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Research Article

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Pharmacognostic and Phytochemical Features of *Andrographis echioides* Whole Plant Powder

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Abstract: *Andrographis echioides* is one of the stress tolerant plant that belongs to the family Acanthaceae commonly called as Kopuramthangi in Tamil, commonly found in central region of Tamilnadu, India. Local peoples used this plant for fever, to relieve griping, irregular stools and loss of appetite. Indian siddha and ayurvedic pharmacopeia provide standardization criteria for herbal drugs. The aim of the study is to provide quality standards for the powder drug from the whole plant of *Andrographis echioides*. In order to provide herbal standards for this plant, the present study was undertaken with the objectives to assess pharmacognostic, physiochemical and phytochemical markers of the drug powder of *Andrographis echioides* whole plant. *Andrographis echioides* whole plant was collected, authenticated and processed for all pharmacognostic activity using standard textual methods. Powder analysis showed the presence of calcium oxalate crystals, trichomes, fibres, vessel elements and parenchyma cells. The powder is yellowish green in colour with 3.6% ash value with higher water extractive (19.4%). Powder contains a smaller number of microbial flora and no pathogens detected. Multiple fluorochromes were detected in fluorescence test. The pharmacognostic results were within the limits of ayurvedic pharmacopeia of India. Whole plant powder showed the presence of terpenoids, flavonoids, Saponins, phenolic compounds and carbohydrates which could be responsible for all biological activities of the plant. Histochemical analysis also confirmed the availability of phytoconstituents like flavonoids and tannins. The observations made through this study would be of immense value in the pharmacological evaluation and standardization of crude powder of the plant materials.

Key words: *Andrographis echioides*, Pharmacognosy, phytochemistry, histochemistry, whole plant.

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1. INTRODUCTION

Medicinal plants play an important role in controlling human health, however there is a problem in documented evidences, authenticated information in a systematic way and stringent quality control measures. It is now quite necessary to generate assurance about the standardization of the plant as well as its parts to be used like a medication. During the process of standardization, advantage of various techniques and methodology are being taken for phase wise approach e.g. pharmacognostic and phytochemical studies. These techniques and methods are helpful in recognition and standardization of the plant material[1, 2]. Pharmacognosy is the word from Seydler used for the assessment of crude drug from the natural source[3]. It is a systematic study of botanical crude drugs morphologically, microscopically, physiochemically and also for quality control[4]. Through pharmacognosy, standardization of crude drug could be done and used as a standard for future study. Some chemicals could be considered as a phytochemical marker to test adulteration of the drug. This method is also used to make the correct identity of the plant material. The aim of the study is to provide first-hand information about the quality standard for the whole plant powder of *Andrographis echioide*s. Data on phytochemicals tested from the crude drug could be used to make the drug through synthetic means[5]. Orhan et al., [6] stated that drug available in US market are from natural or plant origin., ie., it could be semisynthetic or synthetic on the basis of the structure of phytochemicals. Problems of mixing or using similar vernacular name plants for the treatment of particular diseases may leads to various side effects. Safety of herbal medicine is given a top priority, as some of the ingredients interfere with our metabolism[7]. *Andrographis echioides* is an herb belonging to the family Acanthaceae, and are widely available in semi dry places of India and Srilanka[8]. The whole plant is used for the treatment of microbial infections, to control hair fall to control hair colour change, to heal wounds[9,10], used in goiter, cancer and liver problems[11], fertility problems[12,13], acts as an anti-inflammatory[14,15], antioxidant[10] and anti-ulcer agent[16]. Leaf juices of this plant is used to cure fever[17]. Having known the importance of this plant, the pharmacognostic analysis was undertaken for the complete authentication and quality control of *A. echioides* whole plant.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The plant of *A. echioides* was collected from Mannai Rajagopal swami Government Arts College, Mannargudi, Thiruvarur Dt, Tamil Nadu. The plant was identified by local people of that village and authenticated by Professor Dr. John Britto, Taxonomist, Department of Botany, St. Joseph's College, Tiruchirappalli, India. A voucher specimen of the plant material has been submitted to herbarium deposit with reference No. 127/Herb/A/20-21. After authentication, the whole plant was shade dried and then milled into coarse powder by a mechanical grinder.

