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Research Article

Pharmacognostical and Phytochemical evaluation of new species *L. madayiparense* whole plant

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ABSTRACT

Lindernia madayiparense was recently explored new species under the family *Linderniaceae*. Meanwhile mostly lindernia species were considered as weeds in many countries but now only their therapeutic value was identified and investigated in last few decades. Lindernia species occupy a most important place in the traditional medicine used worldwide especially in china. So our current investigation was to produce a standard protocol for the new species by using WHO recommended methods. The macroscopy, microscopy, physiochemical analysis, preliminary phytochemical screening of the whole plant *L.madayiparense* was investigated and reported. Transverse section of leaves confirmed the presence of sunken stomata, poorly developed vascular bundle and parenchyma cells. T. S of quadrangular stem and root showed only few sieve tubes, vessels and trachids in the centre region which is surrounded by large compact aerenchyma cells. Physiochemical constants like ash value, extractive value, fluorescence analysis, behaviour with chemical reagents and its moisture content were studied during the investigation. Qualitative phytochemical analysis of the plant confirms presence of alkaloids, steroidal glycosides, saponins, Coumarins, terpenoids, poly phenol and tannins.

Keywords: *L. madayiparense;* Lindernia; macroscopy; microscopy; physiochemical; phytochemical analysis.

INTRODUCTION

The bio diversity eco system is responsible for variety of plants and animals. Generally, plants and plant derived products play important role in restoring health and heal diseases since ancient time. Plants play an essential role in complementary and alternative medicine due to their capability in forming secondary metabolites. Those phyto constituents make plants a rich source for different type of medicines.

One of rich bio diversity ecosystem found in Madayipara a flat laterite hillock spread in around 365 ha area, located near payangadi in Kannur district, Kerala, South India. This hilly sphere is remarkably rich for valuable medicinal plants which is situated at latitude 12º2' N and longitude 75º16' E and with an altitude of 40-47m from sea level (Madhu et al. 2013). During a recent floristic exploration in the Madayipara hillock area the scientists collected an interesting specimen belonging to the genus Lindernia in 2012 and named as *L. madayiparense* after place where it collected (Ratheesh et al. 2012). *L madayiparense,* aquatic, amphibious annual herb identified in pond sides which is locally used to reduce hyperthermia (excess heat) and burns. Nowhere the plant was reported yet all over the world.

The plant classified under the genus linderniaceae which contributes many medicinally important species. Various species coming under this family Linderniaceae distributed throughout the world. They are incorporated in many medicinal preparations used for heat-clearing, detoxicating, pain relieving, blood cooling, haemostasis, dampness eliminating, and hard mass resolving, bowel relaxing effects. Also used as anti inflammatory and anti tumour agents. Further some of the species report to enhance immunity and normal human resistance against infections and also for the treatment of leathel *Gram (-) coccus, Bacillus tuberculosis, streptococci,* viral infections (Xie et al. 2006 and Kim et al. 1996).

The literature survey revealed that the systemic pharmacognostical and phytochemical studies for this plant was lacking. With this backdrop, the present investigation was aimed to evaluate various pharmacognostical features like microscopy, physiochemical and phytochemical studies for authentication and i dentification of the plant. Hence the standardization processes are consider as an important tool for distinguish the original plant from its adulterants.

MATERIAL AND METHODS

Plant material collection and authentication

A survey was done to find out the *L. madayiparense* in intensive areas of madayipara during the flowering

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season and interrogation with the local people, residential tribal vaidhys etc. Proper methods were followed during the period of plant collection preservation and preparation of herbarium. The collected plant specimen was identified with the help of recent and relevant floras and confirmed after matching with the authentic specimen, housed in M. S. Swaminathan Research Foundation, Kalpetta, Wayanad, Kerala. Collected plant specimen was deposited in the Dept of pharmacy, Annamalai University.

Pharmacognostical studies

Macroscopic and organoleptic characters

Focused knowledge in macroscopy of a plant helps to identify them immediately. The fresh plant and plant material was undergone macroscopic and organoleptic studies for rapid identification.

Fresh plant microscopy (Kokate C K, 1994)

Microscopical studies of leave, stem, root of *L. madayiparense* was done by preparing numerous free thin hand sections. These sections were cleared with chloral hydrate solution and stained with particular staining reagents to identify lignified elements, mucilage and phenolic compounds. After safranin staining, plant sections were mounted in glycerine and placed under various magnifications [10X and 40X] of compound microscope to study the individual anatomical features. Photographs were taken with NIKON camera.

