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ISOLATION OF ANDROGRAPHOLIDES FROM ANDROGRAPHIS PANICULATA: A REVIEW OF ITS THERAPEUTIC BENEFITS, CHEMICAL PROPERTIES, AND PHARMACOLOGICAL EVALUATION

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ABSTRACT:

Andrographis paniculata, a widely used medicinal herb, has been employed for centuries to treat various ailments. This review aims to summarize the literature on A. paniculata, focusing on its therapeutic benefits, chemical properties, pharmacological evaluation, and toxicity. A TLC method has been developed for the determination of Andrographolide in A. paniculata extract.

INTRODUCTION:

Andrographis paniculata, commonly known as the "King of Bitters," is an annual herb used in traditional medicine for its diverse pharmacological activities. This review highlights its medicinal uses, morphology, and scientific classification.

Morphology of Andrographis Paniculata:

A. paniculata is an erect herb with glabrous leaves and white flowers, growing up to 110 cm in height. Its dark green stem, quadrangular in shape, has longitudinal furrows and wings on the angles of the younger parts.

Scientific Classification:

Kingdom: Plantae, Division: Angiospermae, Class: Dicotyledoneae, Order: Tubiflorae, Family: Acanthaceae, Genus: Andrographis, Species: paniculata Nees.

Medicinal Uses:

A. paniculata exhibits a broad range of pharmacological activities, including anti-inflammatory, antidiarrheal, antiviral, antimalarial, hepatoprotective, cardiovascular, anticancer, and immunostimulatory activities.

Pharmacological Evaluation:

Recent research has revealed A. paniculata's potential in treating various diseases, including fever, pain reduction, intestinal disorders, bacterial dysentery, diarrhea, and snake venom.

Toxicity:

Male reproductive toxicity and cytotoxicity of the plant have been reported.

Methodology:

1. COLLECTION OF RAW MATERIAL

The modified leaves of the Kalamegh, were collected fromsonarwada,Karwar in the month of April 2021. The taxon's identity was confirmed as Bougainvillea glabra by Mr. Shivanandh Bhat, a lecturer in the Department of Botany at Government First Grade College and P.G. Centre, Karwar.

2. PHYSICO-CHEMICAL ANALYSIS

Physicochemical values such as the percentage of ash values and extractive values were performed according to official methods prescribed in Indian Pharmacopeia 1996 and WHO guidelines on quality control methods for medicinal plant material.

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Proximate Analysis:

The physico-chemical parameters are helpful in judging the purity and quality of the drug. The percentage of active principles in the plant is determined only in the dry condition. Hence, the moisture lost percentage is very important to decide about the condition of the crude drug. The moisture should be kept minimum to prevent the drug from various kinds of decomposition. The total ash and the acid insoluble ash indicate the presence of any foreign matter, inorganic composition and purity of the drug. Their low value in the bark powder showed that the sample is free of any foreign matter.

Extractive Values

1. Determination of Alcohol Soluble Extractive Value: -

10 gm. of the air-dried coarse powder of A. Paniculata were macerated with 100 ml of 90% ethanol in a closed flask for 24 hours shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air-dried drugs.

2. Determination of Water Soluble Extractive Value: -

Coarsely powdered drug (10 gm) was weighed accurately and macerated with 100 ml of water in a closed flask for 24 hours. It was shaken frequently during the first 6 hours and allowed to stand. After 18 hours it was filtered rapidly. Then 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105°C and weighed. The percentage of water-soluble extractive value was calculated with reference to the air-dried drug.

Ash Values:

Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form. It usually represents the inorganic salts naturally occurring in the drug and adhering to it. Hence, an ash determination furnishes a basis for judging the identity and cleanliness of a drug. Procedure given in Indian Pharmacopoeia was used to determine the different ash values such as total ash, acid insoluble ash, and water-soluble ash etc.

DETERMINATION OF ACID VALUE

The acid value is the number of mg of potassium hydroxide required to neutralize the free acids in 1 g of the substance, when determined by the following method:

About 10 g of the substance is weighed (1 to 5) in the case of a resin into a 250 ml flask and 50ml of a mixture of equal volumes of alcohol and solvent ether is added, which has been neutralized after the addition of 1 mlof solution of phenolphthalein. Heated gently on a water-bath, if necessary until the substance has completely melted, titrated with 0.1 N potassium hydroxide, shaking constantly until a pink colour which persists for fifteen seconds isobtained. Noted the number of ml required. The acid value is calculated from the following formula:

$$\text{Acid Value} = \frac{a \times 0.00561 \times 1000}{W}$$

Where ‘a’ is the number of ml of 0.1 N potassium hydroxide required and ‘W’ is the weight in g of the substance taken.

3. Determination of total ash value: -

Exact 3 grams of air dried powdered drug was taken in a tared silica crucible. It was incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air-dried drug.

$$\% \text{ Total ash} = \frac{\text{wt of total ash}}{\text{wt of crude drug}} \times 100$$

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4. Determination of acid insoluble ash value: -

The ash obtained as directed under total ash value was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then the percentage of acid insoluble ash was calculated with reference to the air-dried drug.

