

Pharmacognostical & TLC Fingerprinting of *Andrographis Paniculata* (Kalmegh)

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Abstract

Kalamegha (*Andrographis paniculata*) is a medicinal plant conventionally used for the treatment of anti-inflammatory, antioxidant, antibacterial, antiparasitic, antispasmodic, antidiabetic, anti carcinogenic, Hepatoprotective, antipyretic, anti-diarrhoeal, nematocidal, anti-HIV and numerous infectious diseases ranging from malaria to dysentery. The plant is extensively used in ayurvedic and homeopathic systems of medicines. The drugs are said to be lord of all plants, as it conquers number of diseases. In Samhita and Chikitsa grantha, Kiratatikta and Kalamegha drugs are very frequently indicated in diseases like Daah, Kasa, Jawaragan, Swans, Udar. In the present work an attempt has been made to emphasize the folk herbal medicine which will aid in the identification of fresh as well as dried crude sample of leaves anatomically and Pharmacognostical. The study deals with macroscopic, microscopic, phytochemical analysis and TLC fingerprinting. These studies will provide referentially information for correct identification and for standardization.

Keywords: Kalamegha (*Andrographis paniculata*), Microscopy, Phytochemical investigations & TLC profiling.

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INTRODUCTION

Herbal drugs play a significant role in health care programs particularly in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants to be potential sources of medicinal substances [1]. *Andrographis paniculata* (Kalmegh) is an herbaceous plant in the family Acanthaceae, local to India and Sri Lanka. The plant is famous in northeastern India as Maha-tita means "king of bitters". As an Ayurveda herb it is known as Kalmegh or Kalamegha, meaning "dark cloud". It is also known as Bhui-neem, meaning "neem of the ground", since the plant, though being a small annual herb, has a similar strong bitter taste as that of the large Neem tree (*Azadirachta indica*) [2]. It has been used for past era in Asia to treat GI tract and upper respiratory infections, fever, herpes, sore throat, and a variety of other chronic and infectious diseases. As per Indian Pharmacopoeia it is a chief constituent of at least 26 Ayurvedic formulations [3].



Andrographis paniculata

Various Ayurvedic properties of action and Pharmacological properties of Kalmegh [4, 5]

Ayurvedic properties	Therapeutic Usage	Pharmacological properties
Rasa : Tikta Guna : Laghu,Ruksa Vipaka : Katu Virya : Sheeta Karma : Deepan, Jwaragna, Krimigan. Kashagna, Pachhana.Kalpa- pittahara.	Arsh, Attisar, Jwar, Kandru, Kaamla, Kustha, Parmeha, Varn, Yakritvikar, Twakvikara.	Antiinflammatory,Antioxidant, Antidiabetic,Antileshmanial, Anti-diahoel and intestinal effects, Antivenom activity,Anti-HIV assay & Antifertility activity.
Important Formulation:- Kalameghaashav		

MATERIALS AND METHODS**Collection**

The sample of Kalamegha was collected from Haridwar, State – Utrakhnad. The sample of Kalmegh was collected by scholar under the guidance of Supervisor after identifying the source of plant as per standard description.

Date of Collection

Andographis panniculata-10/08/2017
(Haridwar).

Authentication

The plant was authentication was done Botanical Survey of India (BSI), Dehradun. Acc.No: 118083



Specimen of *Andographis panniculata*

Microscopic Study

Microscopic study of crude drugs is another aid of Pharmacognosy which can be helpful in the process of standardization of medicinal plants. This

study can be helpful in identifying genuine drug by their known histological characters through Transverse section (T.S.) or Longitudinal Section (L.S.) or Radial Longitudinal Section (R.L.S.) or Tangential Longitudinal Section (T.L.S.) and Powder microscopy which can help in evaluation of different constituents by using different staining reagents. Specimens were soaked in water or other solvents depending upon the hardness of the sample and transverse sections were taken using sharp razor blades. Few microscopic sections were cut by Microtome sectioning. Numerous temporary and permanent mounts of the microscopical sections of the specimen were made and examined microscopically. Different staining reagents were applied on transverse sections so as to differentiate between different cell wall components.

PRELIMINARY PHYSIOCHEMICAL SCREENING OF PLANT EXTRACTS [6-17]**Determination of Solvent Extractive Values****Determination of Water Soluble Extractive Value**

5 g of the air-dried drug, coarsely powdered were macerated with 100 ml of water in closed flask for 24 hours, shaking frequently during the first 6 hours and allow standing for 18 hours. It was filtered rapidly taking precaution against loss of water, then the filtrate was evaporated 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, and dried at 105⁰C then weighed. The percentage of water-soluble extractive with reference to the air dried was calculated.

