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PHARMACOGNOSTIC STUDY OF *LEPIDAGATHIS INCURVA*

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ABSTRACT

Lepidagathis incurva is a common herb which is found in North Estern Indian region-Mizoram and Karnataka Kudajadri hills, Kerala and in Malaysia where it is widely used as a medicinal plant.[1] Traditionally the leaves of *Lepidagathis incurva* were used to stop bleeding (haemostastic), ear infections and the treatment of anemia. The objective of present study was phytochemical screening of ethanol, hexane and aqueous extract, pharmacognostic screening of *L. incurve*. Qualitative microscopic evaluation was carried out by taking transverse sections of fresh Leaf and stem of *L. incurva*.

KEYWORDS: *Lepidagathis incurve*, Acanthaceae, microscopic characters, stomatal number, stomatal index, vein islet number, phytochemical analysis.

INTRODUCTION

The ancient Indian literature, Rigveda contains many references to the curative properties of several herbs, though a more detailed account is found in the Atharvaveda from where Ayurveda, the Indian traditional health care system originated.[2] Medicinal plants have played a significant role in ancient traditional systems of medication in many countries. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (World Health Organization 2002). Traditional Indian medicinal herbs used for strengthening the body immune system

are known to have many essential and nutritional elements.[3] These trace elements constitute a minute fraction in medicinal plant, therefore requiring a sensitive and reliable analytical technique for obtaining precise and accurate data. Their excess or deficiency may disturb normal biochemical functions of the body. Most studies on medicinal plants pertain to their essential oil, glycosides, vitamins, alkaloids, and other active components having pharmacological or therapeutic effects.[4] Besides several phytoconstituents, many trace elements play vital role in cure of disease. Several studies have been reported due to elemental contents in plant extracts, which are consumed by us either as a herbal health drink or medicine.

MATERIALS AND METHODS

The *Lepidagathis Incurva* was received as a gift sample from vaidyamadham, Palakkad dist (Kerala) the plant was authenticated by Dr. Jomy Augustine, Head of the Department of Botany, St. Thomas College, Pala.

2.1 Extraction of plant

Maceration process: In this process, the coarsely powdered leaves of L. incurca was placed in a stoppered container with the solvents such as methanol, distilled water and hexane and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, and clarified by filtration after standing.[5]

2.2 Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods for the analysis of alkaloids, carbohydrates, saponins, phenols, tannins, flavonoids, glycosides, proteins and aminoacids.

2.3 Pharmacognostic study

Fresh leaves and stem were taken for morphological and histological studies. Coarse powder was used to study physicochemical parameters and phytochemical investigation.[6] For the microscopical studies, transverse sections of leaves and stem were prepared and stained as per standard procedure.

2.3.1. Macroscopy

Morphological characters include type of leaf, shape, apex, margin, base, arrangement of leaves on stem; venation, colour, and odour of the leaf of *L.incurva* are studied.[7]

2.3.2 Microscopy

Transverse section of leaf, stem, root and quantitative microscopic characters like vein islet number, stomatal number, stomatal index were determined on fresh leaves of *Lepidagathis incurva*.

Quality control parameters

Coarse powder of the plant leaves has been used to perform quality control test which include determination of ash, extractable matter, moisture content etc. Ash value represents the inorganic salts naturally occurring in the drug and adhering to it. Total ash is the residue remaining after incineration. The acid insoluble ash is the part of total ash which is insoluble in dilute hydrochloric acid. Mixing of sulphuric acid with powdered crude drug before ashing and this sulphated ash is normally less fusible than ordinary ash. The moisture content was determined in reference to air-dried sample by loss on drying method. Extractive value which is an indicative of approximate measures of chemical constituents and nature of the constituents was performed using various solvents.