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

5. Determination of water soluble ash value: -

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited and weighed, then the percentage of acid soluble ash was calculated with reference to the air-dried drug.

$$\text{Ash \%} = \frac{\text{Loss in weight}}{\text{W}} \times 100$$

6. Loss on drying: -

About 5g of drug accurately weighed in different petri dishes and kept in a hot air oven at 100^0 C for four hours. After cooling in a desiccator, the losses in weight are recorded in each case. This procedure is repeated till constant weight is obtained.

Loss of drying (%) =Loss in weight x 100/wW=Weight of drug in gm.

PHYSICO CHEMICAL PARAMETERS:

PARAMETERS	RESULT (% w/w)
Moisture content	7.2%
Total Ash Value	10.2%
Water soluble Ash	1.7%
Acid-insoluble Ash	1.81%
Water soluble Extractive value	12.24%
Alcohol soluble Extractive value	22.8%

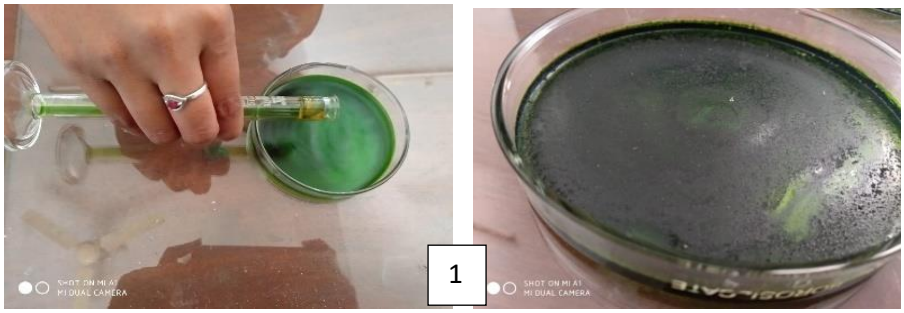
Determination of Extractive values

These are useful for the evaluation of a crude drug. Gives an idea about the nature of the chemical constituents present in the crude drug. Useful for the estimation of constituents extracted with the solvent used for extraction. Employed for material for which as yet no suitable chemical or biological assay exists.

1. Preparations of the extracts

Cold Maceration

All the solvents and reagents used during study were A.R. grade. Macerate 5g of the powdered crude drug of root of curcuma longawith each of 100ml of water, alcohol, chloroform, ethyl acetate, water separately in a closed flask for 24 hours, shaking frequently during first 6 hours and allowing to stand for 18 hours. Filter rapidly taking precaution against loss of alcohol, evaporate 25ml of filtrate to dryness in an evaporating dish to avoid the decomposition of natural metabolites, dry at 105 ^{o}C, and weigh. Calculate the percentage of water, alcohol, chloroform and petroleum ether soluble extractive value with reference to the air-dried drug.



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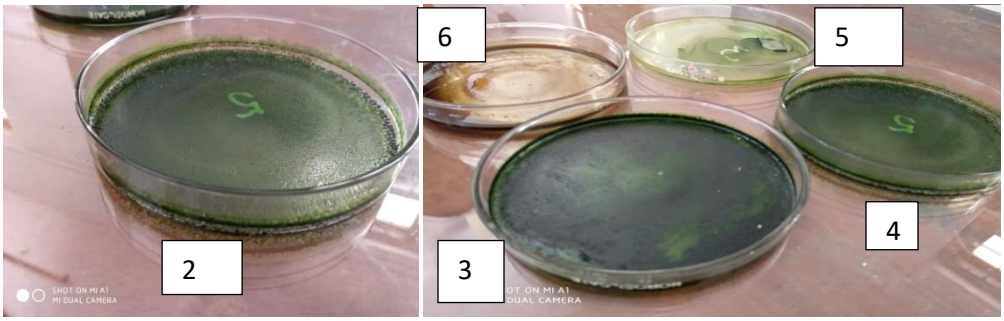

Extractive values of different solvents:

Sr. No.	Solvents for Extraction	Weight of Andrographis leaves (g)	Weight of Extract, (g)	% yield
1.	*n*-hexane	5.01	0.013	0.26
2.	Chloroform	4.8	0.223	4.64
3.	Ethyl acetate	4.98	0.08	1.6
4.	Methanol	5	0.358	7.16
5.	Water	4.79	0.105	2.1

Importance of Extractive values: -

Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The extractive value of the crude drug determines the quality as well as purity of the drug. Thus, alcohol and water soluble extractive values were determined.

Phytochemical Screening

Chemical tests were carried out qualitatively on each extract following standard procedures to identify the phytochemical constituents

Preparation of Extracts of Plant Material

Plant extracts of root powder were prepared using ethanol as extracting solvent. 100g of the dried and powdered plant material (rhizome) was extracted with 50 ml of ethanol at 65°C using Soxhlet extraction method. After filtering and evaporating to dryness, the crude ethanolic extract was obtained.

