WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 9, 961-981. Research Article ISSN 2277– 7105

PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF *ANDROGRAPHIS MACROBOTRYS* (NEES) A FOLK MEDICINAL PLANT

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Article Received on
04 July 2020,

Revised on 25 July 2020,
Accepted on 14 August 2020,

DOI: 10.20959/wjpr20209-18415

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ABSTRACT

Background and Objectives: *Andrographis macrobotrys* (Nees) is an erect, stout herb of Acanthaceae family found in semi-evergreen forest of south India and Srilanka which is used in the treatment of snake bite, diarrhoea, muscle pain, fever, jaundice, liver disorders and skin disease by the tribes of Kerala. The present study has been designed for pharmacognostic and phytochemical study of the above drug. **Materials and Methods:** Drug review, macroscopic, microscopic evaluation and Phytochemical study of plant *Andrographis macrobotrys* (Nees) were carried out. **Results:** Macroscopic study showed many features like dark purplish colour at nodes, cuneate at base etc. Microscopic study of whole plant showed chlorenchyma cells, groups of sclerieds in cortex region of stem, calcium oxalate crystals, starch grains in leaf. Phytochemical study including TLC showed presence of saponins, flavonoids, triterpenoids, glycosides, gums and mucilages, steroids, tannins and phenolic compounds.

KEYWORDS: *Andrographis macrobotrys* Nees., macroscopy, microscopy, phytochemical study, TLC.

1. INTRODUCTION

Since ancient times, plants are given more importance in ethno pharmacology and used as medicine for prevention and treatment of various ailments in India. Depletion of herbs is rapidly increasing all over the world for specific plants which are used in huge quantity. Various other herbs are getting destroyed without proper information about its identification, benefits and utilization.

Andrographis macrobotrys Nees., is a folk medicinal plant found in southern parts of Karnataka and Kerala.[1] It is used by tribes in Kerala for the treatment of snake bite, diarrhoea, muscle pain, fever, jaundice, liver disorders and skin diseases.[2,3] By local people test drug is called as *Gudde Kirathakaddi*, *Gudde Kalamegha* in Karnataka[4] and *Uppali* in Kerala.[5,6] We can find its references in botanical books like flora of Udupi, South Canara, Presidency of Madras, Ceylon, Medicinal plants of Arya Vaidya Sala- herb garden and recent articles. Reported on Western Ghats, South Canara in Karnataka; Tirunelveli, Coimbatore, Madurai, Nilgiri, Anamalai, Pulneys hills in Tamilnadu; Kollam, Kannur, Palakkad, Trissur, Idukki, Kozhikode, Pathanamthitta, hills of Travancore up to 2500ft in Kerala in evergreen forests.[7]

Folk use of *Andrographis macrobotrys* Nees., are - Fresh leaf paste is used in treating skin diseases. Fresh leaf juice is given orally thrice a day for one week to treat liver disorders and as anti-cancerous medication. The root powder mixed with Goat's milk and administered orally to treat jaundice.[2,3,8,9]

2. MATERIALS AND METHODS

Collection and identification of the drug

The drug *Andrographis macrobotrys* Nees., whole plant was collected from Bailur and nearby places in Udupi district, surrounding ALN Rao Ayurvedic medical college campus in Koppa and is authenticated by M. Radhakrishna Rao, Visiting Professor of Botany, ALN Rao memorial Ayurvedic College, Koppa.

In such a way, plant was initially identified and cultivated in MIAMS Herbal garden and was freshly collected.

2.1. Pharmacognostic study

2.1.1. Macroscopic study

Macroscopic study is considered as assessment and evaluation of the plant by its morphological features. This study helps to distinguish species of plant on study with plants with similar morphology and also to find out adulterants.

The external features of the test sample *Andrographis macrobotrys* Nees., was documented using digital camera. The macroscopic features were observed with naked eye and lens. Whole plant was observed with size, shape, fracture and colour as parameters for assessment. Observed morphology of *Andrographis macrobotrys* Nees is compared to local flora for authentication.[10]

2.1.2. Microscopic study

Microscopy is the science of investigating minute objects and structures using microscope as instrument. In the present study, test drug *Andrographis macrobotrys* Nees., is a folk medicinal plant so it is necessary to know the cell types of whole plant and arrangement of cells in each part of the plant is studied.

Materials required

All 5 parts of the test drug *Andrographis macrobotrys* Nees., formalin, acetic acid, ethyl alcohol, Safranin stain, 50% glycerine, water, a sharp razor blade, watch glass, thin painting brush, needles, forceps, glass slides, cover slips, blotting paper, dropper, compound microscope.

Procedure

- Sample was preserved in the fixative solution (Formalin 5 ml + acetic acid 5 ml + 70 % ethyl alcohol 90 ml). The materials were left in FAA for more than 48 hours.
- The drug was held between thumb and index figure of left hand, thin sections were taken with the help of sharp razor blade and put into watch glass containing water.
- Thin, entire and uniform section was selected and transferred onto a clean glass slide with the help of brush.
- Safranin stain 1 drop was put and left for few minutes. Excess stain was removed by washing with water.
- Then section was mounted with 1-2 drops of glycerine and covered with a clean cover glass.

- Excess glycerine was removed using blotting paper and observed under trinocular microscope.
- Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light.
- Magnifications of the figures are indicated by the scale-bars.

2.2.Phytochemical study[11]

Preparation of powder drug

The whole plant *Andrographis macrobotrys* Nees., is properly cleaned, dried in shade and coarsely powdered and sieved using, sieve no. 180 as per WHO standards for medicinal plant materials and stored in clean air tight containers.