Determination of Alcohol Soluble Extractive Value

5 gm of the air dried and coarsely powdered drug was macerated with 100 ml of ethanol of the specific strength in a closed flask for 24 hours, shaking frequently during the first 6 hours and allow standing for 18 hours. There after filter rapidly taking precaution against loss of ethanol. Evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, dry at 105⁰c and weigh. The percentage of ethanol soluble extractive with reference to the air dried drug has to be calculated.

Determination of Moisture Content

Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Method of determination of moisture content include

the loss on drying, the test for loss on drying determines both water and volatile matter in the crude drug. It can be carried out either by heating at 100°C-105°C or in a desiccator over phosphorous pentoxide under atmospheric or reduced pressure at room temperature for specific period of time.

Ash Value

Ash value is helpful in determining the quality and purity of a crude drug, especially in the powdered form. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reflects the care taken in its preparation. A higher limit of acid-insoluble ash is imposed, especially in case where silica may be present or when the calcium oxalate content of the drug is very high.

Total ash value

$$\% \text{ Water soluble ash value} = \frac{\text{Wt. of total ash} - \text{Wt. of water insoluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

Acid Insoluble Ash Value

Boiled the ash for 5 min with 25 ml of 2 M HCL. Filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding 450 °C for 4 h. cooled in a desiccator and weighed. Calculated the percentage of acid insoluble ash with reference to the air -dried drug was calculated by using following formula,

$$\% \text{ Acid insoluble ash value} = \frac{\text{Wt. of acid insoluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

Preliminary Qualitative Test

The various extract of *Andrographis paniculata* was subjected to preliminary qualitative phytochemical investigation. The various tests and reagent used are given below.

ALKALOIDS

Preparation of test solution: The test solution was prepared by dissolving extracts in the dilute hydrochloric acid.

Mayer' test: The acidic test solution with Mayer's reagent (Potassium Mercuric iodide) gave cream colored precipitate.

Hager's test: The acidic test solution with Hager's reagent (Saturated picric acid solution) gave yellow precipitate.

Weighed accurately about 2 to 3 g of the powdered drug in a tared silica crucible. Incinerated at a temperature not exceeding 450 °C for 4 hr, until free from carbon, cooled and weighed. The percentage of ash with reference to air-dried was calculated following formula.

$$\% \text{ Total ash value} = \frac{\text{Wt. of total ash}}{\text{Wt. of crude drugs}} \times 100$$

Water Soluble Ash Value

Boiled the ash with 25 ml of water. Filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding 450 °C for 4 hr. Cooled in a desiccator and weighed. Subtract the weight of insoluble matter from the total weight of ash. The difference in weight represented weight of water soluble ash. Calculated the percentage of water soluble ash with reference to the air- dried drug by using the following formula.

Dragendorff's test: The acidic solution with Dragendorff's reagent (Potassium bismuth iodide) showed reddish brown precipitate.

Wagner's test: The acidic test solution treated with Wagner's reagent (Iodine in potassium iodide) gave brown precipitate.

Tannic acid test: The acidic test solution treated with Tannic acid gave buff colour precipitate.

Picrolonic acid test: Alkaloids gave yellow colour precipitate with picrolonic acid.

AMINO ACID

Millon'test: To the test solution add about 2 ml of millon's reagent white precipitate indicates presence of amino acid.

Ninhydrine test: To the test solution add Ninhydrine solution, boil, violet colour indicates presence of amino acid.

CARBOHYDRATES

Preparation of test solution: The test solution was prepared by dissolving the test extracts with water. Then it was hydrolyzed with 1 volume of 1 N-HCL and subjected to following chemical test.

Molisch's test: Test solution with few drops of Molisch's reagent and 2 ml of conc.H₂SO₄ added slowly from the sides of the test tubes. It showed a purple ring at the junction of two liquids.

Barfoed's test: 1 ml of test solution is heated with 1 ml of Barfoed's reagent on water bath, if red cupric oxide is formed, monosaccharide is present. Disaccharides on prolonged heating (about 10 min.) may also cause reduction, owing to partial hydrolysis to monosaccharide.

Benedict's test: Test solution treated with Benedict's reagent and after boiling on water bath, it showed reddish brown precipitate.

Fehling's test: The test solution when heated with equal volume of Fehling's A and B solution, gave orange red precipitate, indicating the presence of reducing sugars

Flavonoids: The flavonoids are all structurally derived from the parent substance called flavones. The flavonoids occur in the free form as well as bound to sugars as glycosides. For this reason, when analyzing flavonoids it is usually better to examine the flavonoids in hydrolyzed plant extracts.

