

PHYSICO-CHEMICAL EVALUATION, PRELIMINARY PHYTOCHEMICAL INVESTIGATION, FLUORESCENCE AND TLC ANALYSIS OF LEAVES OF THE PLANT *LASIA SPINOSA* (LOUR) THWAITES

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ABSTRACTS

Objective: To investigate the physico-chemical parameter, preliminary phytochemical screening, fluorescence and Thin Layer Chromatographic analysis of the leaves of the plant *Lasia spinosa* (Lour) Thwaites.

Methods: The fresh and powder leaves of the plant *Lasia spinosa* (Lour) Thwaites (Araceae) were studied by morphology, preliminary phytochemical screening, and fluorescence analysis of powdered drug. Other physicochemical parameters were also performed as per WHO guide lines.

Results: The dried powder leaves were investigated by morphology. The results of physico-chemical parameters such as loss on drying and ash values, extractive values, preliminary phyto-chemical screening, percentage of extractive values fluorescence analysis and TLC are in the table no of 1, 2, 3, 4, 5 and 6 respectively.

Conclusion: The present information on the pharmacognostic evaluation of the plant drug *L. spinosa* delivered the qualitative and quantitative parameters serve the important information to the identity and to determine the quality and purity of the plant material in the future. It also signify the important information of the closely related other species and varieties.

Keywords: *Lasia spinosa*, Physico-chemical evaluation, Phyto-chemical screening, Quality control test.

INTRODUCTION

Evaluation of drug means confirmation of its identity and determination of its quality and purity and detection of nature of adulteration. The evaluation of a crude drug is necessary because of these main reasons i) biochemical variation in the drugs ii) deterioration due to treatment and storage, and iii) substitution and adulteration, a result of carelessness, ignorance or fraud.

Over the years the nature and degree of evaluation of crude drugs has undergone a systematic changes. Initially, the crude drugs were identified by comparison only with the standard description available. Due to advancement in the chemical knowledge of crude drugs, at present, evaluation also includes method of estimating active constituents present in the crude drug, in addition to its morphological and microscopic analysis. With the advent of separation techniques and instrumental analysis, it is possible to perform physical evaluation of a crude drug, which could be both of qualitative and quantitative in nature [1]. The plant may be considered a biosynthetic laboratory not only for the chemical compound such as carbohydrate, proteins and lipids that are utilized as food by man but also for a multitude of compounds like glycosides, alkaloids, volatile oils, tannins etc. that exerts a physiologic effect. The compounds that are responsible for therapeutic effect are usually secondary metabolite. The plant material may be subjected to preliminary phyto-chemical screening for the detection of various plant constituents. [2]

Lasia spinosa is belonging to the family Araceae. The family Araceae consists of approximately 115 genera and 16 species are aquatic plants. Plants of family Araceae are perennial herbs with watery, bitter or milky juice and usually an elongated or tuberous rhizome. Leaves are mostly in basal or apical rosettes with a membranous sheath at the base of the petiole. Flowers are small, crowded on a usually fleshy spike and usually enclosed in a large bract. They can be bisexual or unisexual. If they are unisexual, the males in the upper part of the spadix whereas the females below. Fruits are usually a fleshy berry, occasionally a spongy berry, a nut let or a capsule [3].

Lasia spinosa is distributed in India, Sri-Lanka, South-east Asia and Malaysia [3, 4 &5]. It is known as *Chengmora* by the local people of

Assam. Plants are aquatic or terrestrial, shot-stemmed spiny heirs with underground rhizome. *Lasia spinosa* occurs usually in wet forests, open marshes, wetlands or in permanently standing water [4]. *Lasia spinosa* is a large marsh plant with the stem stout 1 m high and the leaves broadly arrow-shaped in outlines, 20-30 cm long deeply divided into 4-6 pairs of narrow side lobes. The petiole is 30-40 cm long, veins beneath the petiole and peduncle prickly [6].