a. **Determination of total ash value**: Accurately weigh a quantity of sample, representing 2-4gm of air dried material in a tared crucible and incinerate, gently at first and gradually increase the temperature to 675+/- 25^0c, until the sample was free from carbon, and determine weight of the ash. Calculate the % of total ash from the weight of the drug taken.[8]
b. **Determination of acid insoluble ash value:** Boil the ash obtained as dried under total ash, with 25 mL of 3N Hcl for 5min. collect the insoluble matter on a tared filtering crucible or ash less filter wash with hot water, ignate and weigh. Determine % of acid insoluble ash calculated from the weight of drug taken.[5]
c. **Determination of water soluble ash:** To the crucible containing the total ash, added 25mL of water and boiled for 5min. collected the insoluble matter in a sintered glass crucible or on an ash less filter paper. Wash with hot water and ignite in a crucible for 15min at a temperature not exceeding 450^{o}C. Subtract the weight of this residue in milligram from the weight of total ash. Calculate the content of water soluble ash in mg/gm of air dried material.[9]

d. **Determination of sulphated ash:** To the crucible containing the total ash added 1mL of sulphuric acid in order to moisten the residue. It then heated gently over water bath till white fumes. The crucible are transferred to a furnace and ignited at temperature 800+/-25^{o}C. Until all the black particles disappear, cooled in a desiccators, weighed and % sulphated ash was calculated.[1]

e. **Determination crude fibre**: Exhaust a weighed quantity of the test sample, representing about 2gm of drug, with ether. Add 200mL of boiling dilute sulphuric acid (1 in 78) to the ether exhausted marc in a 500mL flask, and the connect the flask to a reflux condenser. Reflux the mark for 30minute, accurately timed, then filter through a linen or hardened paper filter, and wash the residue on the filter with boiling water until the effluent washing is no longer acid. Rinse the residue back into the flask with 200mL boiling NaOH solution adjusted to 1.25 percent by titration and free from sodium carbonate. Again reflux the mixture for 30 minutes, then rapidly filter through a tared filter; wash the residue with boiling water until the last washing is neutral and dry it at 110^{o}C to constant weight. Incinerate the dried residue, ignite to constant weight, cool in a desiccators and weigh the ash, the difference between the weight obtained by drying at 110^{o}C and that of the ash represents the weight of the crude fibre.[5]

f. **Extractive values:** Weighed accurately, about 4 gm of coarsely powdered air dried leaves was macerated with 100mL of solvent in glass stoppered conical flask for 24 hrs shaking frequently during 6 hrs and allow to stand for 18 hrs. It was then filtered rapidly, taking precaution against loss of solvent; the filtrate was transferred and evaporated to dryness in a tared flat bottomed dish. Dried at 105^{o}C for 6hrs and cooled in desiccators for 30min and weighed to a constant weight. The % of solvent soluble extractive value was calculated with reference to air dried drug.

g. **Fluorescence analysis:** Powdered leaf and bark material were treated with various chemical reagents and exposed to visible, ultraviolet light (Short UV) to study their fluorescence behavior.[7]

RESULT AND DISCUSSION

Phytochemical screening

The phytochemical profiling of the leaf revealed the presence of carbohydrate, saponins.

Table 1: Phytochemical screening.

Phytoconstituents	observation	Alcoholic extract	Aqueous extract	Hexane extract
Alkaloids Mayer's test:	Yellow colour precipitate	_	_	_
Wagner's test:	Brown/ reddish precipitate	_	_	_
Hager's test:	Yellow coloured precipitate	_	_	_
Dragendroff's Test	Red precipitate	_	_	_
Carbohydrates: Molisch's Test:	Formation of violet ring at the junction of two liquid	++	++	++
Benedict's Test:	Orange red precipitate	+	_	_
Osazone test	needle-shaped yellow crystals	+	+	_
Detection of gycosides: Modified Borntrager's Test:	Rose- pink colour in the ammoniacal layer	–	–	–
Legal's Test	Pink to red blood colour	+	_	_
Detection of Saponins Froth Test	Formation of 1cm layer of foam	+	+	-
Detection of phytosterols Salkowski's Test:	Golden yellow colour	_	_	_
Libermann Burchard's test	Formation of brown ring at the junction	–	–	–
Detection of phenols Ferric Chloride Test	Bluish black colour	_	_	_
Detection of tannins Gelatin Test:	White precipitate	_	_	_
Flavonoids Alkaline Reagent test:	Formation of intense yellow colour, which become colourless on addition of dil acids	–	–	–
Lead acetate Test	Yellow colour precipitate.	_	_	_
Proteins and aminoacids Xanthoproteic Test:	Formation of yellow colour	_	_	_
Ninhydrin Test:	Formation of blue colour	_	_	_
Diterpenes Copper acetate Test	Emerald green colour	–	–	–