A**. Test for alkaloids**

- **Dragendroff's test**: In a test tube containing 1 ml of extract, few drops of Dragendroff's reagent was added and the color developed was noticed. Appearance of orange color indicated the presence of alkaloids.
- **Mayer's test:** To 1 ml of the extract, 2 ml of Mayer's reagent was added, a dull white precipitate indicated the presence of alkaloids.
- **Wagner's test:** To 1 ml of the extract, 2 ml of Wagner's reagent was added. Appearance of a reddish-brown precipitate indicated the presence of alkaloids.
- **Hager's test:** Extracts were dissolved individually in dilute hydrochloric acid and filtered. Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of yellow coloured precipitate.

B. Test for flavonoids

- **Alkaline reagent test:** To the test solution, a few drops of sodium hydroxide solution were added. Formation of intense yellow color which turns to colorless by addition of few drops of dilute acetic acid indicated the presence of flavonoids.
- **Lead acetate test:** To the test solution, a few drops of lead acetate solution were added. Formation of yellow precipitate indicated the presence of flavonoids.

C. **Test for phenolic compounds**

- **Lead acetate test:** To the test solution, a few drops of 10% lead acetate solution were added. Formation of white precipitate indicated the presence of phenolic compounds.
- **Ferric chloride test:** To the test solution, a few drops of ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

D. Test for terpenoids

Salkowski's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken well and allowed to stand. Appearance of red color in the lower layer indicated the presence of steroids. Formation of reddish brown color of interface after.

E. Test for carbohydrates: The extract was dissolved in 10ml of distilled water and filtered through Whatman filter no. 41 then filter it. The filtrate is used for carbohydrate test,

- **Molish test** :2ml of solution in a test tube+ 1 drop of molish reagent + 2ml of conc. HCl from the sides of test tube, it forms the violet ring at the junction of two liquids indicates the presence of carbohydrates.

F. **Test for Saponins:** About 0.2g of extract was shaken with 5ml of distilled water then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

G. **Test for proteins:** Extract + 10ml of distilled water and filtered through Whatman filter no. 41 and filtrate is subjected to test for proteins and amino acids,

- **Millons test:** 2ml of filtrate + few drops of millons reagent are added. The white ppt indicates the presence of proteins.
- **Biuret test:** an aliquot of 2ml filtrate was treated with drop of 2% copper sulphate solution + 1ml of ethanol followed by excess of KOH pallets. The pink colour in ethanol layer indicates presence of proteins.

H. Test for Glycosides:

- **Borntagr's test:** Extract was boiled with dil. Sulfuric acid, filtrate it. Filtrate chloroform is shaken well. Organic layer was separated to which ammonia is added slowly. Presence of glycoside is denoted by pink to red colour in the ammoniacal layer.
- **Legal test:** the test is employed for digitoxin contains glycoside. The extract was dissolved in pyridine, sodium nitroprusside solution was added to it and made alkaline. Pink to red colour indicates presence of glycoside.

SECONDARY METABOLITES	TESTS	RESULT
Alkaloids:	Dragendorff's test	-
Carbohydrates	Molish test	+
Proteins	Millons test	+
Glycosides	Borntagr's test	+
Terpenoids	Lieberman test	+
Flavonoids:	Alkaline test	-
Tannins	Ferric chloride test	+
Saponins	Frothing test	-
Phenols	Lead acetate test	_

Preparation of Andrographis Paniculata Extract

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Kalmegh Dry Extract is obtained by extracting dried Kalmegh leaves withMethanolor ethanol solvent and reflux at 60^0 C for 3 hrs.

Kalmegh Dry Extract contains not less than 90.0 per cent w/wand not more than 120.0 per cent w/w of the labelled amountof andrographolides (sum of andrographolide, neoandrographolideand andrograpanin)