2.2.1. Test for Tannins

a. Ferric chloride reagent

A 5% w/v solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution was added to the test extract taken in test tube. Appearance of dark green or deep blue colour indicate the presence of tannins.

2.2.2. Test for Alkaloids

a. ***Hegar's test*** - 5 ml of extract is taken in a test tube, saturated aqueous solution of picric acid is added. If orange yellow precipitate is obtained it indicate the presence of alkaloids.

b. Mayer's test - Mayer's reagent was prepared. To 5ml of test extract taken in test tube, few drops of Mayer's reagent were added. Formation of cream coloured precipitate shows the presence of alkaloids.

c. Dragendroff's test - A few mg of extract is dissolved in alcohol, to this a few drops of acetic acid and Dragendroff's reagent is added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.

d. Wagner's test - A few mg of extract is dissolved in acetic acid, a few drops of Mayer's reagent will be added. A reddish brown precipitate formed indicates the presence of alkaloids.

2.2.3. Test for Protein

a. Biuret test

Test extract was taken in test tube and 1ml of 4% Sodium hydroxide solution was added to it, followed by a drop of 1% solution of copper sulphate. Development of violet or pink colour indicate the presence of proteins.

b. Xanthoproteic test

A little residue was taken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it. Yellow colour development indicates the presence of proteins.

2.2.4. Test for Steroids

a. Salkowaski reaction

5ml of extract was taken in test tube, 2ml of chloroform is added and 2ml of Conc.Sulphuric acid was added from side of test tube. The test tube was shaken for few minutes. The development of red colour in to chloroform layer indicate the presence of sterols.

b. Libermann- Burchard test

To the extract dissolved in chloroform, 1ml of acetic acid and 1 ml of acetic anhydride will be added, then heat it on water bath and cool. Few drops of Conc. Sulphuric acid should be added along sides of the test tube. Appearance of bluish green colour indicates the presence of steroids.

2.2.5. Test for Triterpenoids - *Liebermann Burchard reaction*

5ml of extract is dissolved in chloroform, add 1ml acetic anhydride, Conc. Sulphuric acid was added to the solution. Formation of reddish violet colour shows the presence of triterpinoids.

2.2.6. Test for Glycoside - *Keller-Kiliani test*

1ml of glacial acetic acid containing traces of ferric chloride and 1ml of Conc. Sulphuric acid was added to the extract and was observed for the formation of reddish brown colour at the junction of 2 layers. Appearance of bluish green colour in the upper layer indicate the presence of glycoside.

2.2.7. Test for Amino-acids -*Ninhydrin test*

The Ninhydrin reagent is 0.1%w/v solution of ninhydrin in n-butanol. Few drops of the reagent is added to the test extract. A violet colour indicate the presence of Amino-acids.

2.2.8. Test for Carbohydrates

a. Fehling's solution test

Fehling's solution is prepared by adding copper sulphate, Sulphuric acid and distilled water. Few ml of this solution is added to 5ml of test drug extract. Formation of red precipitate of cuprous oxide indicate the presence of reducing sugars

b. *Molisch's test*

Extract is taken in the test tube, 1 ml of α-naphthol solution and conc. Sulphuric acid is added to it. Violet colour formed at the junction of two liquids indicates the presence of carbohydrates.

c. *Benedict's test*

5 ml of Benedict's reagent is taken in test tube and few ml of extract is added. Then it is boiled for 2 minutes and cooled. Formation of red precipitate indicates the presence of carbohydrates.

2.2.9. Test for Flavonoids - *Shinoda's test*

Extract was taken in test tube and dissolved in 5ml ethanol. Few turnings of magnesium metal and Conc. HCl is added. Appearance of pink, crimson or magenta colour within a minute or 2 indicate the presence of flavonoids.

2.2.10. Test for Phenol - *Ferric chloride test*

Extract was taken in a test tube, warmed. 2ml of ferric chloride solution was added to this. Observed for the formation of green and blue colour.

2.2.11. Test for Saponin - *Foam test*

Few ml of extract was taken in a test tube with small amount of sodium bicarbonate, add water and shaken vigorously. A stable, characteristic honeycomb like froth indicate the presence of saponins.

2.3.Physicochemical parameters

Loss on drying, total ash, Acid insoluble ash, Water soluble ash, pH value, Water soluble extractive, Alcohol soluble extractive values were conducted according to the pharmaceutical standards of India[12] in R & D department of Muniyal institute of Ayurveda medical sciences, Manipal.

2.4.TLC- Thin layer chromatography

TLC is used for both qualitative and quantitative analysis and evaluation of drugs.

Materials: Plant extract, Pre coated TLC plates, capillary tubes, TLC chambers, Hot air oven, Mobile phase, Spray, UV- chamber.

- Plant extract- 5 gm coarse powder of whole plant taken in screwed cap conical flask with 100 ml methanol and methanol extract of *Andrographis macrobotrys* Nees., is prepared.
- Pre-coated TLC plates.
- Mobile phase.
  - Chloroform: Methanol in 7:1 ratio.
  - Toluene: Ethyl acetate: Acetyl amine in 7:2:1 ratio.
- Spray- Anisaldehyde- Sulphuric acid solution.