2.2 Powder microscopy

Powdered plant material was assessed microscopically for the presence of specific structures. Small quantity of different plant powder was placed separately on slides and each slide was mounted 2-3 drops of chloral hydrate and each slide was covered with cover slip then examined under microscope. Different cell components were noted and photography was done by using digital camera[18].

2.3 Organoleptic Evaluation

Organoleptic evaluation refers to evaluation of the formulation by colour, odour, taste, texture, etc. The organoleptic characters of the samples were evaluated based on the textual methods[19].

2.4 Physicochemical Parameters

The determination of various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, swelling index, foaming index, foreign matter was done by the methods given in the ayurvedic Pharmacopoeia of India[20, 21, 22, 23].

2.5 Fluorescence Analysis

Powder of *A. echioides* were subjected to analyze fluorescence features under ultra violet light and day light after giving treatment for 48 hours with various chemical and organic solvents like ethanol, 50% sulphuric acid, 10% sodium hydroxide and dilute hydrochloric acid[23, 24].

2.6 Microbial Limit Assay

Dissolved 1gm of powdered plant material in 10mL of distilled water. It was serially diluted using phosphate buffer as diluent. The sample was inoculated in Nutrient agar by pour plate, Rose Bengal agar and SS agar by spread plate techniques for Bacteria, Fungi and Salmonella respectively. For bacteria, the plates were incubated at 37°C for 48 hrs and for fungi; the plates were incubated 25°C for 96 hrs[18].

2.7 Qualitative Phytochemical Screening

Preliminary phytochemical characterization was carried out by using standard procedure Sofowara[25], Trease and Evans[26] and Harborne[27,28].

2.8 Quantitative analysis of phytochemicals

2.9 Determination of total phenols by spectrophotometric method

Total phenols estimated by the method of Edeoga *et al*., [29.] Plant powder (2g) was boiled with 50ml of ether for the extraction of the phenolic component for 15min. 5ml of the extract was pipetted out into a 50ml flask, then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30min for colour development. This was measured at 505 nm.

2.10 Determination of Tannin

Tannin was determination by Van-Burden and Robinson method[30]. 500mg of the sample was weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for 1hr in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the

filtrate was pipetted out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferro cyanide. The absorbance was measured at 120nm within 10min

2.11 Determination of Saponin

Saponin determined by the method of Obdoni and Ochuko[31]. Plant powder were ground and 20g of each were put into a conical flask and 100cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4hr with continuous stirring at about 55° C. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90° C. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant.

2.12 Determination of Flavonoid

Flavonoid determined by the method of Boham and Kocipai-Abyazan[32]. 10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

2.13 Histochemical tests [33, 34]

A small quantity of dried and finely powdered bean sample was placed on a grease free microscopic slide and treated with specific chemicals and reagents and waited for 1-2 minutes. A positive result for histochemical analysis was indicated by the appearance of the appropriate colour change after application of the reagent using a light microscope to observe and record any colour changes. The powder sample was treated with diluted ammonia and H_2SO_4 which gave yellow colour that indicated flavonoids. Plant powder treated with Dragant draft reagent gave brown colour indicates alkaloids. Plant powder treated with ferric chloride to give Dark blue to black indicates the presence of tannin. Plant powder treated with 5 drops of acetic anhydride and 5 drops of H_2SO_4 to give Violet to Blue (or) Green colour indicates the presence of steroids. Plant powder treated with Toludine blue to give Blue green/Red colour indicates the presence of polyphenol. Plant powder treated with Dinitrophenol hydrazine (few drops) to give Orange colour indicates the presence of Terpenoids. Plant powder treated with H_2SO_4 (few drops) to give Yellow colour indicates the presence of Saponin. Plant powder treated with Acidic acid, few drops of ferric chloride and H_2SO_4 to give Brown colour indicates the presence of Glycoside.

3. RESULTS

Pharmacognostic study was used for the determination of quality raw materials which need to be validated as a medicine. Good raw materials are needed for better drug efficiency. In India, different medicinal plants were described in single vernacular name, which makes confusion in drug identity. In India herbs are collected from heterogenous sources. To overcome the problems of drug identity, powder analysis, histochemistry, pharmacognosy and preliminary phytochemistry are needed for the confirmation of the plant's identity.