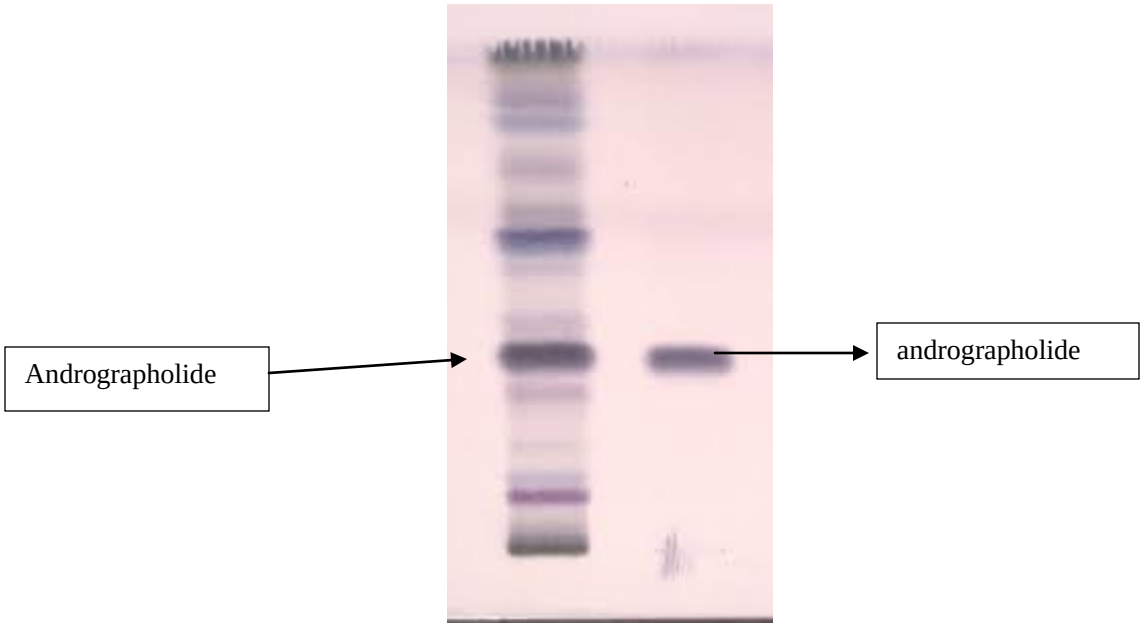
EVALUATION OF ISOLATED ANDROGRAPHOLIDES BY THIN LAYER CHROMATOGRAPHY

Stationary phase: silica gel GF254.

Mobile phase. A mixture of 9 volumes of chloroform and1 volume of methanol.

Test solution. Dissolve 200 mg of the extract under examinationwith 50 ml methanol and filter**.**

Reference solution. A 0.02 per cent w/v solution ofandrographolide RS in methanol. Apply to the plate 10 µl of each solution as bands 10 mm by2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in airand examine in ultraviolet light at 254 nm and 365 nm and alsounder day light. Spray the plate with methanolic sulphuricacid (20 per cent) and heat at 120º for 10 minutes. Thechromatogram obtained with test solution shows a band corresponding to the band obtained using reference solutionindicating the presence of andrographolide



Thin Layer Chromatography of Kalmegh methanol extract

RESULT

The results of preliminary phytochemical evaluation and Study of physico-chemical properties of the Andrographispaniculata will serve as the pioneering project to assess this medicinally important native plant. The results indicated presence of carbohydrates, Proteins andAmino-acids, Alkaloids, Terpenoids, Phenols and Flavonoids. leaf extracts were prepared using different solvents such as water and Methanol as the polar solvents and hexane and chloroform as the nonpolar solvent. The methanolic leaf extract showed the highest andrographolide and other terpenoids content by TLC. Rf value intense spotobtained with test solution was matching with Rf value of the standard Andrographolides. Two more intense spots were observed with testsolution. These spots may be due to the presence of other terpenoidsin the isolated Andrographolides. The chemical structure of Andrographolide is gives idea about various functional groups present the compound.

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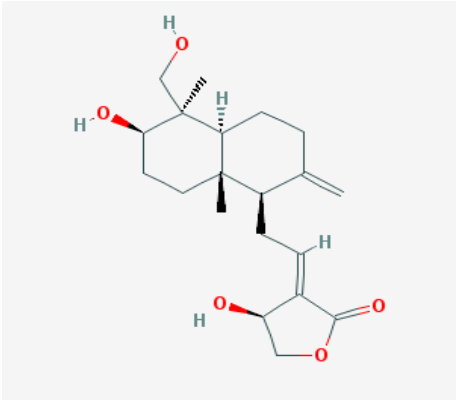

Structure of Andrographolide

PHYTOCHEMISTRY OF ANDROGRAPHIS PANICULATA:

The phytochemical analysis of Andrographis paniculata reveals a diverse range of bioactive compounds, including:

1. Diterpenes (e.g., andrographolide, neoandrographolide)
2. Flavonoids (e.g., 5-hydroxy-7, 8, 2', 3'- tetramethoxyflavone)
3. Polyphenols
4. Steroids (e.g., β-sitosterol, stigmasterol)
5. Phenylpropanoids (e.g., trans-cinnamic acid)

These compounds contribute to the plant's pharmacological benefits, which include:

1. Antimicrobial activity
2. Anti-inflammatory activity
3. Antioxidant activity
4. Anticancer activity
5. Cardiovascular protection
6. Hepatoprotection
7. Antiviral activity (including anti-HIV)
8. Immunomodulatory effects

Andrographolide, a major bioactive compound, has been extensively studied for its therapeutic potential. It exhibits:

1. Antitumor activity
2. Anti-HIV activity
3. Cardioprotective effects
4. Hepatoprotective effects

The plant's extracts and isolated compounds have been investigated for their potential in treating various diseases, including cancer, HIV, and cardiovascular disorders. However, further research is needed to fully understand the mechanisms of action and potential interactions.