Procedure- TLC chamber cleaned and dried, Mobile phase poured into the chamber, lid was closed and kept undisturbed for about an hour for saturation. Activated TLC plates taken out and Plates were spotted with the help of capillary tube. The spotted plates were gently immersed in the TLC chamber containing mobile phase in such a way that solvent had linear contact with the plate. The solvent was allowed to rise up to the required distance. Plate was taken out and distance travelled by the solvent called solvent front is marked with pencil. Plates are visualized in UV chamber under 254 m and 366 m wavelength. And Rf values were noted. Then plates were sprayed with Anisaldehyde-sulphuric acid reagent which is suitable for triterpenoids and bitter compounds and kept in Hot air oven for 15 minutes. Plates were visualised under UV light and observed for spots and marked to find Rf value.

The Rf values of the spots were calculated using the formula.

$$Rf = \frac{Distance\ travelled\ by\ the\ solute}{Distance\ travelled\ by\ the\ solvent}$$

3. RESULTS

3.1. Pharmacognostical Study

3.1.1. Macroscopic observations

Root – Tap root, rooting occurs at the internodes when in contact with soil.

Stems – 4 angled, dark purple at nodes, branches cross armed, erect, stout, spreading horizontal.

Leaves –Simple, opposite, estipulate, short petioled, petiole scarcely 6mm long, leaves up to 8.5 *2.5 cm, oblong-lanceolate, acuminate at apex, cuneate at base, entire margin, coriaceous,

Dark green, glaucous, midrib- dark purplish below, reticulate venation with 5-6 pairs of slender lateral nerves.

Racemes – Terminal as well as axillary horizontal four sided elongated or long second lax flowered sub panicled, up to 20 cm long, glandular hairy, often 1-2 branched, purple in colour.

Flowers – small, bracteates, pedicellate, all turned to one side, distantly placed one at a node, zygomorphic or distinctly 2 lipped, dark purple – red blotches on lower lip;

Pedicel- very short, 3mm long, somewhat glandular, pubescent.

Bracts – from 1.5mm to 2.5mm. linear-lanceolate, Shorter than calyx;

Bracteoles – small or absent.

Calyx – 5-8mm long, deeply five parted, equal lobes, narrow, linear-lanceolate, lobe subulate, glandular hairy.

Corolla – gamopetalous, 2cm long, hairy outside, tube 5mm long, oblique, dilated below the limb, limb prominently lipped for at least half its length, the upper oblong and slightly bifid, deflexed deeply 3 lobed, white colour flower and dark purple-red blotches on lower lip, the segments subacute and imbricate in bud.

Stamen – 2, filaments free, broad or flattened or enlarged at base, hairy upwards; anthers-exerted, blackish, 2-celled, cells parallel, oblong or obovate, flat and muticous, sub equal, base bearded.

Gynoecium – bicarpellary, syncarpus.

Ovary – superior, sub glabrous, or very thinly hairs, elongated, flat and bilocular, with 6-12 ovules in each cell; style slender, terminal ending in a minutely bifid stigma.

Flowering and fruiting- October - January

Fruit – a linear oblong or elliptic, erect, somewhat cylindrical or more often a slightly flattened capsule.

Seeds – the retinacula acute or rounded. 8-10 hard seeds, deeply rugosely pitted, glabrous.

3.1.2. Microscopic observations

i. T.S of Stem

T.S. of the stem of *Andrographis macrobotrys* Nees., shows the following layers.

1. Epidermis: The epidermis is the outermost layer of the stem which is four-winged and made up of ridges and furrows as the stem is angled. It comprises of uniseriate, cuboidal cells which are thin walled. There is an outer waxy layer made up of cutin wax called the cuticle.

2. **Cortex:** The cortex layers are found beneath the epidermis, further divided into hypodermis (outer cortex), middle cortex and inner cortex. The anatomical features are as mentioned below:

a) Hypodermis: It is the layer found just beneath the epidermal layer and outer to the middle cortex. It consists of 8-12 cells thick made up of collenchyma cells.

b) Middle cortex: It is the layer found inner to the outer cortex layer consisting of 4-6 cells thick made up of chlorenchyma cells that are rich in chloroplasts and are green in colour.

c) Inner cortex: It is the innermost layer comprising of 8-10 layers of parenchymatous cells which are polygonal and are thin-walled. There are solitary and groups of 4-6 sclereids followed by endodermis present inner to the inner cortex.

d) Endodermis: It is the innermost layer of the cortex region made up of uniseriate cubical cells which are compactly arranged.

3. **Stelar region:** It is the innermost zone of the stem comprising of vascular tissues and the pith. The stelar region is further categorized into the following layers:

a) Pericycle: Is a layer of polygonal cells inner to the endodermis, outermost layer of stele, with thin walls and are devoid of intercellular spaces.

b) Vascular bundles: They are prominently made up of xylem occupying major portion of the stelar region with solitary vessels arranged in radial rows, smaller in size. The vessels are circular and polygonal in shape. There are many, conspicuous medullary rays.

c) Pith: It is centrally placed with large parenchymatous cells and cells are polygonal in shape.

ii. T. S. of root

A typical T.S. of root of *Andrographis macrobotrys* Nees., shows the following regions:

1. **Cork:** The outermost layer of the root that is quite conspicuous comprising of 8-10 cell layers of which the outermost 2-4 layers are reddish-brown and thick-walled.
2. **Cortex:** Inner to the outer cork, there are 6-8 layers of thin-walled parenchymatous cells that are square-shaped or rectangular-shaped. The cortex forms the major part of the root with a single-layered innermost endodermis layer made out of rectangular cells.
3. **Stelar region:** The xylem vessels predominantly occupy the major portion of the root. They are larger in number mostly solitary and small sized and majority of them are arranged in radial rows. Vessels vary in shape having circular, elliptical or polygonal.

