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Evaluation of pharmacognostic features of aerial parts of *Andrographis paniculata* Wall.

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ABSTRACT
Andrographis paniculata (Burm, f.) Wall. is used traditionally as hepatoprotective, antipyretic and dermatosis. Several phytoconstituents have been reported from different parts of the plant such as glycosides, glycosides, flavonoids and poly-unsaturated fatty acids. As the herb is used widely in the traditional systems of medicine, it was thought worthwhile to undertake the standardization. The present study designed for determination of morphological characters, chemoprofile, physico-chemical parameters and TLC fingerprint profile of aerial parts of *A. paniculata*. The quality control parameters like extractive of plant with different solvents, ash values, foreign organic matter, loss on drying and TLC profile were determined. The results obtained from preliminary pharmacognostic standardization of aerial parts of *A. paniculata* are helpful in determination of quality and purity of the crude drug and its marketed formulation.

Keywords: *Andrographis paniculata,* Acanthaceae, TLC profile, quality standards, WHO guidelines.

1. Introduction

Andrographis paniculata (Burm, f.) Wall. Ex Nees (syn. *A. subspathulata* C.B. Clarke; *Justicia paniculata* Burm. f.) belonging to family-Acanthaceae and commonly known as *Kalmegh.* It is a herbaceous to arborescent annual branched plant, upto 1 m high with linear-lanceolate leaves; growing wild throughout tropical regions in waste lands and as an undergrowth in the deciduous forests of Uttar Pradesh, Assam, West Bengal and South Indian states. The plant is reported as hepatoprotective, antipyretic and dermatosis [10]. Its hepatoprotective activity has been reported in crude extract, purified extract and/or andrographolide by large number of investigators using different test systems *in-vitro* as well as *in-vivo* supported by biochemical, functional and histological evidences [2-4]. Immunostimulant activity in ethanolic extract of plant was also reported [5]. Neoandrographolide (100-150 mg/kg, *p.o.*) was found to possess antiinflammatory activity while andrographolide showed both antiinflammatory [6] and antiulcerogenic [7] activity in different animal models *in-vitro* and *in-vivo.* Andrographolide was reported to possess antiallergic [8] and antidiabetic activity in animal models [8]. The plant was also reported for its antimarial activity [10]. Its major phytoconstituents includes andrographolide, bisandrographolide, ninandrographolide, andropanolide, isoandrographolide, deoxyandrographolide and andrographiside. The present study deals with development of quality standards of aerial parts of *A. paniculata* as per WHO guidelines [11].

2. Materials and Method

2.1. Plant material and chemicals

The aerial parts of *Andrographis paniculata* Wall. were collected from the herbal drug market, Delhi. The sample was identified and a voucher specimen (PRL/2013/09) of the plant was kept for future reference. Silica gel F_{254} HPTLC plates were purchased from Merck, Mumbai, India. Other analytical grade solvents and reagents were obtained from S.D. Fine Chemicals, Mumbai, India.

2.2. Morphological studies

The aerial parts of *A. paniculata* were studies for their morphological characters.

2.3. Preliminary phytochemical screening

The qualitative chemical tests were performed for different extracts according to the methods described by Farnsworth [12] with some modifications.

2.3.1. Determination of alkaloids

The alkaloids were extracted by refluxing the sample with sufficient amount of water for about 2 hr. The extract was concentrated on a rotor vapor, basified with NH_4OH and was extracted with $CHCl_3$ (three times). Then the content was concentrated and 2 drops were spotted separately on a thin layer chromatography (TLC) plate. After development the plate was dried, Dragendorff's reagent was sprayed onto them. Alkaloids give an orange color with Dragendorffs reagent.

2.3.2. Determination of steroid glycosides

The extracts were dissolved in equal volumes of acetic anhydride and $CHCl_3$. The mixture was transferred to a dry test tube and conc. H_2SO_4 acid was added at the bottom of the tube. Formation of a reddish brown or violet brown ring at the interface of the 2 liquids indicates presence of steroids.

