for its physicochemical parameters like total ash, acid insoluble ash, water soluble ash and different extractive values.

The purity of crude drugs could be evaluated by the determination of ash values which represent the content of foreign matter such as inorganic salts or silica present as a form of adulterant in the drug sample. In the present investigation, an analytical result for total ash was formed to be 10.84%. The total ash includes both 'physiological ash' which is derived from the plant tissue itself, a 'nonphysiological ash' which is the residue of the extraneous matter adhering to the plant surface. The amount of acid insoluble ash and water soluble ash were found to be 3.62% and 5.28% respectively. Acid insoluble ash is a part of total ash and measures the amount of silica

Table 3: Preliminary phytochemical screening of whole plant of *A.echioides*

Test	Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol
Alkaloid	+	+	+	+	+
Anthraquinone	-	-	-	-	-
Catechin	–	–	–	–	–
Coumarin	+	+	+	+	+
Flavonoid	+	+	+	+	+
Phenol	+	+	+	+	+
Quinone	+	+	+	+	+
Saponin	+	+	+	-	-
Steroid	+	+	+	+	+
Tannin	+	+	+	+	+
Terpenoids	+	+	+	+	+
Sugar	+	+	+	+	+
Glycoside	+	+	+	+	+
Xanthoprotien	+	+	+	+	+
Fixed oil	+	+	+	+	+

+ Presence - Absence

present especially as sand and siliceous earth. Water soluble ash is the water soluble portion of the total ash[14,15]. Form the results; it is clear that the amount of water soluble ash is higher than that of acid insoluble ash, whereas the amount of total ash was almost double the quantity of water soluble ash. The ash content given an idea about the inorganic content of powdered whole plant under investigation and thus the quality of the drugs can be assessed. The crude drugs owe their biological activity mainly due to active chemical constituents. These constituents may be in different polar, semi polar or non polar solvents. Total soluble constituents of the drug in any particular solvent or mixture of solvents may be called as extractive value[16]. The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Taking into consideration the diversity in chemical nature and properties of contents of drugs, various solvents are used for determination of extractives. The solvent used for extraction is in a position to dissolve appreciable quantities of substances desired[16]. The preliminary phytochemical screening of whole plant methanol and ethanol extract of *A. echioides* has revealed the presence of alkaloid, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotein in them for treating different ailments and having a potential of providing useful drugs of human use. This is because; the pharmacological activity of any plant is usually traced to a particular compound. Therapeutically terpenoids exert a wide spectrum of activities such as antiseptic, stimulant, diuretic, anthelmintic, analgesic and counter-irritant[17]. Many tannin containing drugs are used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering[18]. They are also medically used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns and piles and as antidote[19]. Saponins, a group of natural products, occur in both the investigated plants namely *A. echioides.* In plants, the presence of steroidal saponins like cardiac glycosides appear to be confined to many families and these saponins have great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisones, diuretic steroids, vitamin D etc.,[20]. A synthetic steroid by name sapogenin is prepared from plants and is used to treat a wide variety of diseases such as rheumatoid arthritis, collagen disorders, allergic and asthmatic conditions[21]. Saponin reduces the uptake of certain nutrients including glucose and cholesterol at the gut through intraluminal physicochemical interactions. Hence, it has been reported to have hypocholesterolemic effect and thus may aid lessening metabolic burden that would have been placed in the liver[22]. Several authors have reported that flavonoids, sterols/terpenoids and phenolic acidsare known to be bioactive anti-diabetic principles[23,24]. Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats[25]. Flavonoids act as insulin secretagogues[26]. Most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, and flavonoids etc., which are frequently implicated as having anti-diabetic effects[27]. To understand the nature of the fluorescence emission from these crude preparations under different conditions, the preliminary phytochemical analysis of these crude preparations were compared. The comparative analysis clearly showed a correlation between a compound present in it and their fluorescent behaviour under different conditions. The major bioactive compounds present in these crude preparations are the coumarins, flavonones, tannins, alkaloids and saponins. Coumarin especially hydroxyl amino acid derivatives like o-coumaric acid appears yellowish green in alkaline condition under short UV radiation. Flavonones which are light yellow in aqueous condition, under UV light, turns to bright yellow under alkaline conditions. Similarly, the phytosterols, when treated with 50% H_2SO_4 show green fluorescence under UV light. Terpenoids, especially sapogenins, exhibit yellow green fluorescence under short UV light[28]. Quinine, aconitin, berberin and emetin show specific colours of fluorescence (Aconitin - light blue; berberin – light yellow; emetin - orange). Fixed oils and fats fluoresce least, waxes more strongly and mineral salts most of all[29]. Haydon[30] studied the photophysical

characters of coumarins. Hydroxy methyl coumarin fluoresced at 420–440 nm when observed in different solvents with increasing polarity[31]. The fluorescence analysis of the crude drugs prepared from the whole plants of *A. echioides* exhibited clear fluorescence behaviours at different radiations which can be taken as standard fluorescence pattern.

CONCLUSION

The macroscopic as well as microscopic studies of any drug material are the preliminary steps for establishing the botanical parameters prior to any kind of study. As per WHO guidelines, botanical standards are to be proposed as a protocol for the diagnosis of the herbal drug. The quantitative determinations of some physicochemical parameters are useful for setting standards for crude drugs. The physical constant evaluation is an important parameter in detecting adulteration or improper handing of the drug. Since the plant *Andrographis echioides* (L). Nees is useful in the traditional system of medicine in treatment of various diseases, it is important to standardize it for use as a drug. The macroscopic studies, microscopic studies and physicochemical parameters reported in this work could be useful for the compilation of a suitable monograph for its proper identification.