Medullary rays are very conspicuous and many in number. At the centre there is large pith made up of compactly arranged parenchymatous cells.

iii. T. S. of leaf

The leaf is dorsi-ventrally differentiated with distinct adaxial and abaxial surfaces. The lamina is flattened with a distinct midrib having projections on two corners and a shallow furrow on the adaxial surface.

A typical T.S. of leaf of *Andrographis macrobotrys* Nees., shows the following regions

1. **Epidermis:** The epidermis is divisible into upper epidermis on the adaxial surface and lower epidermis on the abaxial surface made up of uniseriate, rectangular cells. Cuticle layer is present on both the surfaces. A few stomata cells were present in both surfaces. **Hypodermis:** It is composed of 6-8 layers of collenchyma cells followed by 2-3 layers of chlorenchyma cells. The ground tissue is parenchymatous.
2. **Mesophyll tissue:** The lamina comprises of upper palisade parenchyma that is single layer while the spongy parenchyma are present towards abaxial surface that are 3-layered with large intercellular spaces.
3. **Vascular tissues:** The xylem vessels are arranged in between the ground tissue in 5-6 radial rows while the phloem tissue is arranged on the abaxial surface.

iv. T. S. of fruit

T.S. of the *Andrographis macrobotrys* Nees., fruit reveals the following features.

1. **Epidermis:** The outermost layer of the pericarp is the epidermis that is one-cell thick cuboidal cells without cuticle. Inner to the epidermis is the 3-4 layers of spongy parenchyma, inner to which lies vascular bundles comprising of both xylem and phloem.
2. **Aleurone layer:** Aleurone layers present on the seeds as a protein source. These are the protein molecules present in the cell cytoplasm as ergastic substance.
3. **Embryo:** The embryo is found situated at the centre of the fruit/ seed covered by the layer of perisperm followed by the endosperm and parenchymatous ground tissue towards the outer surface.

3.2.Phytochemical Study

Preliminary phytochemical analysis

Table No 1: Results of Preliminary Phytochemical Tests.

Sl no	Phyto-chemicals	Tests	Part	Aqueous extract	Methanol extract	Chloroform extract	Petroleum ether extract
1	Tannin	Ferric chloride test	Whole	+	+	+	+
			Leaves	+	+	+	+
2	Alkaloid	Hager's test	Whole	-	-	-	-
			Leaves	-	-	-	-
		Mayer's test	Whole	-	-	-	-
			Leaves	-	-	-	-
		Dragendroff's test	Whole	-	+	-	-
			Leaves	-	+	-	-
		Wagner's test	Whole	-	-	-	-
			Leaves	-	-	-	-
3	Protein	Biuret test	Whole	-	-	-	-
			Leaves	-	-	-	-
		Xanthoproteic test	Whole	-	-	-	-
			Leaves	-	-	-	-
4	Steroid	Salkowski test	Whole	+	+	+	+
			Leaves	+	+	+	+
		Liebermann Buchard test	Whole	+	+	+	+
			Leaves	+	+	+	+
5	Triterpinoid	Conc. Sulphuric acid	whole	+	+	+	+
			Leaves	+	+	+	+
6	Glycoside	Keller-Kiliani test	Whole	-	+	-	+
			Leaves	-	-	+	-
7	Amino acids	Ninhydrin test	Whole	-	-	-	-
			Leaves	-	-	-	-
8	Carbohydrate	Fehling's test	Whole	+	-	-	-
			Leaves	+	-	-	-
		Benedict's test	Whole	+	-	-	-
			Leaves	+	-	-	-
		Molisch's test	Whole	+	-	-	-
			Leaves	+	-	-	-
9	Flavonoid	Shinoda's test	Whole	+	+	+	+
			Leaves	+	+	+	+
10	Phenol	Ferric chloride test	Whole	+	+	+	+
			Leaves	+	+	+	+
11	Saponin	Foam test	Whole	-	-	-	-
			Leaves	-	-	-	-

'+' – Present and '-' – Absent

3.3. Physico-Chemical study

Table no. 2: Results of Physico-Chemical Parameters.

Parameter	Whole plant	Leaves
Loss on drying	9.72%w/w	10.13%w/w
Total ash	5.84% w/w	10.86% w/w
Acid Insoluble Ash	0.59% w/w	1.74% w/w
Water soluble Ash	70.05% w/w	68% w/w
Water soluble extractive	19.86%w/v	26.33%w/v
Alcohol soluble extractive	17.77%w/v	26.91%w/v
pH	6.74	6.42

3.4. TLC

Table no 3: Rf. value of TLC in methanol extract of whole plant *Andrographis macrobotrys* Nees.

Short UV	Long UV	Post derivatization
-	-	0.08(Violet)
-	-	0.22(Violet)
0.57(Green)	-	-
0.81(Green)	-	-
0.96(Green)	-	-

Table no 4: Rf. value of TLC in methanol extract of whole plant *Andrographis macrobotrys* Nees.

Short UV	Long UV	Post derivatization
-	-	0.071(Violet)
-	0.116(F. yellow)	-
-	-	0.473(Violet)
-	0.714(Green)	-
-	0.767(Green)	-
-	0.973(F. Red)	-

F- Fluorescent

1. Annexure

Macroscopic view of Andrographis macrobotrys Nees.

Fig. no.1: Stem showing nodes; Fig. no. 2: Leaf arrangement; Fig. no. 3: Flower.

Microscopic structure of stem of Andrographis macrobotrys Nees.

Figure no. 4: T.S of stem.