Powder microscopy (Kokate C K et al,1995**)**

To find out the different type of structures individually, the shade dried plant material was pulverized in to coarse powder by using electric grinder, then passed through sieve No. 60 and studied microscopically.

Physiochemical parameters and phytochemical analysis (WHO, 1998)

These parameters were done to prepare a monograph for the plant material. Moisture content, ash value and extractive values were evaluated according to IP. Fluroscecene analysis was also carried out for the powder as per standard procedures.

Determination of moisture content (Sahu1 S K et al, 2012**)**

The moisture content of the powdered plant material was evaluated by loss on drying method using hot air oven. In a thin flat porcelain dish, 5 gm of plant material was transferred and kept in an oven at 105°C. Then the content was weighed for every 1 hr interval until the constant weight was obtained. Moisture content of the plant powder was calculated as a reference to the air dried material.

Fluroscecene analysis (Pravin Sopan Borhade et al, 2014**)**

The plant material was treated with various chemical reagents and the colour change was noticed under ultraviolet light (long and short wave length) and in day light.

Ash value (Yamini K et al, 2011, Harborne J B, 2007 and Kokate C K, 1994)

The dried plant material as transferred to a clean weighed silica crucible. The controlled incineration gradually turns out the plant material into ash residue, which is the mixture of inorganic metallic salts and silica. Once the incineration completed, the crucible was cooled and weighed to determine the total ash value. Then the ash was again subjected to find acid insoluble and water soluble ash value. Ash value directly proportional to the adulterants present in the plant material.

Determination of extraction values (Dhongade H J et al, 2013, Harborne J B, 2007 and Kokate C K, 1994**)**

Presence of diverse phytochemicals insists to estimate the extractive value of plant material with four different solvents viz water, ethanol, ethyl acetate and petroleum ether. Powdered plant material undergone two different extraction procedures i.e. Decoction process water extraction done, continuous soxhlet extraction process was carried out with petroleum ether, ethyl acetate, ethanol solvents. All the extracts were concentrated by using rotary vacuum evaporator. Then all extracts were freeze dried /air dried and weighed to find out the Percentage yield of each extracts.

Preliminary phytochemical analysis (Thakur Nirmala Devi et al, 2011)

The chemical constituents present in the plant make it therapeutically valued. So it is necessary to identify the presence and absence of the primary and secondary phytoconstituents in the different extracts using standard methods.

RESULTS AND DISCUSSION

Macroscopic and organoleptic characters

Morphologically all the lindernia species are in more or less same height app. 20-28cm and having erect four angled stem; whorls are observed in the stem of current investigated plant *L.madayiparense* was not reported in other lindernia species. *L.madayiparense* have sessile and dimorphic leaves with acute or obtuse margins, flowers are axillary, solitary in whitish blue shades with two perfect stamina. An organoleptic character of plant powder was evaluated and the results were given in table no: 1.

Microscopical characters

Anatomy of leaflet

Outermost layer is epidermis composed of tangentially elongated isodiametric cells and covered by thin cuticle. Beneath the epidermal cells a layer of palisade tissue was observed. Cortex is consists of parenchyma cells and partially developed vascular bundle sheath.