3.1 Scientific Classification of Andrographis echioides[10]

Kingdom	: Plantae,
Subkingdom	: Tracheobionta – Vascular plants
Super division	: Spermatophyta – Seed plants
Division	: Magnoliophyta – Flowering plants
Class	: Magnoliopsida – Dicotyledons
Subclass	: Asteridae
Order	: Scrophulariales
Family	: Acanthaceae
Genus	: Andrographis
Species	: *Andrographis echioides* (L.) Nees

3.2 Habitat

Andrographis echioides is a herb abundantly found in dry area (Fig.1) of southern Asian countries like India, Srilanka, Pakistan, Myanmer etc. Flowering season is July to October. This plant is propagated via seeds[15, 35].

3.3 Morphology

Plate I

***Andrographis echinoides*-Plant habit and Dried**

A. echioides plant grows up to 50 cm, whereas plants collected for the present study are 18.5cm in height (Fig.2). Powder was made from dried portions of whole plant (Fig. 3). Stem of the plant are angular with dense hairy structures. The stem is slightly quadrangular with hairs on its surface. Leaves are oblong shape with small hairs on both sides. The plant showed raceme type of inflorescence not exceeding the leaves and is scarcely branched. The calyx of the flower is with sub equal lobes, lanceolate with glandular hairs. Corolla is white with brown tinge. It is tubular, showing the 2+3 lipped condition, which are unequal. Stamens-2, exserted and straight, style slender, with capitate stigma. The capsules are ovoid, sparsely hairy, pointed above and narrowed below. The average number of the capsule per plant is 38, seed are yellow in colour and ovoid. Four seeds per capsule, 1.5mm across and glabarous. By the free hand sections, the anatomical characters of root stem and leaf were observed (Plate I). The epidermal peeling showed thick walled, polygonal epidermal cells and large stomata (Fig. 4). The stomata are paracytic type, with two subsidiary cells with their common walls lying at right to the guard cells. The guard cells are elliptic in shape with wide stomatal pore. There are large and thick cylindrical calcium oxalate crystals lying in horizontal position in the mesophyll tissue. Different types of trichomes are seen in the powder. There are long, cylindrical conical unbranched trichomes are seen in the powder (Fig. 5, 6). There is long stalk with three cells with large spherical terminal gland (Fig. 6). The stalk cells were wide and cylindrical and thin walled. The glandular head is spherical multicellular and thin with prominent nucleus. The gland proper are secretary in function. The stalk of the glandular trichome has differently modified stalk (Fig. 7, 8, 9). The cells are shrunken stalked cells at the subterminal or basal region (Fig. 9). There is a bundle of vertically elongated rectangular parenchyma cells. The cells are attached with axile fiber (Fig. 10). There are also isolated parenchyma cells consists of two cells one below another. The cells have thick walled and dense protoplast and prominent nuclei. The cells are 100 µm long and 30 µm thick (Fig.11). There are also group of parenchyma cells which are longitudinally elongated and occur in group of four cells. The cell wall of some parenchyma cells are thick walled and other parenchyma cells are thin walled (Fig. 12). The vessel elements are short cylindrical vessel element with thick tail and lateral wall elliptical pits (Fig 13 and 14) and large cylindrical vessel element with thick long, cylindrical vessel element with thick long tail with small dot like central pit (Fig. 15). Size of the trichomes, stalk, fibre, vessel element, parenchyma cells were in Table 1.

Table 1: Microscopic characters of Powder of *Andrographis echioides* whole plant powder

Trichomes	410 µm long and 30 µm thick.
The stalk	300 µm long and 80 µm thick
The fiber	850 µm long and 20 µm thick
The vessel element	350 µm long and 90 µm wide
The Parenchyma cells	100 µm long and 30 µm thick
The glandular head	Spherical and Multicellular 850 µm long and 20 µm thick walled with prominent nucleus.
Calcium oxalate crystals	Rectangualr shape