Preparation of test solution: To a small amount of extract added equal volume of 2 M HCL and heated in a test tube for 30 to 40 min at 100°C. The cooled extract was filtered, and extracted with ethyl acetate. The ethyl acetate was concentrated to dryness, and used to test for flavonoids.

Shinoda test: Test solution with few fragments of magnesium ribbon and conc. HCL showed pink to magenta red colour. To a small quantity of test solution when lead acetate solution was added, it formed yellow colored precipitate.

Alkaline reagent test: Test solution when treated with sodium hydroxide solution showed increase in the intensity of yellow colour, which becomes colorless on addition of few drops of dilute acid.

GLYCOSIDES

Preparation of test solution: The test solution was prepared by dissolving extract in the alcohol or hydro-alcoholic solution.

TEST FOR CARDIAC GLYCOSIDES

Kedde' test: Add one drop of 90% alcohol and 2 drops of 2% 3, 5-dinitro benzoic acid in 90% alcohol. Make alkaline with 20% sodium hydroxide solution, purple colour is produced. The colour reaction with 3, 5-dinitro benzoic acid depends on the presence of α , β -unsaturated lactones in the aglycone.

Baljet's test: The test solution treated with sodium picrate gave yellow to orange colour.

Raymond's test: Test solution treated with hot methanolic alkali, violet colour is produced.

Bromine water test: Test solution dissolve in bromine water give yellow precipitate.

Keller-killani test for digitoxose: The test solution treated with few drops of FeCl₃ solution and mixed, then H₂SO₄ containing FeCl₃ solution was added, it formed two layers. Lower layer reddish brown, upper layer turns bluish green.

Legal's test: Test solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gave pink to red colour.

Test for Anthraquinone glycosides

Borntrager's Test

Boiled powdered drug with 5 ml of 10% sulphuric acid for five minutes. Filtered while hot, cooled the filtrate shaken gently with equal volume of benzene. Benzene layer was separated and then treated with half of its volume solution ammonia (10%). Allowed to separate it. The ammoniacal layer acquired rose pink colour due to presence of anthraquinones.

Proteins

Preparation of Test Solution: The test solution was prepared by dissolving the extract in water.

Millon's Test: Test solution was treated with millon's reagent and heated on a water bath. The proteins were stained red

Biuret test: Test solution was treated with 40% sodium hydroxide and dilute copper sulphate solution gave blue colour.

Xanthoproteic test: Test solution was treated with conc. HNO₃ and boiled which gave yellow precipitate.

Modified Borntrager's test: C-glycosides of anthraquinones require more drastic conditions for hydrolysis. Hydrolysis of the drug was carried out with 5 ml of dilute HCL and 5 ml of 5% solution of FeCl₃. For hydrolyzed extract procedure was carried out as described under Borntrager's test.

TEST FOR STEROIDS

Preparation of test extract solution: The extract was refluxed separately with alcoholic solution of potassium hydroxide till complete saponification. The saponified extract was diluted with water and unsaponifiable matter was extracted with diethyl ether. The ethereal extract was evaporated and the residue (saponifiable matter) was subjected to the following test by dissolving the residue in the chloroform.

Salkowski test: To the test extract solution add few drops of conc. H₂SO₄ shaken and allowed to stand, lower layer turned red indicating the presence of steroids.

Libermann - Burchard test: The test solution treated with few drops of acetic anhydride and mixed, when conc. H₂SO₄ was added from the sides of the test tubes, it showed a brown ring at the junction of the two layers and the upper layers turned green. Added few drops of concentrated H₂SO₄. Blue colour appeared.

Sulphur test: Sulphur test when added in to the test solution, it sank it.

Tannins and phenol compound: To 2-3 ml of alcoholic or aqueous extract, added few drops of following reagents.

5% FeCl₃ solution: Deep blue- black colour.

Lead acetate solution: White precipitate.

Bromine water: Discoloration of bromine water

Acetic acid solution: Red colour solution.

Dilute iodine solution: Transient red colour. One drop of NH₄OH, excess 10% AGNO₃ solution. Heated for 20 min in boiling water bath. White precipitate was observed, then dark silver mirror deposited on wall of test tube.

TRITERPENOIDS

Preparation of test extract solution: The test extract solution was prepared by dissolving extract in the chloroform.

Salkowski test: Few drops of concentrated sulphuric acid were added to the test solution, shaken and on standing lower layer turned golden yellow.

TLC analysis for different Phytochemicals

T.L.C. plate coated with 0.25 mm layer of silica gel GF 254 with fluorescent indicator, (Mercks) were used. (Each plate dimension is 10 cm long and 2 cm width).