REFERENCES

1. Nadkarni AK, Nadkarni KM. Indian Materia Medica, Bombay: Popular Prakashan 1976; 1: 9-10.
2. Qadrie ZL, Beena Jacob, Anandan R, Rajkapoor B, Rahamathulla M. Antibacterial activity of ethanol extract of *Indoneesiella echioides* evaluated by the filter paper disc method. Pak J Pharm Sci 2009; 22: 123-5.
3. Krithikar KR, Basu RD. Indian Medicinal Plants, Vol II, 4th edition, International Book. Distributors, Dehradun, 1975; 1325-27.
4. Nazar S, Ravikumar S, Williams GP, Syed Ali M, Suganthi P. Screening of Indian coastal plant extracts for larvicidal activity of *Culex quinquefasciatus*. Indian J Sci Technol 2009; 2: 24-7.
5. Pandi kumar P, Ayyanar M, Ignacimuthu S. Medicinal plants used by Malasar tribes of Coimbatore district, Tamil Nadu. Indian J Trad Knowl 2007; 6: 579-582.
6. Johanson DA. Plant Microtechnique. Mc Graw Hill Book Co, New York, 1940, 523.
7. O'Brien TP, Feder N, Mc Cull ME. Polychromatic staining of plant cell walls by toluidine blue-O. Protoplasma 1964; 59: 364-373.
8. Lala PK. Lab Manuals of Pharmacognosy. Edn 5, CSI Publishers and Distributors, Culcutta, 1993.
9. Anonymous (1996). Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, the Controller of Publications, Civil Lines, Delhi – 110 054. Vol.I & II.
10. Brindha P, Sasikala P, Purushothaman KK. Pharmacognostic studies on *merugan kizhangu*. Bulletin of Medico Ethnobotanical Research 1981; 3: 84-96.
11. Thomas S, Patil DA, Patil AG, Naresh Chandra. Pharmacognistical evaluation of physicochemical analysis of *Averrhoa carambola* L. fruil. J.Herbal Med. Toxicol 2008; 2: 51- 54.
12. Ahmad, I, Aqil, F. and Owais, M. Modern Phytomedicine: Turning Medicinal Plants into Drugs, John Wiley & Sons. 2007; p. 34.
13. Pratima, H. and Pratima, M. Pharmacognostic evaluation and phytochemical analysis of leaves of *Cajanus cajan* L. J. Advan. Develop Res 2011; 2: 181-185.
14. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Nirali Prakashan; 38th edition, Pune, 2007.
15. Evans WC. Trease and Evans Pharmacognosy. 15th ed. Edinburgh: W.B. Saunders; 2002.
16. Kokate CK, Purohit AP, Gokhule SB. Pharmacognosy 43rd ed. Nirali Prakashan, Pune. 2009; 6.17- 6.20.
17. Gokhale SG, Kokate CK, Purohit AP. 2003. A Text Book of Pharmacognosy, Nirali Prakashan, Pune - 21. Handa, S.S. Kapoor, V.K. (Edn.) 1992.
18. Handa SS, Kapoor VK. (Eds): Pharmacognosy. Vallabah Prakashan Publications, New Delhi, 1992.
19. Ali M. Text book of Pharmacognosy. CBS Publishers and Distributors, New Delhi, 1994, 405.
20. Evans WC, Saunders WB. (Eds.). Trease and Evan's Pharmacognosy Tokyo. 2001; 1 -579.
21. Claus EP. Pharmacognosy. (Ed.). Lea and Febiger, Philadelphia, 1956: 1- 697.
22. Price KR, Johnson LT, Ferewick H. The chemical and biological significant of saponins in food and feeding staffs CRC. Critical Rev. Food. Sci. Nut. 1987; 26: 127 - 135.
23. Oliver-Bever B. Medicinal plants in tropical West Africa, Cambridge University press, London. 1986; 45 - 267.
24. Rhemann AV, Zaman K. Medicinal plants with hypoglycemic activity. J. Ethnopharmacol 1989; 26**:** 1 - 55.
25. Chakravarthy BK, Gupta S, Gambir SS, Gode KD. Pancreatic beta cell regeneration. A novel anti-diabetic mechanism of *Pterocarpus marsupium* Roxb. Ind. J. Pharmacol 1980; 12: 123 - 127.
26. Geetha BS, Mathew BC. Augusti KT. Hypoglycemic effects of leucodelphinidin derivative isolated from *Ficus bengalensis* Linn. Indian J. Physiol. Pharmacol 1994; 38: 220 -222.
27. Loew D, Kaszkin M. Approaching the problem of bioequivalence of herbal medicinal products. Phytother. Res 2002; 16: 705 – 711.
28. Horborne JB: Phytochemical methods. Chapman & Hall, New York.1976; 1- 288.
29. Evans WC. In: Trease and Evans' Pharmacognosy. Singapore, Harcourt Baraco and Company Asia Pvt. Ltd, 1996; 1 –437.
30. Haydon, S.C. 1975. Spectroscopic letters. 8: 815 – 826.
31. Chaltopudhyay N, Mallick A, Sengupt S. Phytophysical studies of hydroxyl methyl coumarin: A new fluorescent chemosensor for Zinc and Nickel ions in water. J. Photochem. Photobiol 2006; 177: 55 – 60.