Plate II

Powder Microscopy of *Andrographis echioides* whole plant Powder

Fig .4. Stomata of the leaf in surface view showing paracytic subsidiary cells

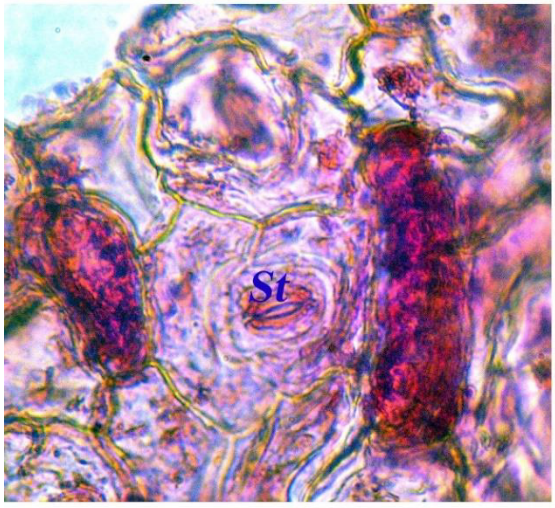
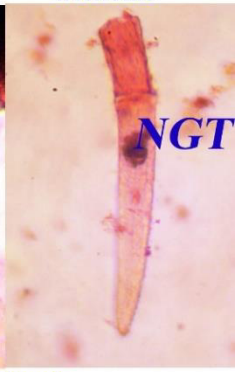


Fig. 5. Glandular trichome with three stalk cell with spherical terminal gland

6. Non glandular epidermal trichome



St- Stomata; Gl - Gland;
NGT - Non Glandular trichome

Fig 7, 8 - Glandular trichome with shrunken stalk cells

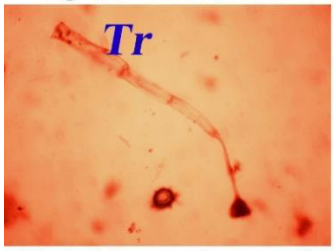
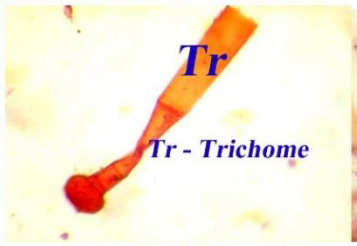
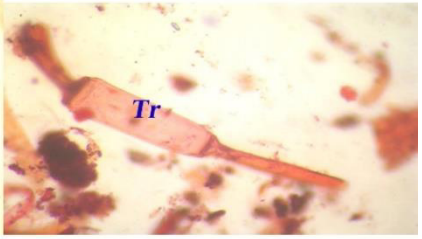


Fig. 9. Glandular trichome with shrunken stalk cells

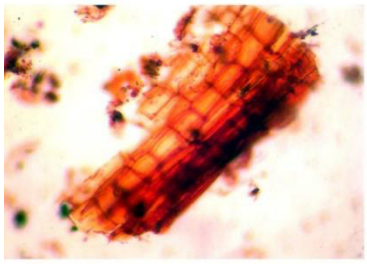


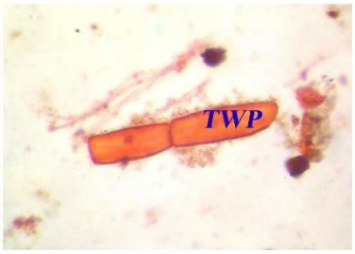


10. Axial parenchyma cells in vertical row

Fig. 11. Thick wall parenchyma cells

Fig. 12. A pair of thin wall parenchyma cells





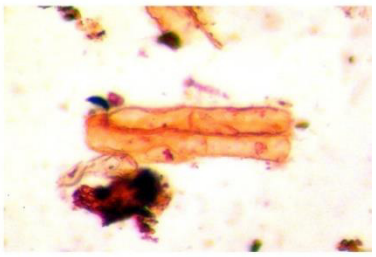
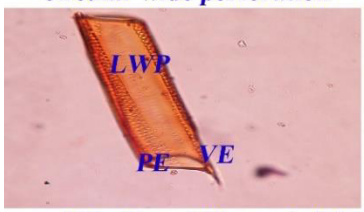
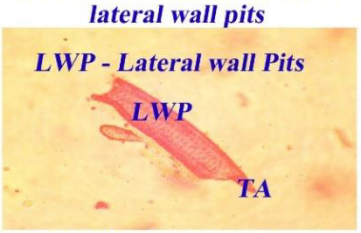


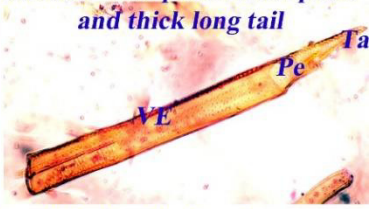
Fig.13. A vessel element with tailed end circular wide perforation

14. A vessel element with tail perforation and multicerate lateral wall pits

Fig.15. Long cylindrical vessel element with circular wide perforation plate and thick long tail







VE - Vessel Element; TWp - Thick Walled Parenchyma; Ta - Tail; Pe - Perforation

Table 2- Organoleptic characters of mixed powder preparation of *Andrographis echioides*

S. No	Character	Observation/ Result
1	Colour	Yellowish Green
2	Odour	Characteristic
3	Taste	Bitter
4	Texture	Rough

Processed whole plant powder of *Andrographis echioides* was assessed for its organoleptic characters, it revealed that the powder was yellowish green in colour, characteristic odour, bitter taste and rough texture (Table 2).