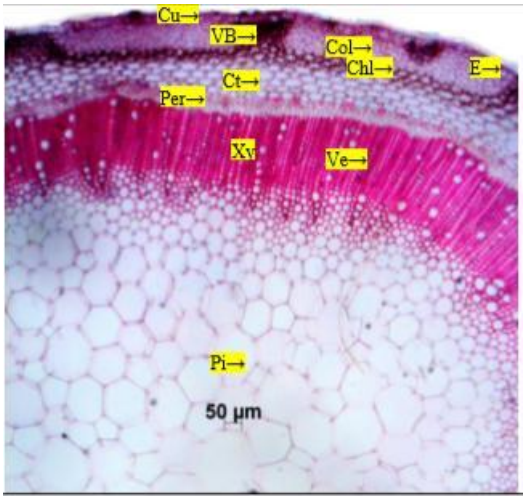


Fig. no. 5: A portion of stem detailed view.

Cam–cambium; Chl – chlorenchyma; Col–collenchyma; Cu – cuticle; E – epidermis; Per – pericycle; Ph – phloem; Pi – pith; Scl–sclerenchyma; SG – starch grains; Ve – vessel; VB– vascular bundle; XR – xylem ray; XY – xylem.

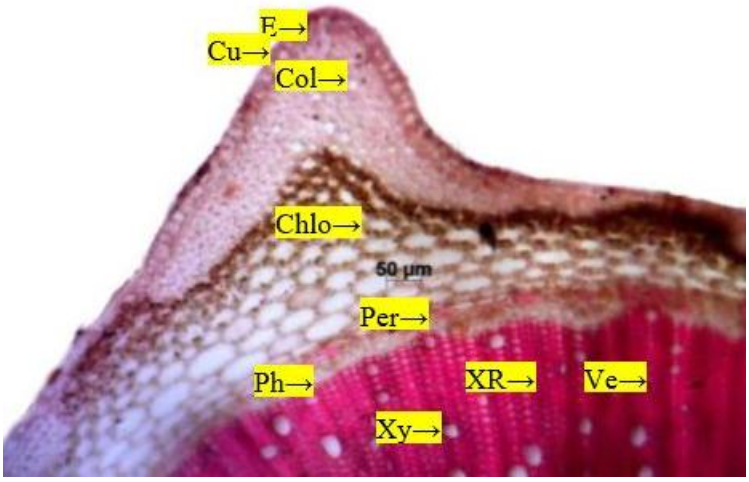


Figure no. 6: Wing of stem.

Chl – chlorenchyma; Col–collenchymas; Cu – cuticle; E – epidermis; Per – pericycle; Ph – phloem; Ve – vessel; VB– vascular bundle; XR – xylem ray; XY – xylem

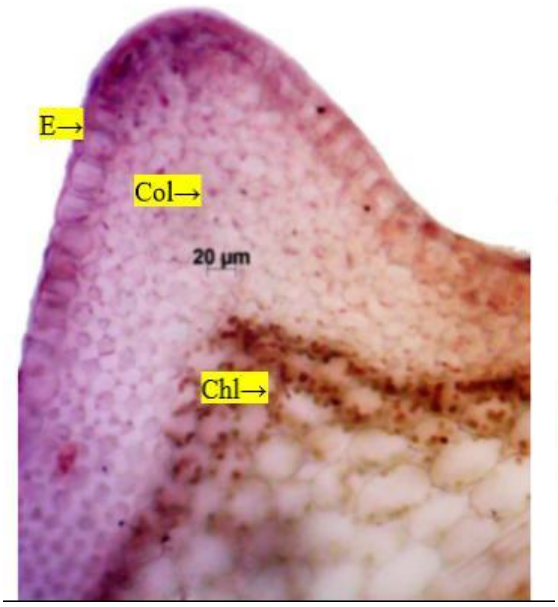


Figure no. 7: A portion of enlarged wing.

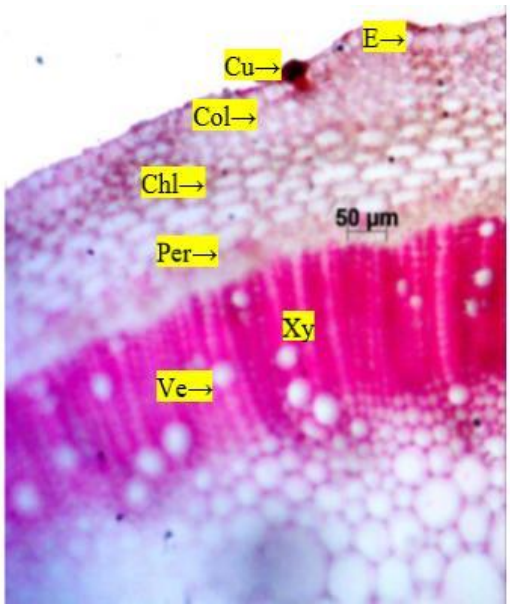


Figure no. 8: Pericycle and xylem.

Cam–cambium; Chl – chlorenchyma; Col–collenchymas; Cu – cuticle; E – epidermis; Pa– parenchyma Per – pericycle; Ph – phloem; Pi – pith; Ve – vessel; XR – xylem ray; XY – xylem.

Microscopy of root of Andrographis macrobotrys Nees.

