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## PHYSICOCHEMICAL STANDARDIZATION OF *ANDROGRAPHIS PANICULATA* (NEES) : AN AYURVEDIC DRUG

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### ABSTRACT

*Andrographis paniculata* Nees popularly known as “Kalmegh” used in indigenous system of medicine as a hepatoprotective drug. In the present investigation, pharmacognostical, physicochemical characteristics, thin layer chromatography, and HPLC studies of *Andrographis paniculata* Nees were studied. In order to ensure the use of only genuine and uniform material of such herbal remedies, work on standardization assumes vital significance. Preliminary phytochemical analysis showed that alkaloids, flavonoids, glycosides, saponins, steroids, tannins, were present. The results of the pharmacognostical studies, features of powdered drug include response to U.V. Light exposure, some physicochemical constants, results of TLC, and chromatogram of HPLC, would serve as standard reference for identifications of *Andrographis paniculata* (Nees).

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### Key Words

*Andrographis paniculata* Nees, Hepatoprotective, fluorescence characters, colour reactions, TLC, HPLC.

## INTRODUCTION

*Andrographis paniculata* (Acanthaceae) popularly known as Kalmegh or King of Bitters is a well known drug in the Indian system of medicines and widely cultivated in India. It is used as a wonder drug in traditional Sidha and Ayurvedic system of medicine as well as tribal medicine in India for multiple clinical applications<sup>1,2</sup>. It is a component of over 50% of the multi ingredient herbal formulations available in India for the treatment of liver ailments<sup>3</sup>. The plant has been reported to possess antipyretic<sup>4</sup>, analgesic<sup>4</sup>, antihepatotoxic<sup>5,6,7</sup>, antidiabetic<sup>8</sup>, antimalarial<sup>9</sup>, antibacterial<sup>10,11,12</sup>, antifertility<sup>13</sup>, anti-inflammatory<sup>14</sup> and immunosuppressive<sup>15</sup> properties due to bitter content. Most of biological actions of *Andrographis paniculata* has been due to the presence of andrographolide, which is a bicyclic a diterpene lactone<sup>16-26</sup>. About 26 different poly herbal formulations of this plant are mentioned in Ayurveda as a popular remedy for the treatment of various liver disorders. In traditional Chinese medicine (TCM) *Andrographis* is considered as the herb possessing an important cold property useful to treat the heat of body in fever and to dispel toxins from the body. In Scandinavian countries, it is commonly used to prevent and treat common colds<sup>27</sup>.

In recent years focus on use of non traditional approaches to treat disease has been revived worldwide, and there is an urgent need that adulterants of herbal drugs should be defined in terms of botanical and chemical standards. Ultimately such standards can be used as marker by the pharmaceutical industries in order to detect adulterant from the genuine crude drug for their quality control and to obtain therapeutic effect of particular drug.

The evidence collected till now shows immense potential of this medicinal plant used in traditional systems. In present studies an attempt has been made to provide referential information for the correct identification and standardisation of this plant material for qualitative evaluation.

## EXPERIMENTAL SECTION

**Plant Material:** The plant material were collected from local market of Ghaziabad, (U.P.) India, and identified in

Department of Pharmacognosy, Pharmacopoeia Laboratory of Indian Medicine, Ghaziabad and confirmed by comparison with reference herbarium specimens. The herbarium sheet was deposited in the herbarium.

## METHODS

The anatomical studies were performed on aerial parts of plant. The relevant parts were fixed in a solution of FAA (70% EtOH- Ac-CH<sub>2</sub>O 90:5:5) All parts were cuts across sectioned with free hand and rotatory microtome. These sections were prepared and permanent staining was done to study the anatomical features.

**Phytochemical Studies:** The plant materials were dried under shade and were powdered using a homogenizer and whole powder was considered as drug. Various chemical tests were performed according to conventional methods<sup>28, 29, 30</sup>. The Fluorescence behaviour was determined according to the methods of Chase and Pratt<sup>31</sup>. Preliminary phytochemical screening, physicochemical constants like ash value, water soluble extracts, alcoholic extracts, sulphated ash, loss on drying and pH values were determined as per method described in Indian Pharmacopoeia<sup>17</sup>. Thin layer Chromatographic studies were performed for Rf values in various extracts the spots new detected by exposing the plates with iodine vapours, UV light, ordinary light(354 nm), using silica gel F<sup>254</sup> precoated plates.. Qualitative and quantitative estimation of inorganic element was determined by atomic absorption spectrophotometer Varian spectra A-10. Successive extraction of the powder was done in the soxhlet extractor.