Table 3 - Physicochemical constant and extractives of mixed powder preparation of *Andrographis echioides*

S. No	Parameter	Results
1	Foreign matter	Nil
2	Total ash	03.6%
3	Acid insoluble ash	00.8%
4	Water soluble ash	01.6%
5	Water extractive	19.8%
6	Alcohol extractive	12.4%
7	Chloroform extractive	06.3%
8	Ethyl acetate extractive	01.6%
9	Hexane extractive	03.7%

When the plant powder was analyzed for its physicochemical parameters, it showed 3.6% total ash content followed by 1.6% water soluble ash and 0.8% acid insoluble ash. This is within the limits of ayurvedic pharmacopeia of India (Table 3). Extractive phytocompounds play a vital role in biological potentials. Whole plant powder showed 19.8% water extractives followed by 12.4% ethanol extractive, 6.3% chloroform extractive, 3.7% hexane extractives and 1.6% ethyl acetate extractives. Higher water extractive indicated that the presence of polar phytochemicals in the powder. This was done successively with increased polar solvents. This is also in line with ayurvedic pharmacopoeia of India.

Table 4 - Fluorescence analysis of *Andrographis echioides* powder

S. No	Test	Visible Light	Short UV (254 nm)	Long UV (365 nm)
1	Plant powder	Green	Green	Black
2	Plant powder +Water	Green	Light green	Black
3	Plant powder+Hexane	Green	Green	Black
4	Plant powder+Chloroform	Green	Yellowish green	Black
5	Plant powder+Methanol	Green	Green	Black
6	Plant powder +Acetone	Dark green	Green	Black
7	Plant powder+1N NaOH	Yellowish green	Light green	Black
8	Plant powder+1N HCL	Blackish brown	Black green	Greenish black
9	Plant powder+H2SO4+Water	Yellowish brown	Yellow	Black
10	Plant powder+HNO3+Water	Brown	Black	Black

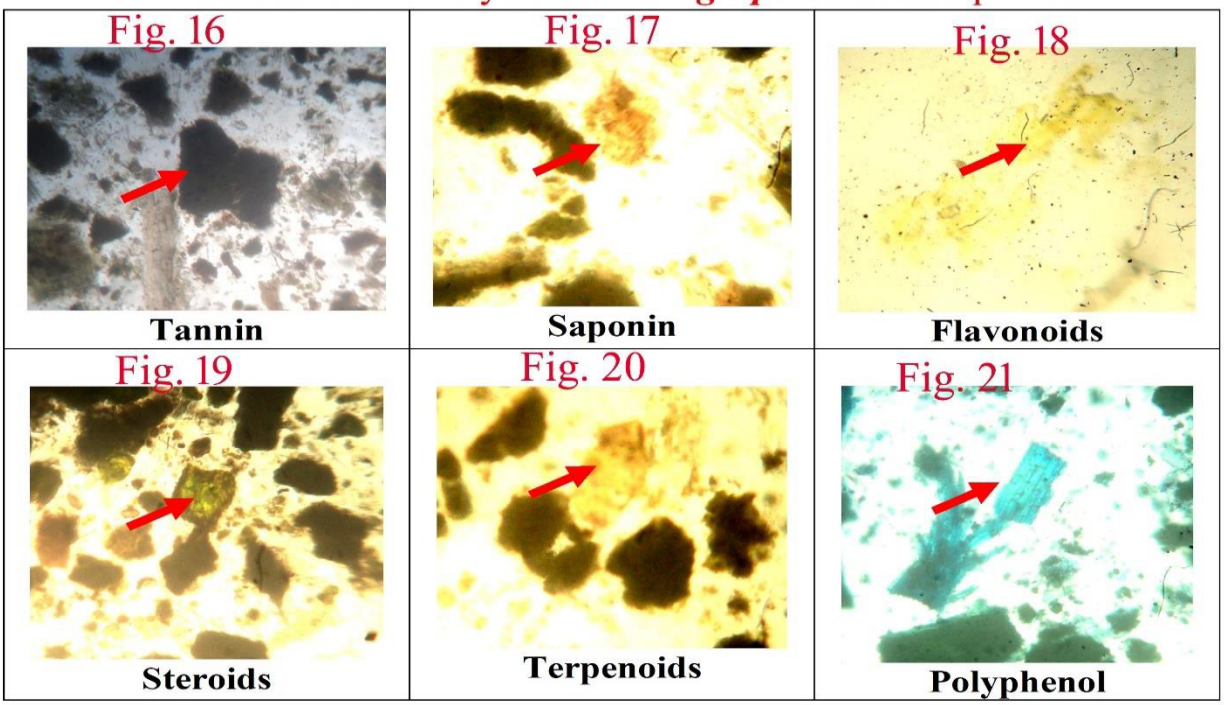
The fluorescence analysis is one of the tools for the determination of constituents in herbal drugs and it provides an idea about the chemical nature of the plant material. The powder of *Andrographis echioides* showed mostly black colouration under long wavelength of UV (365 nm). Powder preparations observed under visible light showed characteristic green, dark green, yellowish green, blackish brown, yellowish brown and brown colouration (Table 4).