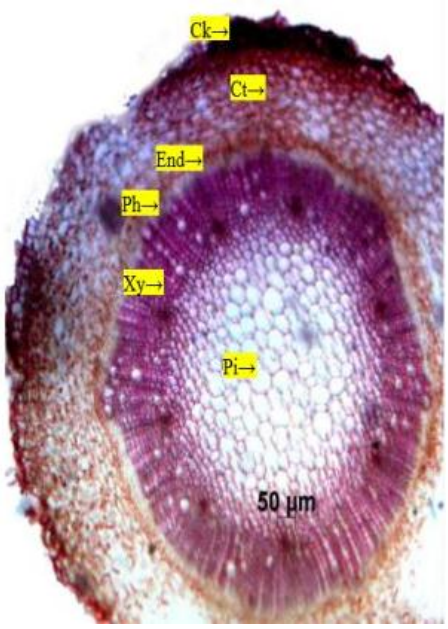


Figure no. 9: A portion of root enlarged.

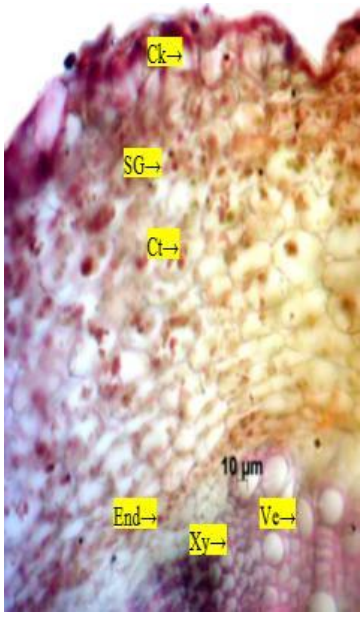


Figure no. 10: Cork and cortex.

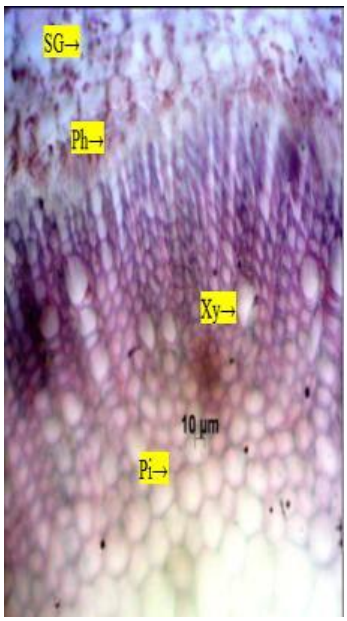


Figure no. 11: Xylem and pith.

Ph –phloem; Pi–pith; SG–starch grains; Ve–vessels; Xy–xylem. Ck–cork; Ct–cortex; End–endodermis; Ph –phloem; Pi–pith; Xy–xylem

Microscopy of leaf of Andrographis macrobotrys Nees.

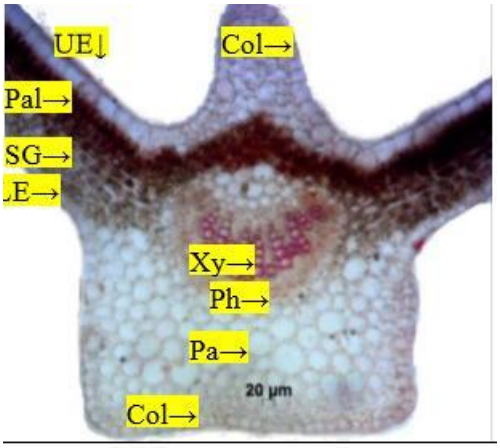


Figure no. 12: T.S of leaf.

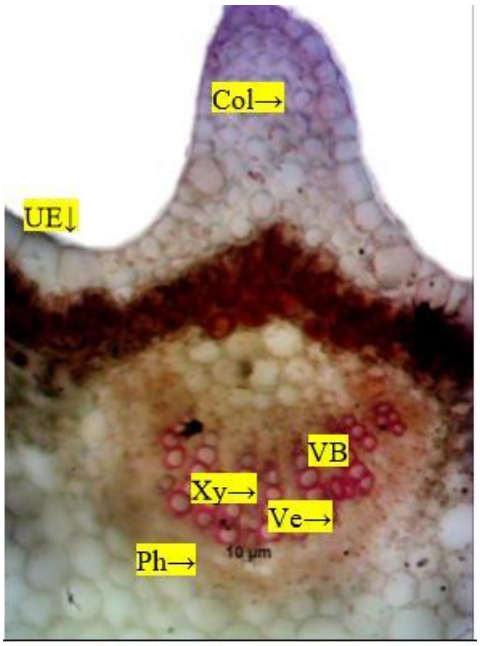


Figure no. 13: A portion of leaf enlarged.

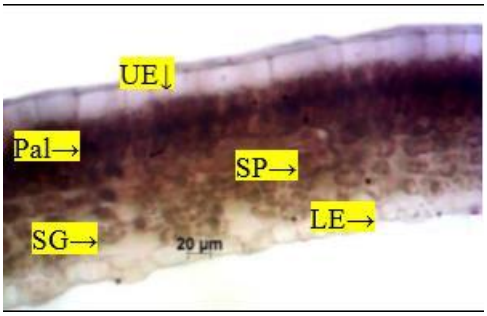


Figure no. 14: T.S of Lamina.

Figure no. 15: Abaxial surface.

Col–collenchymas; LE –lower epidermis; Pal – palisade; Ph–phloem; SG–starch grains; SP–spongy parenchyma; UE–upper epidermis; VB– vascular bundle; Ve–vessels; Xy – xylem.

Microscopy of fruit of *Andrographis macrobotrys* Nees.