Figure 1: T. S of Leaf

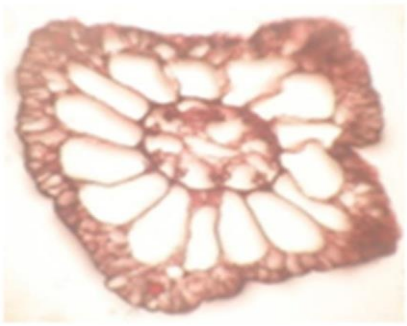

Figure 2: T.S of stem

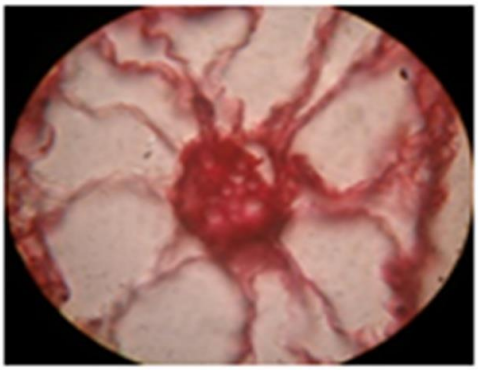

Figure 3: T.S of root

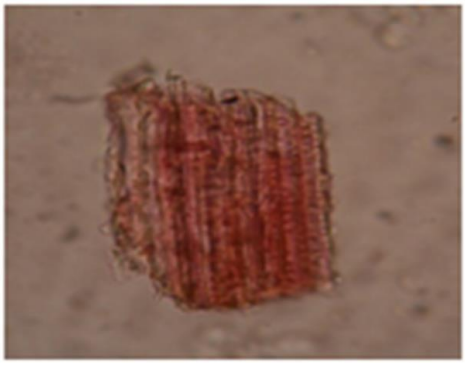

Figure 4: Vascular bundle

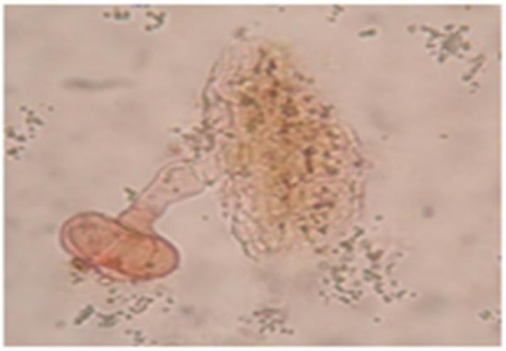

Figure 5: Glandular trichome

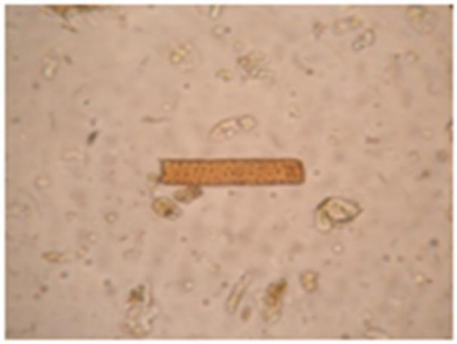

Figure 6: Single sieve tube

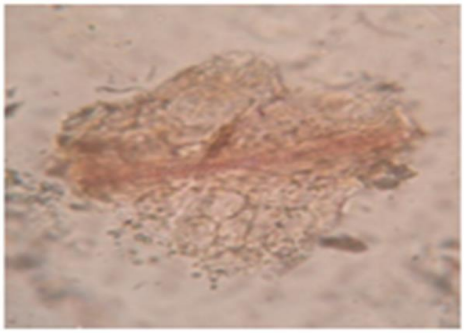

Figure 7: Epidermis

Table 1: organoleptic characteristics of dried plant powder

S.No	Physical properties	Observation
1	Colour	Brown
2	Odour	Unpleasant
3	Taste	Bitter
4	pH	5.8

Table 2: Behaviour of powdered drug with different chemical reagents

S.No	Acid/ Reagent	Observation (Day light)
Acid(concentrated & diluted)		
1	Conc. HCl	Yellow
2	Conc.HNO_3	Yellowish brown
3	Conc.H_2SO_4	Dark brown
4	Glacial acetic acid	Slight yellowish brown
5	Dil. HCl	Watery
Alkali		
8	NaOH(5N)	Slight yellow
9	KOH(5N)	watery yellow
Others		
10	Iodine (N/20)	Slight yellowish brown
11	Picric acid	Yellow
12	$FeCl_3$ (5%)	Brown

Table 3: Fluorescence analysis

S.No	Reagent	Long wave length (366nm)	Short wave length (254nm)
1	Powder + 1N NaOH	Brownish yellow	Brown
2	Powder + 1N NaOH in methanol	Brown	Brown
3	Powder + Ethanol	Slight brown	Slight brown
4	Powder + 50% HNO_3	Yellowish brown	Brown
5	Powder + 1N HCl	Slight yellowish brown	Brown
6	Powder + 50% H_2SO_4	Brown	Brown
7	Powder + Gl. Acetic acid	Brownish yellow	Brown
8	Powder + HNO_3+ NH_3	Dark brownish yellow	Brown

Table 4.1: Ash value analysis

S. No	Parameters	Observation (% W/W)
1.	Total Ash value	4.82
2.	Water soluble ash value	3.42
3.	Acid insoluble ash value	1.40
4.	Sulphated ash value	1.78