Activation of pre-coated Silica gel G60F₂₅₄
Dry in hot oven at 105⁰ C for one to two hour.

Sample: Alcoholic Extract of test sample.

Solvent system: Toluene, Ethyl Acetate: Formic acid [[5:4:1]

Visualization: Iodine Vapor & UV Short Wave Length.

RESULT AND DISCUSSION

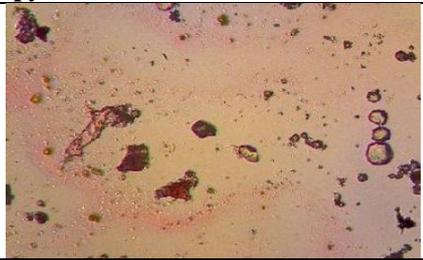
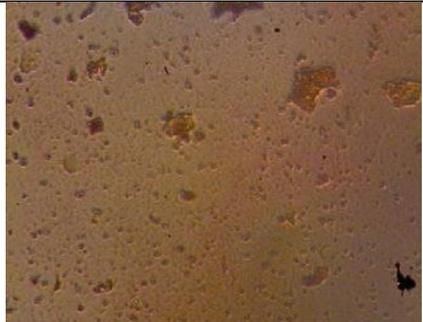
Kalamegha is one of the ingredients of many important formulations like kwath, Varunadi Ghrit, Satviryadi Kwath, and many others, following observations were found. In Charaka Samhita, properties of Kiratatikta and Kalamegha drugs have been mentioned as best Jwarchikitsa, Stanyajanan, Kusht chikitsa (C.S.Su. 07/146) signifying the usage of drug in any sort of disorders because of having skin disorder pacifying activity. The collected drug were studied organoleptically with naked eye & magnifying lens, with the help of different Organoleptic features i.e. Colour, Odour, Taste, and Appearance were recorded (Table-1). The microscopic characteristics study revealed the presence of parenchymatic cells, lignified fibres, nitrogenous compound, starch, scleroids & trichome (Table-2). Various physiochemical parameters were studied and result was tabulated in the Table-3. The extractive value was found to be 10.76% (Alcohol Extractive Value) and 13.55% (Aqueous Extractive Value).The phytochemical screening (Table-4) revealed the presence of the carbohydrate, alkaloid, glycoside, saponin, flavonoids, tannins, Aminoacid & protein. TLC fingerprinting showed 5 spots having R_f values 0.10, 0.20, 0.42, 0.78 and 0.92 at 366 nm. At 254 nm the sample showed 4 spots having R_f values 0.19, 0.41, 0.74 and 0.92 and at Visible Light: showed 3 spots having R_f values 0.20, 0.51 and 0.87. TLC fingerprinting revealed the presence of phytoceuticals showed the therapeutic utility of the plant (Table-5).

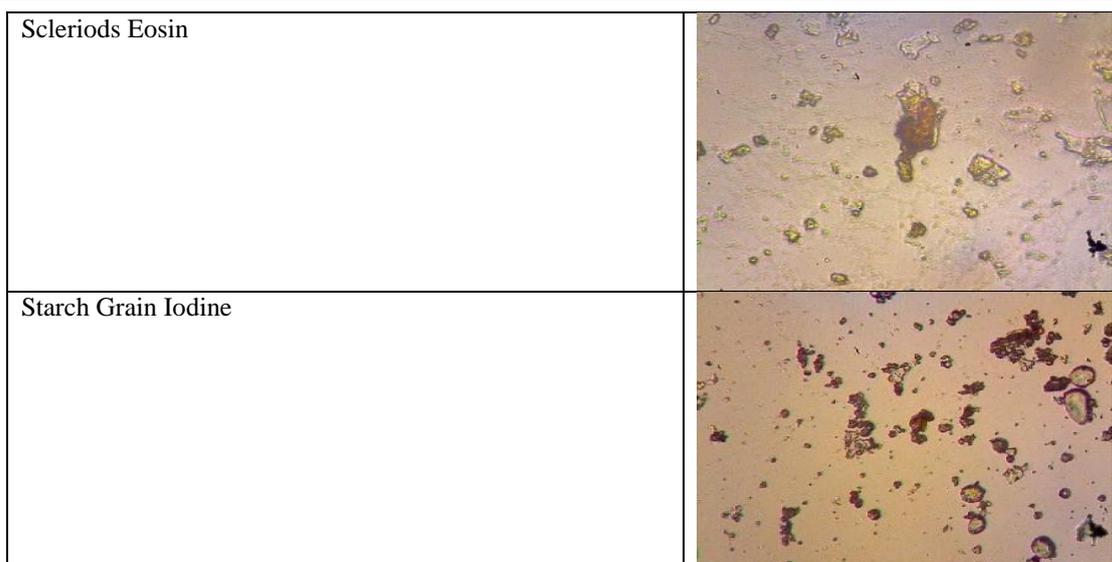
Table-1: Macroscopy of Kalmegh

Appearance	<i>Andrographis paniculata</i>	Organoleptic characters
General appearance and stem	Acutely 4 angled, nodose, jointed and nearly glabrous, branches cross armed, 4 sided, spreading or horizontal.	