1. Pharmacognostic screening

Macroscopy of leaves

Macroscopically, the fresh leaf of *L.incurva* is 12-15cm long, 4-5cm wide. *L. incurva* leaves are simple, astipulate with opposite phyllotaxy. Shape of the leaf is lanceolate (pointed at both ends), apex is acute - apex ending in a sharp point, base is cuneate - wedge-shaped and it has reticulate system of veins (also called pinnate-netted, penniribbed, penninerved, or

penniveined) – the veins arise pinnately from a single mid-vein and subdivide into veinlets. Leaf margin is entire-undulate; ventral and dorsal surfaces puberulous. Colour of the leaf is dark green and is bitter in taste with slight aromatic odour.

Microscopy of leaves (T.S)

L.incurva leaves are dorsiventral. It contains single layer of upper and lower epidermis with thin cuticle. Mesophyll differentiated into one to two layers of cylindrical cells closely packed with their long axis at right angle to epidermis and spongy parenchyma containing oval, rounded cells loosely arranged towards the lower epidermis, Vascular bundles consist of Xylem and Phloem tisuue. (Figure1)

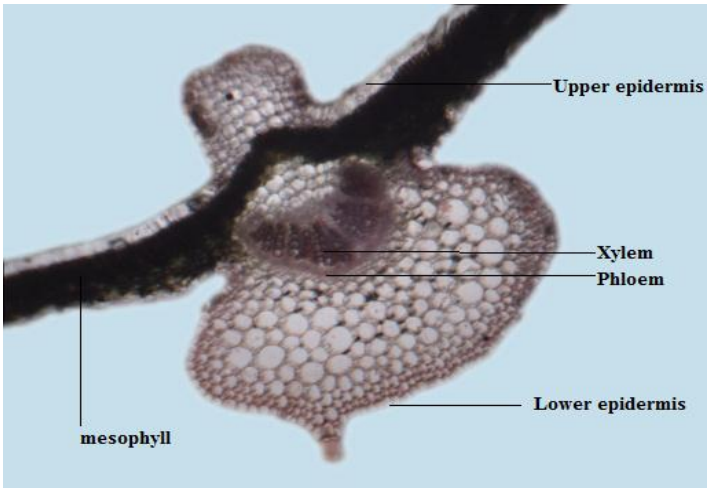


Fig. 1: TS of *L.incurva* leaf.

Leaf constants such as stomatal number, stomatal index, palisade ratio, vein-islet number were measured using camera lucida (table 2). Each stomata is surrounded by 2 subsidiary cells having their long axes perpendicular to the pore, so it is diacytic type, caryophyllaceae.

Table 2: Vein islet number, stomatal number, stomatal index of *L. incurve.*

Plant	Stomatal number	Stomatal index	Vein islet number
Lepidagathis incurva	187	46	13

Microscopy of stem (T.S)

A transverse section of stem of L.incurva showed that outer epidermis consists of single layer of tangentially elongated parenchymatous cells. It was covered with thin cuticle. Cortex was just below the epidermal cell; consist of collenchymatous and parenchymatous cells. Below this 1-2 layerd endodermis followed by thin 1-3 layerd pericycle was seen. Phloem was composed of 2-3 layers of small sized closely arranged cells. Xylem vessels are arranged in

radial row. Lignified xylem parenchyma was also seen. Pith was made up of rounded parenchymatous cells.