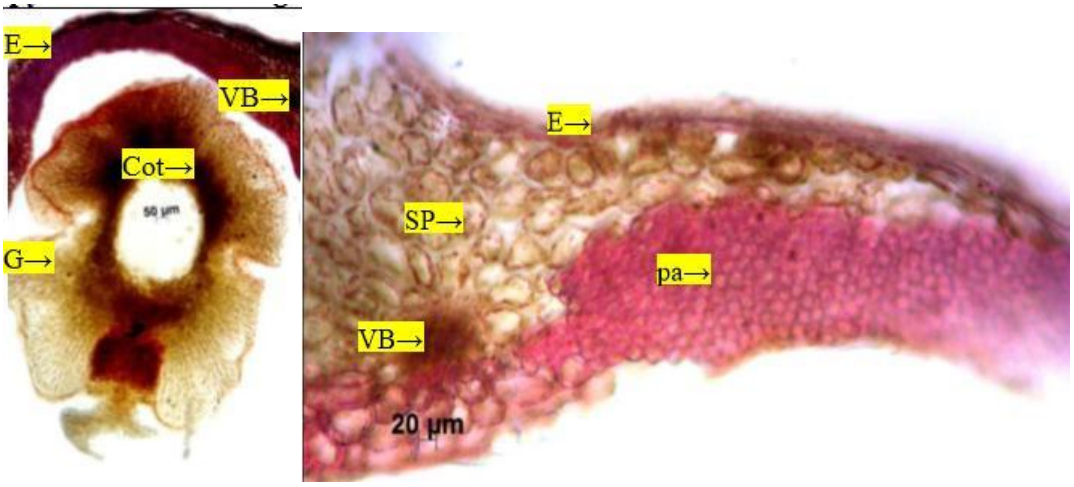
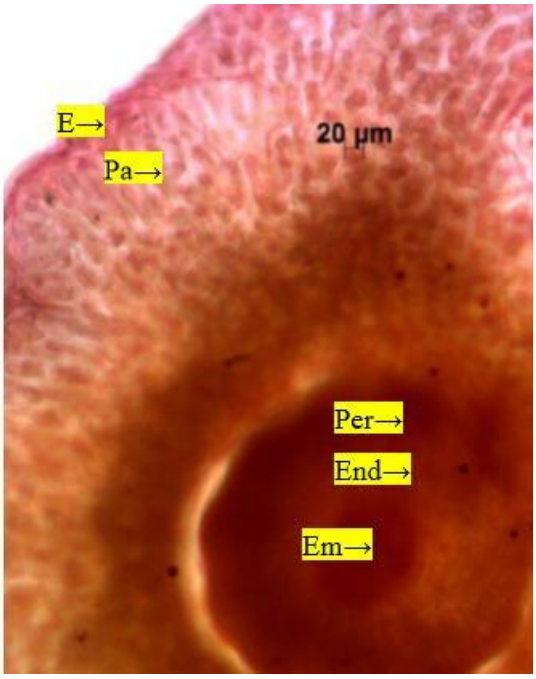


Figure no. 16*:* Section of fruit. Figure no. 17*:* Vascular bundle and lignified parenchyma

AG – aleurone grains; E – epidermis; SP–Spongy parenchyma; VB–vascular bundle; Xy–xylem; Cot – cotyledon.



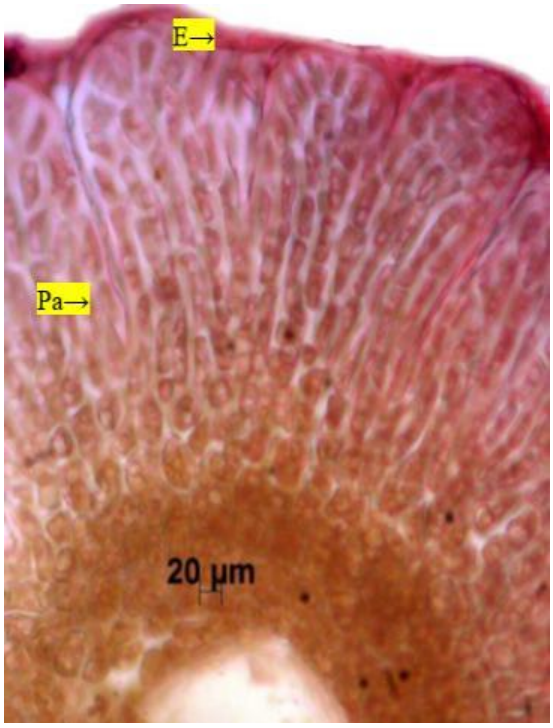


Figure no. 18: Cotyledon and embryo. Figure no. 19: Epidermis and Parenchyma.

AG – aleurone grains; E – epidermis; End–endosperm; Em–embryo; Pa–parenchyma; Per–perisperm.

TLC

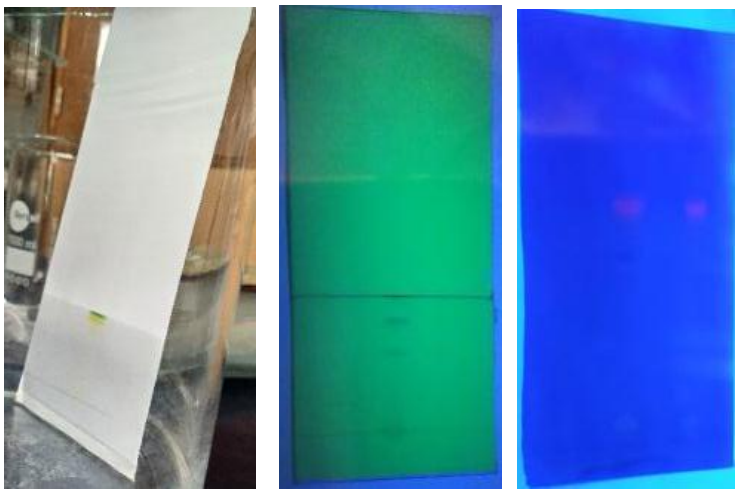


Figure no. 20: TLC Plate, Under short and long UV.