Table 5 - Nature of microbial availability in mixed powder preparation of *Andrographis echioides*

S. No	Test organism	Microbial count
1	Total aerobic Bacteria	96×10^2 CFU/g
2	Total Fungal count	12 CFU/g
3	Total Enteric Bacteria	Nil
4	Total *E. coli*	Nil
5	*Salmonella*	Nil
6	*Shigella*	Nil

Microbial assessment of powdered *Andrographis echioides* whole plant indicated low level of microbial population. It contains 96×10^2 CFU/g of total count of aerobic bacteria and 12 CFU/g of total fungus. There are no enteric bacteria like *Escherichia coli, Salmonella* and *Shigella* (Table 5). This is also within the limits of ayurvedic pharmacopoeia of India.

Plate III
Histochemical Analysis of ***Andrographis ecioides*** powder



The formation of yellow colouration when treated with the powder with diluted ammonia and H_2SO_4. The presence of tannin indicted by dark blue to black colouration when treated with ferric chloride. Plant powder treated with 5 drops of acetic anhydride and 5 drops of H_2SO_4 to give Violet to Blue (or) Green colour indicates the presence of steroids. Toludine blue treatment of plant powder produces Blue green colouration showed the presence of polyphenol. On the other hand, treatment of plant powder with Dinitrophenol hydrazine (few drops) to give Orange colour indicates the presence of Terpenoids. Similarly, plant powder treated with H_2SO_4 (few drops) to give Yellow colour indicates the presence of Saponin (Plate III – Fig. 16-21 and Table 6).

Table 6: Histochemical analysis of *Andrographis echioides* powder

S. No	Phytochemicals	Histochemical Results	
		Colour observation	Results
1	Tannin	Black	++
2	Saponin	Yellow	+
3	Flavonoids	Yellow	++
4	Steroids	Green	+
5	Terpenoids	Orange /Yellow	+
6	Polyphenol	Blue/ green	++

(-) Absent, (+) Present and (++) high concentration

Table 7: Qualitative analysis of phytochemicals constituents of plant powder *Andrographis echioides*

S. No	Phytochemicals	Result
1	Tannin	++
2	Saponin	++
3	Flavonoids	++
4	Steroids	++
5	Terpenoids	+
6	Triterpenoids	+
7	Alkaloids	+
8	Anthroquinone	+
9	Polyphenol	++
10	Glycoside	+
11	Coumarins	++
12	Emodins	+
13	Anthocyanins	+

(-) Absent, (+) Present and (++) high concentration

Table 8 Quantitative analysis of *Andrographis echioides* powder

S. No	Phytochemicals	Results (mg/gm)
1	Polyphenol	167.43 ± 3.68
2	Flavonoids	089.28 ± 1.67
3	Tannin	077.21 ± 4.88
4	Saponin	032.54 ± 0.23

Values expressed as Mean ± SD for triplicate

The results of preliminary quantitative phytochemical screening of whole plant extracts of *Andrographis echioides* revealed the presence of multiple polar and non-polar chemical constituents (Table 7). Plant powder contains all the 13 phytocompounds tested, which includes tannins, saponins, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, anthroquinones, polyphenols, glycoside, coumarins, emodins and anthocyanins. Table 8 expresses quantity of important phytochemicals available in the powder. Among the phytocompounds both extracts showed higher concentrations of polyphenols followed by tannins, flavonoids and saponins.