Extraction of andrographolids

Extraction of andrographolide from *Andrographis paniculata:*

Raw material:

Part used: Andrographis Paniculata leaf powder

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Apparatus and equipments used:

Beakers, separating funnel, conical flask, glass rod, muslin cloth, funnel and filtration flask. Weighing machine, Stirrer, Rotary evaporator etc.

Chemicals used:

Methanol, hexane, D.M water, Chloroform

Methanol extraction:

PROCEDURE:

STEP 1: 500g of powdered raw material (*Andrographis paniculata*) is taken in a beaker. To these 1.5 liters of methanol is added then kept it on water bath under stirring at 55ºc- 60ºc.

STEP 2: Filter the extract and again add 1.5 liter of Methanol and repeat the same procedure on Three more time.

STEP 3: Combine and pass the methanol extract through celite bed. The obtained filtrate is concentrated to about 100 to 200ml. by using Soxhlet Distillation method. This process is called distillation.

STEP 4: The Methanol is evaporated and we get the concentrated Methanol extract.

STEP 5: To this Methanol extract equal vol. of Hexane is added and it is then taken in a separating funnel and 4 times Hexane wash is given to it.

STEP 6: The Methanol layer (lower layer) is separated and Hexane layer (upper layer) is discarded.

STEP 7: To the Methanol layer equal volume of D.M water is added and 4 times chloroform wash is given to it.

STEP 8: Then chloroform layer (lower layer) is separated and Charcolisation is carried out.

STEP 9: Then again, the above extract undergoes Hi-flow filtration and the filtrate is concentrated to about 30ml using Rotary evaporator.

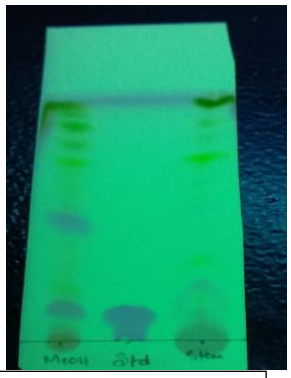
STEP 10: The concentrated extract is cooled in a refrigerator to get crystals of Andrographolide.

STEP 11: Again, this extract is filtered and Methanol wash is given to the crystals.

STEP 12: After 1 hr. dried the crystals in an Vacuum tray drier. Weighed and yield is noted down.

RESUL

Sample



TLC of sample and standard

Yield: 7.3g

Percentage yield: 3.46%

Color of the compound: Pale green

In TLC: Shows impurities

PURIFICATION OF ANDROGRAPHOLIDES :

There are two methods of purification

1. Crystallization method.
2. Column purification

1. Purification of Andrographolide by crystallization method:

Procedure:

STEP 1: 4g of extract is taken in a beaker and dissolved in minimum amount of Methanol
STEP 2: 1g of charcoal is added to that solution and kept in a water bath stirred for half an hour maintained at 45^{o}c
STEP 3: Then it undergoes hi- flow filtration.
STEP 4: Filtrate is concentrated in a Rotary evaporator.
STEP 5: Then the concentrated solution is allowed for crystallization in a refrigerator.
STEP 6: Crystals are filtered and washed with cold Methanol
STEP 7: Crystals are dried and yield is noted down.

OBSERVATION

Yield: 2.7 g

Percentage yield: 67.5%

Colour of the crystals: white

TLC: Single Spot

2. COLUMN CHROMATOGRAPHY

Column purification of andrographolide

- Glass Column was packed with slurry of silica gel (mesh size, 60-120) with chloroform.
- Sample (4 gm) of A. paniculata was first dissolved in Methanol and carefully applied by pipette at the top of prepared column. Immediately after application of sample, run with chloroform upto 1 to 20 fractions
- Run with a gradient of Chloroform: Methanol (98:2, 95:5, 90:10, 80:20, 70:30) finally Methanol and 50 fractions (F1-F12) were collected. 70:30 shows pure andrographolide on TLC
- Thereafter, (from 70: 30) collected fractions solvent was removed by evaporation at room temperature.

- After evaporation of solvent from the fractions F30 to F 40, colourless crystals were isolated. The crystals of fractions were concentrated at 60^0 C. obtained concentrated fraction kept in freezer at 4 to 6^0 C. The identity of crystalswas confirmed by spectroscopic analysis.

Identification of isolated crystals

The isolated constituent of A. paniculata (colourless crystal), were identified through Thin layer chromatography.

Thin Layer Chromatography:

Preparation of mobile phase:
9 ml Chloroform + 1 ml Methanol
Reagent used: Methanol sulfuric acid
OR
Detector: UV Light

Procedure:

Freshly prepared mobile phase is taken in a clean and dry 250 ml beaker which is covered with a foil. Small amount of standard Andrographolide compound dissolved in minimum amount of methanol is taken in a small test tube. In another test tube sample dissolved in minimum amount of methanol is taken. TLC plate of suitable length and width is taken. On that plate marked two spots, one for standard and another one for sample. Then two drops of standard and sample solution is dropped on the spot with the help of capillary tube. TLC plate is kept in the mobile phase. Adsorption of mobile phase on the TLC plate takes place. Taken out the TLC plate from mobile phase and dipped it in Dragendorff's reagent. Dried the plate by drier.