2.3.3. Determination of polyphenolics

Two to three drops of 1% $FeCl_3$ solution was added to 2 ml portions (1%) of each extract. Phenolic compounds produce a deep violet color with ferric ions.

2.3.4. Determination of saponins

The extract is taken in test tube with small amount of water and shaken vigorously for one minute and observed for formation of rich lather, which is stable for more than ten minutes.

2.3.5. Determination of flavonoids

The extracts were dissolved in methanol (50%, 1-2 ml) by heating. Then metal magnesium and 5-6 drops of concentrated hydrochloride acid (HCl) were added. The solution turns red when flavonoids are present.

Other chemical tests for phytoconstituents were performed as per method described by Mukherjee [13].

2.4. Determination of physico-chemical parameters

Physicochemical parameters were determined for aerial parts of *A. paniculata* according to methods described in WHO guidelines.

2.4.1. Determination of total ash

The powdered material (2 g) was accurately weighed and placed in a crucible. The material was spread in an even layer and it was ignited to a constant weight by gradually increasing the heat to 500-600 °C until it was white indicating the absence of carbon. The residual ash was allowed to cool in a desiccator. The content of total ash (in mg/g) of air-dried material was calculated as follows:

$$\text{Total ash } (\% \text{ w/w}) = \frac{(\text{weight of ash}) \times 100}{\text{weight of samle}}$$

2.4.2. Determination of acid insoluble ash

HCl (2 N; 25 ml) was added to the crucible containing the total ash, covered with a watch glass, and boiled gently for 5 min. The watch glass was rinsed with 5 ml of hot water and the rinsed contents were added to the crucible. The acid insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing acid insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The residue was allowed to cool in a desiccator and weighed. The content of the acid insoluble ash (in mg/g) of air-dried material was calculated as follows:

$$\text{Acid insoluble ash } (\% \text{ w/w}) = \frac{(\text{weight of ash}) \times 100}{\text{weight of samle}}$$

2.4.3. Determination of water soluble ash

Water (25 ml) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 ml of hot water and added to the crucible. The water insoluble matter was collected on an ash less filter paper and washed with hot water. The filter paper containing the water insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The water soluble ash content was calculated using the following equation.

$$\text{Water soluble ash}(\% \text{ w/w}) = \frac{(\text{total ash} - \text{water insoluble residue in total ash}) \times 100}{\text{weight of samle}}$$

2.4.4. Determination of alcohol soluble extractive

Accurately weighed powdered material (4 g) and was placed in a glass stoppered round bottle flask (RBF). Ethanol (100 ml) was added to the RBF and then, it was shaken well and allowed to stand for 1 h. A reflux condenser was attached and boiled gently for 1 h, and then it was cooled and filtered. The flask was shaken well and filtered rapidly through a dry filter paper. After that, 25 ml of the filtrate was transferred to a tarred flat bottomed dish and evaporated to dryness on a water bath. Then the dish was dried at 105 °C for 6 h and cooled in a desiccator and weighed. The content of extractable matter (% w/w) air-dried material was calculated as follows:

$$\text{Alcohol soluble extractives } (\% \text{ w/w}) = \frac{(\text{weight of residue}) \times 4 \times 100}{\text{weight of samle}}$$

2.4.5. Determination of hydro-alcoholic and water soluble extractives

The same procedure as described for the ethanol soluble extractive matter was followed for the determination of hydro-alcoholic and water soluble extractive matter using ethanol-water (1:1) and distilled water instead of ethanol.

2.4.6. Determination of moisture content

The powdered material (10 g) was placed in a moisture dish and dried to a constant weight in an oven at 100-105 ^{0}C. The loss of weight (in mg/g) of air dried was calculated as follows:

$$\text{Moisture content } (\% \text{ w/w}) = \frac{(\text{Initial weight of sample} - \text{final weight of sample}) \times 100}{\text{weight of samle}}$$

2.4.7. Foreign matter analysis

Foreign matter presence may be due to faulty collection of crude drug or due to deliberate mixing. It was separated from the drug so that results obtained from analysis of the drug gives accuracy. Its percentage in the crude drug was calculated.