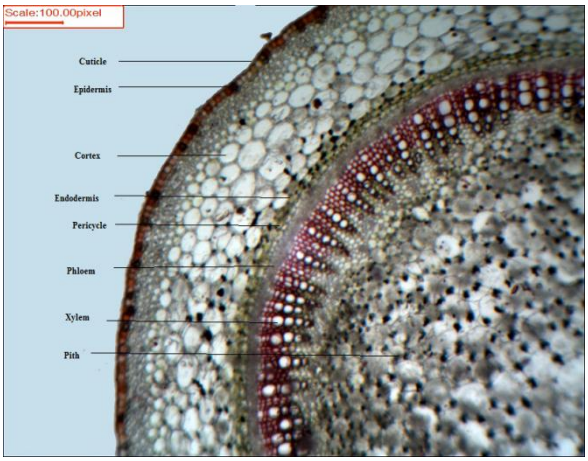
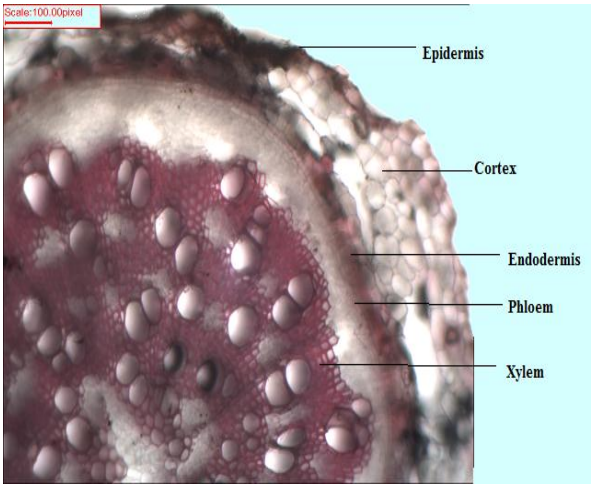


Fig. 2: TS of the stem *L. incurva*.

Microscopy of root (T.S)

Transverse section of root of L.incurva showed an outer one layer epidermis and elongated parenchymatous cortex just below the epidermis. Endodermis consist of one layerd collenchymatous cells. Pericycle was made up of 1-2 layers. Phloem was composed of 1-2 layers of parenchymatous cells. Xylem vessels were reticulated and in between these xylem vessels 3-4 layers of elongated parenchymatous cells were seen.



Physicochemical evaluation: Physicochemical analysis of leaf powder, loss on drying, swelling index, ash value and extractive value are presented in Table 3. The fluorescence analysis of L. incurva leaf under day light and UV (Short and long wave length) light is recorded in Table 4.

Table 3: Loss on drying, swelling index, ash value and extractive value.

Parameters	Values w/w
Loss on drying	11.5
Crude fibre content	12.565
Ash value	
Total ash	21
Acid insoluble ash	6
Water soluble ash	16
Sulphated ash	14.2

Table 4: Extractive values.

Solvents	Plant power taken (gm)	Extractive value (%)W/W
Petroleum ether	4	0.293
Chloroform	4	0.886
n- hexane	4	0.5856
Benzene	4	0.974

Table 5: Fluorescence analysis.

Treatment	Colour in Day light	Colour in short wave length	Colour in long wave length
Powdered leaves	Brown	Dark green	Black
Leaves + H_2SO_4	Dark brown	Green	Black
Leaves + con.HCl	Green	Green	Black
Leaves+ con. HNO_3	Brown	Green	Black
Leaves + $CuSO_4$	Light green	Green	Black
Leaves+ $FeCl_3$	Green	Green	Green
Leaves + iodine	Yellow	Light green	Black
Leaves+ 1N NaOH	Light green	Green	Black
Leaves+leadacetate	Light green	Green	Black
Leaves + KOH	Brown	Green	Brown
Leaves + acetone	Brown	Brown	Black

DISCUSSION

In present study an attempt has been made to establish evaluation of L.incurva through physicochemical and pharmacognostic parameters which could be helpful in identification of the authentic plant. Varieties of adulterants are found in natural drugs. This study describes different laboratory methods for their detection.

The leaves of the plant Lepidagathis incurva were studied for Pharmacognostic characteristics namely, morphology, microscopy, physicochemical parameters like moisture content, ash value such as total ash, acid insoluble ash, Sulfated ash, water soluble ash. Extractive value such as water soluble extractive value and alcohol soluble extractive value,

total fibre content were determined. These help in formulating pharmacopoeial standards for the drug. Ash values of drug give an idea of earthy matter or the inorganic composition and other impurities present along with drug. Extractive values give information about the nature of chemical constituents present in the plant.

The preliminary phytochemical screenings of the crude extracts of leaf of Lepidagathis incurva were carried out in order to ascertain the presence of various secondary metabolites.

CONCLUSION

Presence of carbohydrates, glycosaponins, and iron in lepidagathis incurve were confirmed and estimated. The pharmacognostic standards for leaves, stem and root of L.incurva are carried out for the first time in this study. The macroscopical characters of leaf can serve as diagnostic parameters.

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