**HPLC Analysis:** A simple, rapid and precise reverse phase high performance liquid chromatographic method has been done for identification, as per the method given in I.P.<sup>17</sup>. 2010. Sample prepared by using 0.25 gm of the coarsely powdered substance in 50 ml of methanol on water bath for 15 minutes cool and filter. Reflux the residue further with 2+50 ml of methanol, cool and filter. combine all the filterates and

concentrate to 50.0 ml. Standard prepared by dissolving 0.10 mg of Ref. Std Andrographolide (Natural Remedies) in 100 ml methanol. The analytes were resolved by using C-18 column (25 cm x 4.6 mm 5  $\mu$ m), a mixture of 65 volumes of methanol and 35 volumes of water as mobile phase, flow rate 1 ml per min, and detection was 223 nm using photodiode array detector (Agilent 1100 series).

## RESULT SECTION

**Plant material of interest: dried aerial part**

**Organoleptic Properties:** Odour, slight, characteristic, taste intensely bitter.

**Macroscopic Characteristics:** Mixture of crisp, dark green coloured broken leaves, quadrangular stems, leaves brittle. Stem fracture short and fibrous.

**Microscopic Characteristics:** Stems quadrangular with collenchymas strands at angles and on side, small acicular crystals of calcium oxalate present in pith and cortex. Trichomes 1-3 celled, glandular hair disc shaped and multicellular.

**Study of the Powder Materials:** The green powder was treated with routinely used reagents and characteristic changes were observed and summarized in Table -1.

**Table-1** Behaviour of drug with different reagents

S. No.	Chemical Treatment	Observation
1.	Powder treated with conc. HCl	Powder settles down slowly Colour: Brownish
2.	Powder treated with Conc. HNO <sub>3</sub>	Powder float on the surface. Colour: Yellowish brown
3.	Powder treated with Conc. H <sub>2</sub> SO <sub>4</sub>	Powder settles down slowly. Colour: Brownish black
4.	Powder treated with 5% aqueous NaOH	Powder settles down slowly. Colour: Brownish
5.	Powder treated with 5% aqueous FeCl <sub>3</sub>	Powder settles down immediately. Colour: Greenish
6.	Powder pressed between two filter papers for 24 hours	Stained
7.	Powder treated with iodine solution	Powder settles down immediately. Colour: Brownish
8.	Powder treated with 5% aqueous KOH	Powder settles down slowly. Colour: Dark brown
9.	Powder treated with Glacial Acetic Acid	Powder settles down immediately. Colour: Brownish
10.	Organoleptic Character	Foreign matter – Nil Powder Coarse, odour slight, characteristic Taste – Intensely bitter

**Fluorescence characteristics:** Fluorescence characteristics of the powder as such and after treating with some chemical reagents were observed in day light

as well as in ultraviolet radiation. The results were recorded in Table-2 and Table-3.

**Table-2** Florescence behaviour of different extracts

S. No.	Extractives	Values in %	Ordinary Light	L – UV Light(254nm)	S.U.V. Light(360nm)
1.	Carbon Tetra Chloride	2.62	Green	Light Green	Dark Green
2.	Benzene	3.24	Colourless	Colourless	Colourless
3.	Pet. Ether	2.42	Yellowish	Light Green	Colourless
4.	Chloroform	3.88	Yellowish	Light Green	Colourless
5.	Ethyl Acetate	5.14	Dark Green	Dark Green	Light Brown
6.	Acetone	1.9	Green	Light Green	Umber
7.	Ethyl Alcohol	12.8	Green	Dark Green	Umber
8.	Methyl Alcohol	10.06	Green	Dark Green	Umber
9.	Dist. Water	19.62	Green	Pale Green	Umber

**Table -3** Fluorescence characteristics

S. No.	Chemical Treatment	Ordinary Light	Observation under UV Light 254nm	Observation under UV Light 366m
1.	Powder as such	Green	Green	Silver Green
2.	Powder treated with 1 N NaOH in Methanol	Dull Green	Greenish White	Cream
3.	Powder treated with 1 N NaOH in Water	Yellowish	Green	Dark Greenish White
4.	Powder treated with 50% HNO <sub>3</sub>	Dark Yellow	Green	Greenish Yellow
5.	Powder treated with 50% H <sub>2</sub> SO <sub>4</sub>	Yellowish Brown	Yellowish Green	Greenish White

**Preliminary Phytochemical analysis:** Preliminary phytochemical analysis of *Andrographis paniculata* show various types of chemical compounds which provide the base line for the occurrence of the medicinally active constituents like terpenoids, steroids, tannins, flavanoids, amino acids, glycosides, saponin and alkaloids. The test of extraneous material was also carried out. The results were recorded in Table -4 and 5.