2.5. TLC studies of methanolic extract of *A. paniculata*

Preparation of test solution

Extracted 10 g powdered drug with 250 ml of methanol in a

Soxhlet apparatus for 6 h filter and concentrated the extract under vacuum and make up the volume to 20 ml with methanol.

Procedure

Apply the test sample through fine capillary tube on a precoated silica gel 60 F_{254} TLC plate. Developed the plate in the mobile phase in a twin-trough chamber to a distance of 8 cm using Toluene: Ethyl acetate: Methanol (6: 3: 1 *v/v*) as mobile phase.

Visualization:

The plate was air dried and sprayed with anisaldehyde-sulphuric acid reagent and then heated the plate in oven at 105 °C till the colour of the spots appears. Recorded the R_f value and colour of the resolved spots.

3. Results

3.1. Morphological characters

The aerial parts of *A. paniculata* consisting of the following morphological characters (Figure 1):

Fig 1: Dried specimens of aerial parts of *Andrographis paniculata* Wall.

Stem: woody to semiwoody, bearing numerous cross armed branches, upper being distinctly quadrangular, with swollen nodes and 4 winged projections, lower basal somewhat spherical and bearing thin, slender, hard adventitious roots at the nodes; fracture short, colour greenish.

Leaf: simple, opposite, exstipulate, thin, membranous, lanceolate, 3 to 8 cm in length and 1 to 2 cm in width, entire to somewhat undulated, acute to acuminate, upper surface glabrous, dark green, lower granular, shows 4 to 6 pairs of lateral veins; petiole short, winged, 5 to 8 mm in length.

Flower: about 1 cm long, bracteates, pedicillate, zygomorphic, two-lipped, white, pink to purple coloured with purple dots, lower lip darker; bracks, calyx and corolla are pubescent; pedicel very short upto 4 mm long, gland dotted, arranged on about 10 cm long armed, terminal to axillary semipaniculate racemes producing flowers on one side of their ultimate branches.

Fruit: a capsule, narrowing at both ends, slightly flattened to cylindrical with a septum at the centre, elliptic to linear-oblong, gland dotted when young, glabrous when fully mature, 2 cm long, 3 mm wide, encloses 6 to 12 seeds attached to flattened retinacula.

3.2. Phytochemical Screening

The results of phytochemical screening of aerial parts of *A. paniculata* were depicted in Table 1.

Table 1: Results of phytochemical screening of aerial parts of *A. paniculata*

Extract constituents	Methanolic extract	Hydro-alcoholic extract	Aqueous extract
Glycosides	+	++	++
Alkaloids	+	++	+
Carbohydrates	+	+	+
Tannin	+	+	+
Phenolics	+	+	-
Flavonoids	+	++	+
Proteins & amino acids	+	+	+
Resins	+	+	-
Lipids/fats	-	+	-

(Where, – absent and + present)

3.3. Physico-chemical parameters
Results of physico-chemical parameters of aerial parts of *A. paniculata* were summarized in Table 2.

3.4. TLC finger printing analysis of methanolic extract of *A.* Paniculata: The results of TLC identify test were presented in Table 3 and Figure 2. The methanolic extract showed 7 prominent spots with toluene: ethyl acetate: methanol (6: 3: 1 *v/v*) as mobile phase. The results of TLC identity test helps in determination of chemical profile of plant material.