OBSERVATION

Result: Clear spots are observed for column fraction (70:30) along with the standard. There is no impurity.

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HPLC ANALYSIS REPORT

Assay – Andrographolide by HPLC:

HPLC condition:-		
Column	:	Ascentis Express C18 (100 x 4.6) mm / particle size: 2.7 µ
Wave length	:	226nm
Flow rate	:	1.0 ml/minute
Volume of injection	:	10 µl
Mobile phase	:	Solvent-A : 0.1%Trifluoro acetic acid in purified water Solvent-B : 0.1% TFA in Acetonitrile: water(70:30) (Gradient) **Gradient program:** Time(Minutes) B.Conc 0.01 20 15.00 35 18.00 80 24.00 80 25.00 20 30.00 20 30.01 Stop
Run time	:	30 minutes
Approximate retention time	:	about 12 minutes
Column temperature	:	40^{0}C
Diluent	:	70% Ethanol

Preparation of 0.1% w/v Trifluro acetic acid: Pipette out 1 ml of trifluro acetic acid in to 1000 ml volumetric flask containing 500 ml purified water, mix well and make up the volume to 1000 ml with purified water and sonicate for 5 minutes.

Preparation of diluent (70% ethanol): Measure accurately about 700 ml of absolute ethanol in to 1000 ml volumetric flask and make the volume up to 1000 ml with purified water, mix well and sonicate for 5 minutes.

Standard Stock solution of Andrographolide (1mg/ml): weigh accurately about 10 mg of standard Andrographolide in a 10 ml volumetric flask. Add 7 ml of diluent and sonicate for 10 minutes. Make the volume up to the mark with same diluent.

Working standard solution(0.3mg/ml): Pipetted out 3 mL of Standard stock solution into 10mL volumetric flask and make the volume upto the mark with diluent. Filter the solution through 0.45µ syringe filter. Discard the first 2mL of the filtrate and use subsequent filtrate for the analysis.

Sample preparation (5 mg/ml): Weigh accurately about 0.5g of finely powdered test substance in a 100 ml beaker add 50 ml of diluent and sonicate for five minutes. Decant the solution in to a 100 ml volumetric flask. Repeat the same process with 2 X 20 ml of fresh

diluent. Finally rinse the beaker with 5 ml of diluent and transfer into the same volumetric flask. Make the volume up to the mark with diluent. Filter the solution through 0.45µ syringe filter. Discard the first 2mL of the filtrate and use subsequent filtrate for the analysis.

Chromatographic procedure: Stabilize the instrument with the mobile phase till the baseline is satisfactory. Inject the standard solution of five times and record the chromatogram. The % RSD between the results should be less than 2 %. Inject the sample solutions and record the chromatogram.

The analysis should comply for the following system suitability parameters. Theoretical plate: more than 2000; and tailing factor: less than 2.

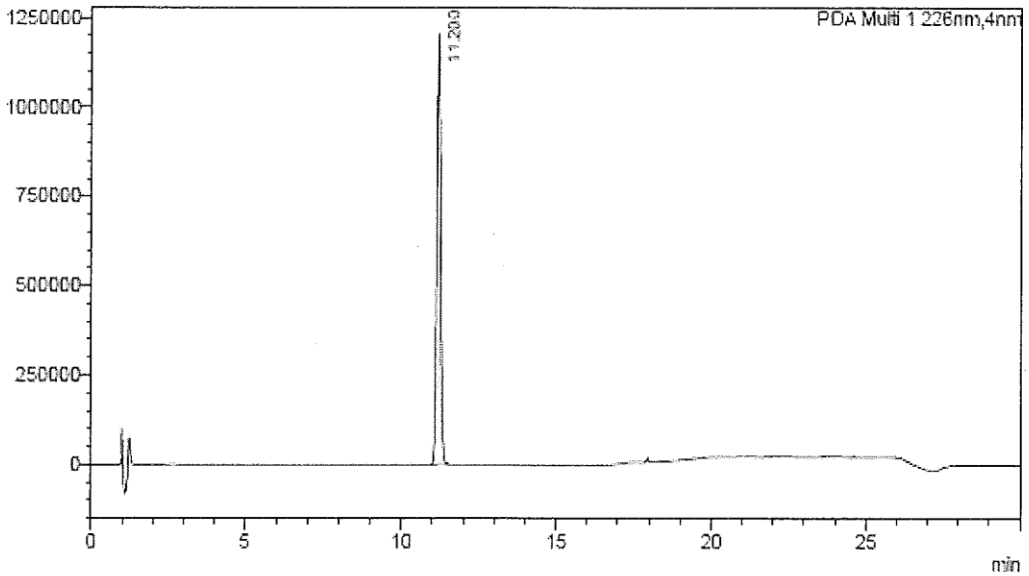
Calculation:-

Calculate the content of % of Andrographolide using below formula

$$\%\ w/w\ \text{Andrographolide} = \frac{A_1 \times W_2 \times 3 \times 100}{A_2 \times 10 \times 10 \times W_1} \times \%\ \text{Purity of Andrographolide}$$