Table 4.2: Extractive value analysis

S. No	Extracts	% Extractability
1.	Water (Decoction)	14.42
2.	Ethanol	10.40
3.	Ethyl acetate	5.6
3.	Ether	4.78

Table 5: Qualitative Phytochemical screening

L.madayiparense extracts	Alkaloids	Carbohydrates	Glycosides	Flavanoids	Proteins / Amino acids	Coumarins	Di terpene	Tannins	Phyto sterol	Saponins	Oil / Resins	Poly phenols	Fat
Decoction method													
Aqueous	+	+	+	+	-	+	+	+	+	+	-	+	-
Continuous extraction method													
Pet ether	-	-	-	-	-	+	-	-	-	+	-	-	+
Ethyl acetate	+	+	+	+	-	+	+	+	+	+	-	+	-
Ethanol	+	+	+	+	-	+	+	+	+	+	-	+	-

Vascular system was embedded within the parenchymatous cell and shows only few vessels and sieve tubes confirmed the above fact. Secretary hairs also found along with trichomes also observed. Epidermis consists of sunken stomata which special feature in maximum number of resurrection species.

Anatomy of stem

The stem is quadrangular in outline. Outermost layer is epidermis composed of small vertically oblong cells which are covered by thin cuticle. Single layer hypodermis with compact large aerenchymatous cells was observed underneath the epidermis. Vascul ar bundle was poorly developed with few large vessels and sieve tubes which embedded in the centre of the stem region.

Anatomy of root

Outer layer composed of single layered epidermis covered with thin cuticle. Below the epidermal layer a ring of large compact aerenchyma cells were present. At the centre, the partially developed dense vascular bundle was observed with few vessels, tracheids and small number of sieve tubes.

Powder microscopy

The whole plant powder shows, epidermal fragments, sieve tube and fragments of vascular bundles. The epidermal cells are rectangular in shape. The epidermal peelings are common in the powder consists of glandular trichomes. Pieces of vascular bundles are scattered in the powder and the bundle pattern was exhibit through Microscopical observation.

Physiochemical constant and phytochemical analysis

Determination of physiochemical parameters of a crude drug is essential as it helps in identification and estimation of mishandling, adulteration and also in setting proper standards.

Moisture content of the plant material was estimated and found out as 10.95%W/W.

An ash value gives an idea about the earthy matter or inorganic composition and other impurities or contamination present along with the drug. Ash values were calculated and tabulated in table. Moisture content was also found out and given in table 4.1.

Extractive value was primarily useful for the determination of exhausted or adulterated drug and to evaluate the chemical constituents of crude drug. Extractive capacity of different solvents gives variable extractive value according to the polarity and amount of phytoconstituents present in the plant material. The percentage yield of each extracts were calculated and given in table 4.2.

Generally the phytoconstituents treated with different reagents colour change was observed in ultraviolet light. This colour change was observed due to the chemical reaction between the particular reagents with the chemical constituents present in the sample. Colours observed for each reagent were observed and mentioned in the table 2 and 3.

The solvents are solubilise the chemical constituents present in the broken plant tissues and extract them based on their chemical nature .The amount of phytoconstituents is present in each extract was di rectly proportional to extractability and polar nature of the solvents used.

Pet ether chooses to be the first for extraction process which highly extracts the non polar compounds because to minimise the interference of such compounds from further extraction. The extraction process extended based on the polarity of solvents i.e. low polar to high polar in continuous hot percolation method. High and medium polar constituents are completely extracted in decoction process using universal solvent above 100°C.

Pet ether extract shows positive results for Coumarins, saponins, terpinoids and fat identification tests. The ethanol, ethyl acetate and aqueous extracts were reveals the presence of various phyto constituents like alkaloids, steroidal glycosides, saponins, di-terpenes, Coumarins, tannins, phyto- sterol and poly phenols but percentage yield of each extract was varied.

CONCLUSION

Standardization techniques play an important role in identification and authentication of the original therapeutic plant/ plant material/ crude drug through simple methods. Lack of perfect protocol for the identification and authentication of *L. Madayiparense* encourage us to investigate the same by microscopy and chemical analysis. The Microscopical analysis helps to set micro morphological standards and physiochemical analysis helps to authenticate genuine plant material as per WHO guidelines. Thus the present investigation was aimed and results were found to be significant which helps in identification, standardisation and authentication of plant in future research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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