Table 2: Results of physico-chemical standards of aerial parts of *A. paniculata*

Parameters	Results
Total ash	11.64 ± 0.92%
Acid insoluble ash	1.3 ± 0.27%
Water soluble ash	0.8 ± 0.06%
Alcoholic extractive value	13.18 ± 1.38%
Hydro-alcoholic extractive value	15.68 ± 0.92%
Aqueous extractive value	19.43 ± 1.42%
Loss on drying (moisture content)	4.73 ± 1.64%
Foreign matter	Nil

(Data expressed as mean ± SD, n =3)

Table 3: TLC Details of methanolic extract of *A. paniculata* aerial parts

R_f Value	Colour of the band
0.21	Purple
0.30	purple
0.45	Purple
0.52	Light purple
0.62	Light green
0.68	Light purple
0.80	Light green

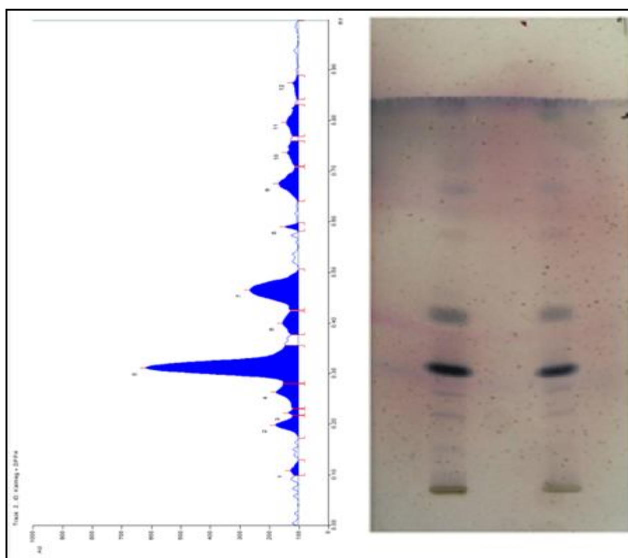

Fig 2: TLC profile of methanolic extract of *A. paniculata* aerial parts.

4. Discussion
The major hindrance in the acceptance of herbal medicines into modern medical practices is the lack of scientific and clinical data on safety and efficacy of the herbal products. To ensure the quality, safety and efficacy of herbal products, standardization is of vital importance [14]. The safety problems emerging with herbal medicinal products are due to a largely unregulated growing market where there is lack of effective quality control, lack of strict guidelines on the assessment of safety and efficacy monitoring on tradition medicine and alternative medicine. Standardization of crude drug is an integral part of establishing its correct identity. The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. Phytochemical screening revealed the presence of glycosides, alkaloids, flavonoids and amino acids in ethanolic, hydro-alcoholic and aqueous extracts mainly. The physicochemical analysis of plant drugs is an important for detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity and quality of drugs. The ash value was determined by 3 different methods, which measured total ash, acid insoluble ash, and water soluble ash. The total ash method is employed to measure the total amount of material remaining after

ignition. The total ash usually consists of carbonates, phosphates, silicates and silica, which include both physiologic ash and nonphysiologic ash. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the crude drug for marketing. Acid insoluble ash indicates contamination with silica, for example, earth and sand. Comparison of this with the total ash value of the same sample will differentiate between contaminating materials and variations of the natural ash of the drug. Water soluble ash is that part of the total ash content, which is soluble in water. It is a good indicator of the water soluble salts in the drug. Extractive values are representative of the presence of the polar or nonpolar extractable compounds in a plant material. Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Insufficient drying leads to spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles [13]. The results of TLC identify test helps in determination of chemical profile of plant crude plant material and quick detection of any adulteration in drug sample [15]. All these parameters, which are being reported, could be useful in identification of distinctiveness features of the crude drug and used for establishing quality of aerial parts of *A. paniculata*. In conclusion, the results obtained from phytochemical screening studies, physico-chemical parameters and TLC identity test can be used to standardize aerial parts of *A. paniculata.*

5. Conclusion

The results of physico-chemical analysis, phytochemical screening and TLC identity test are useful in determining quality, safety and efficacy of aerial parts of *A. paniculata* for its use as potential drug candidate.

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