leaves	Simple, opposite, exstipulate, short petioled scarcely 6mm long, blade entire, slightly undulate, glabrous, membranous, lanceolate, narrowed at both ends, the base attenuate to the petiole, dark green and glabrous above, paler and slightly glaucous or finely granular beneath from 2.5-7.5 cm long and 12-18 mm wide with 4-6 pairs of slender lateral nerves.	
Inflorescence.	Terminal as well as axillary, horizontal, 4 sided, elongate, sub paniced, racemes 2.5 to 10 cm long bear only one flower at each node.	
Flower	Small, bracteate, pedicillate, all turned to one side, distantly placed one only at one node, zygomorphic	

Table-2: Powder Microscopy

Parenchymatic cells Lignified Fibres Safranin reagent	
Mucilaginous cells & Trichome Methylene blue	
Nitrogenous compound or amorphous mass of protein Eosin	

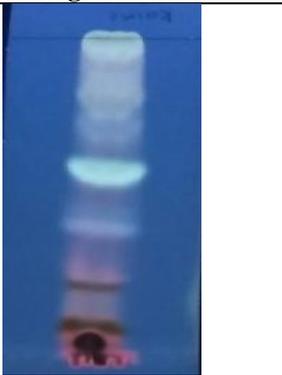
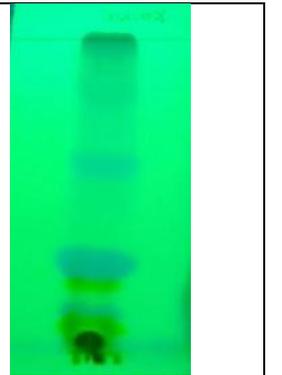
**Table-3: Physiochemical study of Parameters**

S. No	Tests	<i>Andographis paniculata</i> (S1)
1	Moisture content	8.70% w/w
2	pH	7.97
3	Alcohol Extractive Value	10.76% w/w
4	Aqueous Extractive Value	13.55% w/w
5	Foreign matter	1.34% w/w
6	Total Ash	8.62% w/w
7	Acid Insoluble Ash	1.72% w/w
8	Water Soluble Ash	3.42% w/w

Table-4: Results of preliminary phytochemical analysis

Sr. No.	Name of the Test	Observation		
		Aqueous Extract	Alcoholic Extract	Pet.ether Extract
1	Tests for sterols			
	1. Salkowski's Test	+	+	+
	2. Libermann Burchard's Test	+	+	+
2	Test for glycosides			
	1. Baljet's Test	+	-	-
	2. Brontrager Test	+	-	-
3	Tests for saponins			
	1. Foam Test	+	+	-
4	Test for carbohydrates			
	1. Molish's Test	+	-	-
	2. Barfoed's Test	+	-	-
	3. Benedict's Test	+	-	-
5	Tests for alkaloids			
	1. Mayer's Test.	+	+	-
	2. Wagner's Test.	+	+	-
	3. Dragendorff's Test	+	+	-
6	Tests for flavonoids			
	1. Ferric chloride Test.	+	+	+
	2. Shinoda Test.	+	+	+
	3. Alkaline Reagent Test.	+	+	+
	4. Lead Acetate Test.	+	+	+
7	Tests for tannins			
	1. Ferric chloride Test.	-	+	-
	2. Gelatin Test	-	+	-
8	Test for amino acid and protein			
	1. Biurete test	+	-	-

Table-5: TLC Fingerprinting

Solvent system: Toluene, Ethyl Acetate: Formic acid [5:4.5:0.5]			
	TLC IN DAY LIGHT	TLC at 366 nm	TLC at 254 nm
R_f value	0.20, 0.51 and 0.87.	0.10, 0.20, 0.42, 0.78 and 0.92 at 366 nm	0.19, 0.41, 0.74 and 0.92

CONCLUSION

The present study deals with macroscopic, microscopic, phytochemical analysis and TLC fingerprinting. These studied will provide referentially information for correct identification and for standardization. This study also aid in the assessing of adulteration and substitution in market sample of the plant.

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