4. DISCUSSION

This study provides authentic information on the whole plant of *Andrographis echioides*. This plant is traditionally used to cure inflammatory diseases, fever, dysentery etc.,[35,13, 12, 10]. Pharmacognostic methods are useful to differentiate the powder of *Andrographis echioides* from the closely related species like *Andrographis paniculata*. Pharmacognostic standards were determined through this study and are also useful in detecting adulterations. Though this plant is used for various ailments, there are no proper pharmacological and pharmacognostic standard for this plant. Hence this study was undertaken to assess its quality standards, the results of this study could be considered as a standard for this plant material. Low quantity of ash value indicated the plant materials were free from soil or any other metal-based (Inorganic) contamination[24,26]. Mostly these plants are available in road sides where its environment is semidried and dried, and it grows abundantly. The plant materials used in this study were collected after flowering. Similar kind of plants botanical description were also provided by Mathivannan and Suzeem[15]. In the present study, the plant powder showed very less ash content indicating the purity of the sample. The higher water extractive values of the powdered plant materials indicated the presence of flavonoids[36]. In our study, the plant sample reveled higher water and ethanol extractive value. Hexane extractable value indicated the presence of straight chain fatty acid, which showed antimicrobial potentials of this plant. Presence of polyphenols and tannins in this plant supports chemically the antidiarrhoeal and antimicrobial activity, which was in agreement with various earlier reports[21,37]. Behavior of drug materials under UV radiation and visible light exhibited different colour depending up on the various chromophores present in the material. Under long UV range, plant drug produces black colouration under all type of chemical treatment, which could be due to long chain fatty acids[23, 24]. The results of preliminary phytochemical analysis revealed the presence of alkaloids, terpenoids, flavonoids, tannins, saponins and carbohydrates in aqueous extract. Polyphenols are used in the treatment of burns as they precipitate the proteins of exposed tissue to form a protective covering[38]. These compounds also interfere with microbial growth thereby producing antimicrobial activity. They are also used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and as antidote[39,40]. The availability of similar phytochemicals also indicated by few scientists from India and abroad[41- 44.] In the present investigation *A. echioides* powder revealed therapeutically important phytochemicals both qualitatively and quantitatively. Microbial limit assay indicated quality of raw or crude drug powder, number of microbial cells are directly proportional to the quality of the plant materials. This study is in line with the quality parameters prescribed in Ayurvedic Pharmacopeia of India and also standards set by other international agencies. This work provides qualitative and quantitative standards for the identification of plant powder and which can be valuable information to carry out further research in elucidation of new drugs after carrying out systematic clinical trials. Phytochemicals of the plant materials could be considered as a biomarker for the authentication and also for the certification of plant materials4[5, 46, 47.] All the pharmacognostic reports were within the limits of common Ayurvedhic pharmacopeia of India. This study could be worthwhile for the correct identification of plant materials and differentiation of this plant with taxonomically similar plants like *Andrographis paniculate*[48]. These observations made in this study could be make as a standard for the plant Andrographis echioides.

5. CONCLUSION

The present study indicated the importance of pharmacognosy and phytochemistry in controlling raw drug as per ayurvedic phamacopoeia. The pharmacognostic results were within the limits of ayurvedic pharmacopeia of India. Whole plant powder showed the presence of terpenoids, flavonoids, Saponins, phenolic compounds and carbohydrates which could be responsible for all biological activities of the plant. Histochemical analysis also confirmed the availability of phytoconstituents like flavonoids and tannins. The observations made through this study would be of immense value in the pharmacological evaluation and standardization of crude powder of the plant materials.

6. AUTHOR CONTRIBUTION STATEMENT

Mrs. Subhashini and Mrs Chitradevi hypothesized the study and performed all experiments, Dr. S. Rajan analyzed all data and discussed all study matter and Dr. S. K. Sundar is designed all methodology described in the study. All the authors read this article and approved it.

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8. CONFLICT OF INTEREST

Conflict of interest declared none.

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