4. DISCUSSION

1.1.Pharmacognostical study

1.1.1. Macroscopic study

Plants which belongs to Family Acanthaceae is identified with quadrangular stem, angles prominent and narrow winged.

The test drug *Andrographis macrobotrys* Nees., can be differentiated from other species plants of same family as it has quadrangular stem with dark purple colouration at nodes, hairs throughout the plant, leaves opposite in arrangement with short petiole, entire margin, coriaceous, racemes terminal or axillary, purple colour, glandular hairy, flower has dark purple blotches on lower lip.

4.1.2. Microscopic study

i. **T.S of stem-** It contains Epidermis, cortex and stellar region. Cortex has layers of Collenchyma, chlorenchyma, parenchymatous and sclerenchyma cells. Stellar region comprises of pericycle, vascular bundle and pith.

ii. **T.S of root-** It contains cork, cortex and stellar region. It is made up of thick walled cells in the cork. Parenchymatous cells loosely arranged in cortex. Stellar region contains numerous xylem vessels, medullary rays and parenchymatous cells compactly arranged in pith.

iii. **T.S of leaf-** It contains epidermis, hypodermis, mesophyll and vascular tissue. Epidermis contains rectangular cells. Cuticle present over both upper and lower epidermis.

Hypodermis has layers of collenchyma, chlorenchyma cells and parenchyma cells as ground tissue. Mesophyll tissue- Lamina contains palisade parenchyma and spongy parenchyma. Vascular tissue comprised by xylem and phloem vessels.

iv. **T.S of fruit-** It contains Epidermis, aleurone layer and embryo. Epidermis has outer cuboidal cells, inner spongy parenchyma and vascular bundles. No cuticle layer. Aleurone layer present on seeds. Embryo has perisperm, endosperm and parenchymatous tissue.

v. **Powder microscopy**

Powder microscopy was performed with powder of *Andrographis macrobotrys* Nees., whole plant. This reveals the presence of epidermis with stomata, Starch grains, Xylem vessels, trichomes, Calcium oxalate crystals and spiral thickening.

1.2. Phytochemical study

Preliminary phytochemical study

Phytochemical analysis of Whole plant and leaves of *Andrographis macrobotrys* Nees., has been carried out in aqueous, Methanol, chloroform and petroleum ether extract. Study shows that- Tannin, steroid, flavonoid, phenol were present in all 4 extract of whole plant and leaves. Alkaloid present only in methanol extract, Glycoside present in whole plant methanol and Petroleum extract, leaves chloroform extract, carbohydrates present only in aqueous extract. Protein, Amino acids and saponin was absent in all 4 extracts. Hence, *Andrographis macrobotrys* Nees., contains tannin, steroid, flavonoid, phenol, alkaloid, glycoside and carbohydrates. *Andrographis macrobotrys* Nees., does not contain Protein, amino acid and saponin.

1.3. Physico-chemical study

Loss on drying test is used to estimate the amount of water and other volatile materials present in the test sample and it showed 9.72%w/w and 10.73%w/w of the whole plant and leaves respectively. Total ash is performed to find the amount of inorganic salts and inorganic matter present in sample and it showed 5.84% and 10.86% of the whole plant and leaves respectively. Acid insoluble ash is performed as a part of total ash that is insoluble in Dil. Hydrochloric acid and it showed 0.59% and 1.74% of the whole plant and leaves respectively. Water soluble ash is a test performed to know the solubility of the sample in water and it showed 70.05% and 68% solubility of the whole plant and leaves respectively. Water soluble extractive is performed to determine the amount of active constituents extracted with water from the given amount of drug and it showed 19.86% and 26.33% of the

whole plant and leaves respectively. Alcohol soluble extractive is performed to determine the amount of active constituents extracted with alcohol from the given amount of drug and it showed 17.77% and 26.91% of the whole plant and leaves respectively.

1.4. TLC

Under UV, plate shown major spots at Rf 0.8, 0.22- violet; 0.57, 0.81, 0.96- green in study with chloroform:methanol as mobile phase. Plates shown major spots as Rf 0.071-violet 0.116-Fluorescent yellow; 0.473-violet; 0.714, 0.767- green; 0.973-Fluorescent red in study with Toluene: Ethyl acetate: Acetyl amine as mobile phase. These spots corresponds to either of the phytoconstituents. Alkaloids may be present which gives blue-green, yellow fluorescence. Flavonoids are which mainly causes fluorescence as it showed yellow fluorescence it can be a flavonoid. Red fluorescence is present which is indicating the presence of anthraquinone, a type of glycoside. Violet colour indicates the presence of triterpene. Thus, TLC study of *Andrographis macrobotrys* Nees., indicates the presence of alkaloids, flavonoids, glycosides, triterpene

2. CONCLUSION

Herbal drugs which are further used as raw materials in preparation needs to be standardized to maintain authenticity of drug. Macroscopic and microscopic evaluation of the drug is important for its botanical identification. Present work provides authentic pharmacognostic features of folk medicinal plant *Andrographis macrobotrys* Nees. The preliminary phytochemical, physicochemical and chromatographic assays were performed and analysed. Phytochemical study revealed presence of phytoconstituents like tannin, steroid, triterpenoids, flavonoid, phenol, alkaloid, glycoside and carbohydrates. TLC indicates the presence of alkaloids, flavonoids, glycosides and triterpenes.

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