A_1- Area of sample peak corresponds to standard Andrographolide peak
A_2- Area of standard Andrographolide peak
W_1- Weight of sample (mg)
W_2- Weight of standard (mg)

Standard chromatogram:



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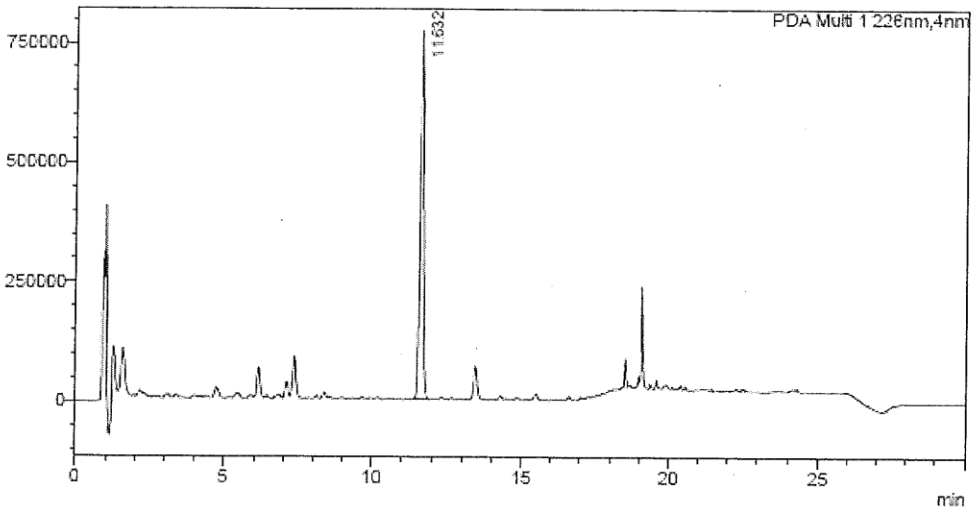

TLC fingerprint:

Preparation of dip reagent:

Preparation of Anisaldehyde reagent:
Mix in the following order, 0.5 ml of anisaldehyde, 10.0 ml of glacial acetic acid, 85.0 ml of methanol and 5.0 ml of sulphuric acid.

Chromatographic system:

TLC plate type	:	Pre coated thin layer silica plate 60 F_{254}, 10 x 10 cm, E-Merck
Mobile phase	:	Toluene: Acetone: Formic acid (60:40:0.4)
Spotting volume	:	10 µl

Sample preparation: Weigh accurately about 0.5 g of finely powdered sample in a 100 ml beaker. Add 10 ml of methanol and sonicate for 5 minutes. Filter the extract through Whatman No.1 filter paper.

Standard Andrographolide preparation: Weigh accurately about 10mg of standard Andrographolide in a 10 ml volumetric flask. Add 8 ml of methanol and sonicate for 5 minutes. Make the volume up to the mark with same solvent .Pipette out 1 ml of this solution in to a 10 ml volumetric flask make the volume upto the mark with same solvent.

Application: Apply the sample and standard solutions as 10 – 12 mm band, in a distance of 12 mm from the bottom of TLC plate. Make a mark up to a distance of 8.5 cm from the application point as a development mark using pencil.

Preparation of development tank: Use Camag twin trough development tank (10 x 10 cm). Cover one side of the inside chamber with required size of Whatman No.1filter paper. Measure about 20 ml of mobile phase and transfer into the chamber along the side of the filter paper.

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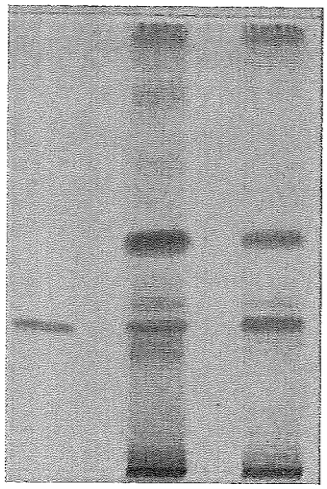
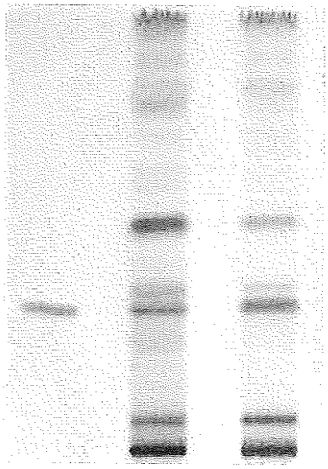
Tank saturation: Cover the development tank with a lid to saturate the chamber for 30 minutes. At the end of 30 minutes, open the lid to keep the spotted plate. Develop the plate up to the pencil marking in the solvent system. Soon after the development, remove the plate and dry in air at room temperature (27 ± 2^{0}C) for about 10 minutes.