**Table-4** Test for Extraneous Material

Foreign matter	0.1%
Sand & Silica	Absent
Insect infestation	Nil
Rodent Contamination	Nil

**Table -5** Preliminary phytochemical tests

S. No.	Nature Product	Test Performed	Result
1.	Steroid	Liebermann's Reagent	+ ve
2.	Flavonoid	Shinoda Test	+ ve
3.	Tannin	Neutral FeCl <sub>3</sub>	+ ve
4.	Carbohydrate	Molesch Test	+ ve
5.	Starch	Iodine Solution	+ ve
6.	Protein	Million's Solution	- ve
7.	Saponin	NaOH Solution	- ve
8.	Mucilage	Swelling in Water	- ve
9.	Alkaloid	Mayer's Test, Dregandroff's Test, Wanger Test, Hager's Test	+ ve
10.	Amino Acid	Ninhydrine	- ve
11.	Coumarine Glycoside	Alkaline Solution	+ ve
12.	Fat and Oil	Pt. ether ext.	Trace
13.	Diterpenoids	Picric acid (alkaline)	+ ve

**Phytochemical Screening of extracts:** Phytochemical screening of plant extracts was carried out qualitatively

for the presence of terpenoids, steroids, tannins, flavonoids, amino acid, glycosides, saponin and alkaloids. The results were recorded in Table-6

**Table-6** Phytochemical Screening of Different Extractions of *Andrographis peniculata*

S. No.	Constituents	Dichloromethane Extract	Methanolic Extract	Aqueous Extract
1.	Alkaloids	+	+	+
2.	Amino Acids	-	+	+
3.	Flavonoids	+	+	+
4.	Glycosides	-	+	+
5.	Saponins	-	+	+
6.	Steroids	+	+	-
7.	Tannins	-	+	+
8.	Terpenoids	+	+	-

**Microbial Contamination:** The crude drugs were screened for the presence of microbial contamination containing *E.Coli*, *Salmonella*, Total aerobic bacteria and Enterobacteria, as per the method laid down in Indian pharmacopoeia<sup>17</sup> (2010). The results are recorded in Table -7

**Table-7** Microbial bio burden observation

Micro Organism	Observation	Limit (Raw Material)
<i>E. Coli</i>	Absent	10 <sup>1</sup>
<i>Salmonella</i>	Absent	---
Total aerobic bacteria	<2500/gm CFU	10 <sup>5</sup>
Enterobacteria	<200/gm CFU	10 <sup>3</sup>

**Physicochemical Studies:** Various physicochemical constants of the powdered of the were determined and recorded in Table –8

**Table-8** Physico-chemical observations

S. No.	Parameter	Results
1.	Organoleptic Character	
	(a) Appearance	Powder
	(b) Colour	Green
	(c) Smell	Slight, Characteristic
	(d) Taste	Bitter
2.	Loss in Weight on drying at 105° C (%)	8.2%
3.	Alcohol Soluble matter (%)	12.85%
4.	Water Soluble matter (%)	21.85%
5.	pH Value	
	(a) pH of 1% aqueous solution	5.94%
	(b) pH of 10% aqueous solution	5.22%
6.	Ash Value	
	(a) Total ash	12.0%
	(b) Water soluble ash	1.8%
	(c) Acid insoluble ash	0.85%
	(d) Sulphated ash	0.95%
7.	Successive extractives (%)	
	(a) Petroleum ether	10.96%
	(b) Chloroform	2.48%
	(c) Acetone	2.91%
	(d) Absorbed alcohol	12.92%
	(e) Distilled water	12.92%

**Inorganic Elements:** Inorganic elements (Fe, Zn, Cd, K and Ca) have been measured by AAS in plant samples and the results were presented in Table-9.