Visualization and documentation: Visualize the dried plate under UV 254 nm and 366 nm using UV cabinet. Dip the plate in Anisaldehyde reagent, heat at 105 ± 5^{0}C for about 5 minutes. The image of the plate to be captured under UV 254 nm and 366 nm before dipping and in white light after dipping.

Observation: The Rf value of one of the band in the sample should match with corresponding



@254nm



@white light after spray

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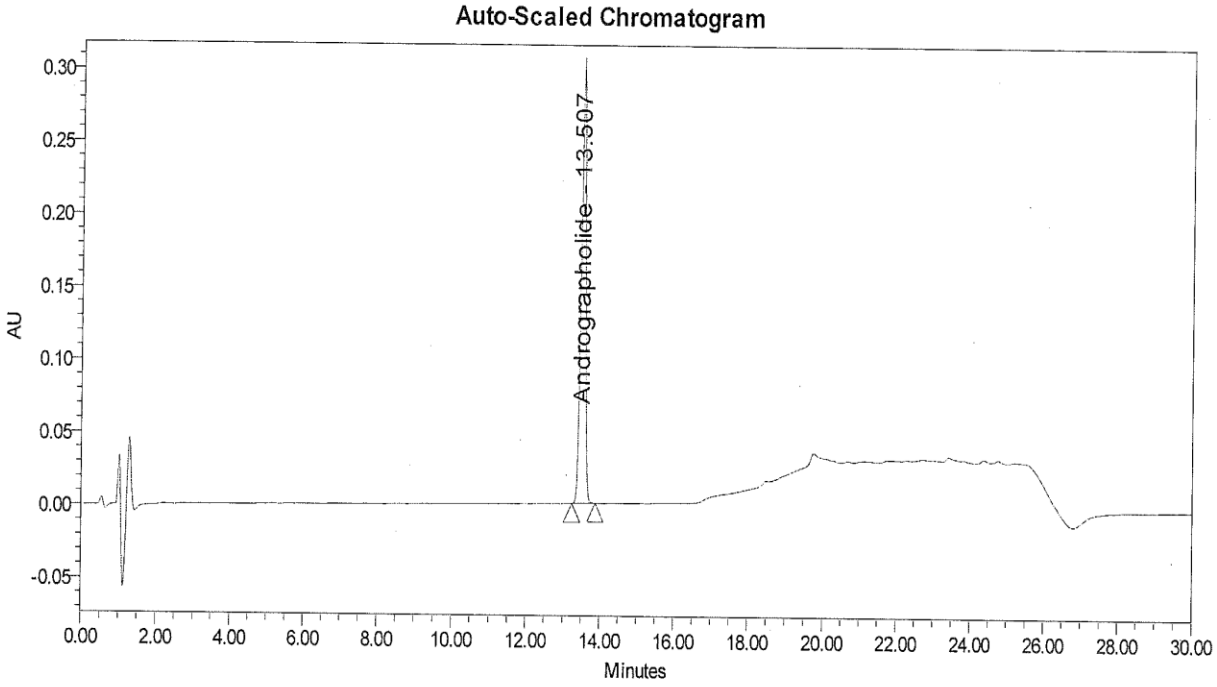
SAMPLE INFORMATION

Sample Type:	Unknown		
Vial:	35		
Injection #:	1	Processing Methoc	Andrographolide_PM
Injection Volume:	10.00 ul	Channel Name:	2998 Ch1 226nm@1.2nm
Run Time:	30.0 Minutes	Proc. Chnl. Descr.:	2998 Ch1 226nm@1.2nm



Peak Results

	Name	RT	Area	USP Plate Count	USP Tailing	Resolution
1	Andrographolide	13.5	2667376	50690	1.00	

Result and discussion:

Andrographolide is confirmed by NMR and HPLC, thatandrographolide with 96% purity. Solubility study at the different temperature of Andrographolide was carried out in methanol. Purified andrographolide was effectively characterized by LCMS and Melting Point. The process parameters of crystallization were studied in terms of such as supersaturation (□y), supersaturation ratio and temperature potential . To obtain substantial yield of andrographolide, 20°C super cooling was found to be sufficient practically. The simple and novel approach based on extraction followed by clarification of extract and crystallization suggested in the present work might be one of the most promising techniques for this kind of natural bitter separation and purification.

The developed HPLC method can be utilized for the quantitative determination of Andrographolide. The method developed is simple, sensitive and statistically validated.

Conclusion:

In conclusion, Andrographis paniculata (Kalmegh) is a versatile and valuable medicinal plant with a wide range of health benefits, backed by traditional uses, phytochemical analysis, and experimental results. Its immunomodulatory, anti-inflammatory, and antioxidant properties make it an effective remedy for various ailments, from fever and infections to cardiovascular and liver issues. However, its potential impact on fertility should be considered. Overall, Andrographis paniculata is a promising medicinal plant that warrants further research, quality control, and responsible usage to unlock its full therapeutic potential and ensure its safe use for the benefit of humanity**.**

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