**Table – 9** Inorganic Analysis

S. No.	Name and part of the Plants	Fe	Zn	Cd	K	Ca
1.	<i>Andrographis peniculata</i> (Aerial parts)	0.46	0.15	Not detected less than 0.005 ppm	1.02	0.82

**TLC Analysis of Different Extractives:** 10 gm powder of crude drug was separately extracted with petroleum

ether, acetone and alcohol, which gave 0.21, 3.2 and 2.14 percentage yield respectively. These extracts were loaded on silica plate (Merck Aluminium sheet – silica

gel 60 F<sup>254</sup>). The best separation was achieved using chloroform : methanol (7:3) as a solvent system. The TLC plate was kept in iodine chamber for one minute and

under UV light (254). The Rf value and colour of the spots under UV light are given in Table-10

**Table-10** TLC Data of *Andrographis paniculata*

S. No.	Part	Solvent System	Spraying Reagent	Spot Colour Under UV	Rf Value (Rf x 100)
1.	Pet ether extract	Chloroform Methanol (7:3)	Iodine Vapour	Yellow	50
2.	Acetone extract	Chloroform Methanol (7:3)	Iodine Vapour	Brown Brown Brown Brown	68 58 42 25
3.	Alcoholic extract	Chloroform Methanol (7:3)	Iodine Vapour	Light Brown Violet Light Brown Light Brown Violet	88 62 45 30 20

**HPLC Analysis:** The peak corresponding to Andrographolide in the sample was confirmed by comparing the spectrum obtained by photodiode

array detector, which was completely in agreement with the standard (Fig-1).

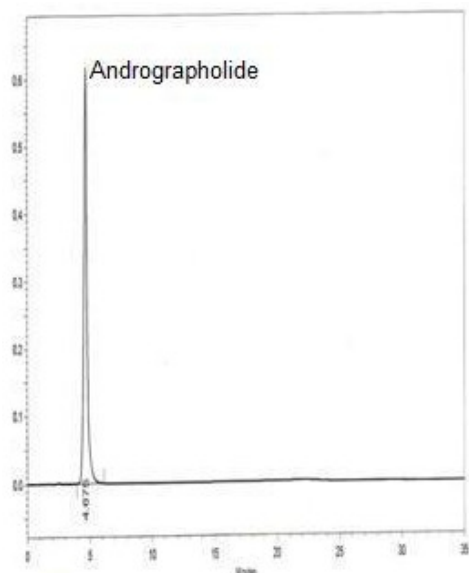


Fig-1A

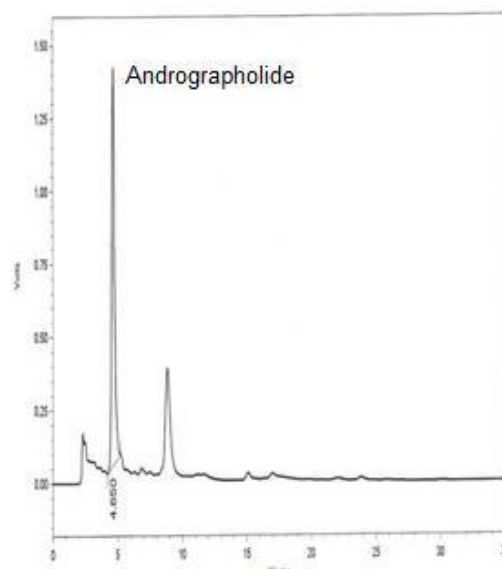


Fig-1B

The observations in the present studies have brought out several diagnostics features of the *Andrographis*

*paniculata*. On the basis of which identification of the crude drug can easily ascertained. The microscopic

characters physicochemical constants, fluorescence behaviour, TLC analysis, HPLC chromatogram not only provide criteria for the correct identification but would also serve as future standard data for quality assessment of the pharmaceutical preparations from the crude drug.

It has been concluded from these studies it is highly essential for raw drugs on plant parts used for the preparation of compound formulation drugs. The periodic assessment is essential for quality assurance and safer use of herbal drugs. Efficacy of any drug depends on the genuine raw material is the main cause for deterioration of the desired therapeutic effect of a particular drug. Additionally, sometimes it may cause deleterious effect on human health. Therefore, before using any crude drug material for manufacturing medicines authenticity and correct identify of the crude material must be ensured.

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