The tuber of *Lasia spinosa* (L.) Thwaites are used for treatment of rheumatoid arthritis, constipation, and to purify blood in Rajshahi and Natore district of Bangladesh [7]. The young tender leaves of *L. spinosa* are used to treat intestinal worms' infections in folk medicine of Naga tribes of India [8]. *Lasia spinosa* rhizome possessed a wide-ranging antioxidant capacity [9], antimicrobial property and cytotoxic activities [10]. The leaves are used as anticestodal agent. [11]. Scientific parameters are not yet available to identify the exact plant material to confirm its quality, purity and various pharmacognostic parameters. The present study on this plant was therefore undertaken to determine the pharmacognostical standards for evaluating the plant material. Various investigation like organoleptic parameter, various physico-chemical evaluation like ash value, extractive value, loss on drying and phyto-chemical screening test. Thin layer chromatography profiling and fluorescence analysis of powdered crude drug were carried out and some salient qualitative as quantitative parameter were mentioned.

MATERIAL AND METHOD

Plant material

The whole plants of *Lasia spinosa* were collected from the nearby forest of Dibrugarh (Assam) in the month of August-September 2012. The rhizome, shoot and leaves were dried under shade for 15 days, coarsely powdered and stored in air tight container for the further study.

Plant profile

Taxonomical Classification-[12]

Botanical name: *Lasia spinosa*

Kingdom: Plantae

Phylum: Maglinophyta

Class: Liliopsida

Order: Alismatales

Family: Araceae

Genus : Lasia lour

Species: spinosa

Common name

Assamese: Chengmora

Bengali: Kata-kachu

Manipuri: Janum-saru

Mizoram : Zawangzang

Sanskrit : Lakshmana



Fig. 1: It shows the leaf shoot of the plant *L. spinosa* (L.)

Reagent and Chemicals

All reagents and chemicals used for pharmacognostic evaluation and phyto-chemical screening were analytical grade obtained from SRL Chemical, Rankem, Otto, Himedia Pvt Ltd.India.

Organoleptic Evaluation

The leaves and rhizomes are collected from the surrounding environment. The fresh leaves are deep green in color, the rhizomes are grayish brown in color. The leaves and rhizomes were separated from the other parts of the plant (stem & shoot of leaf) cleaned manually and kept over dry plastic sheet to investigated different organoleptic features. The magnifying glass and scale were used to measure the parameter like morphology, length, width etc. The dried rhizomes were also subjected for the organoleptic evaluation.

Physico-chemical evaluation

Physico-chemical parameters such as the percentage of loss on drying (LOD), total ash, acid insoluble ash, water soluble ash were determined as per the Indian Pharmacopoeia. [13]

Water and alcohol soluble extractive were estimated by cold maceration according to the method prescribed by WHO [14]. All the parameters were taken in triplicate and the result which was obtained presented as mean \pm standard error of mean (SEM).

Phyto-chemical Screening

The dried and powdered leaves were subjected to preliminary phyto-chemical screening for qualitative detection of phytoconstituents. The dried and coarsely leaves sample (50 gm) was extracted successively with petroleum ether (60-80°C), chloroform, ethyl acetate, methanol and water in a soxhlet extractor by continuous hot percolation. Finally the marc was macerated with chloroform water. Each time before extracting with the next solvent of higher polarity the powder drug (marc) was dried in a hot air oven below 50°C for 10 minutes. Each extract was concentrated by distilling off the solvent, which was recovered subsequently. The concentrated extracts were evaporated to dryness and the extracts obtained with each solvent were weighed. Their percentage was calculated in terms of initial air dried plant material. The colors of

extracts were observed. The successive extract, as mentioned above, were subjected to various qualitative phyto-chemical test for the identification of chemical constituents present in the plant material. [2,15]

Fluorescence Analysis

A small quantity of dried and finely powdered leaves sample was placed on a grease free microscopic slide and added 1-2 drops of freshly prepared solution, mixed by gentle tilting the slide and waited for 1-2 minutes.

Then the slide was placed inside the UV viewer chamber and viewed in day light, short (254 nm) and long (365 nm) ultraviolet radiations. The colors observed by application of different reagents in various radiations were recorded [16, 17].

Thin Layer Chromatographic Analysis

The chromatographic methods which are presently available, Thin Layer Chromatography are widely used for the rapid analysis of drugs and drug preparations. There are several reasons for this.

- The time required for the demonstrations of most of the characteristic constituent of a drug by TLC is very short.
- In addition to qualitative detection, TLC also provides semi-quantitative information on the major active constituent of a drug or drug preparation, thus enabling an assessment of drug quality.
- TLC provides a chromatographic drug finger print. It is therefore suitable for monitoring the identity and purity of drugs and for detecting adulterations and substitutions.
- With the aid of appropriate separation procedures, TLC can be used to analyze drug combinations and phytochemical preparations [18].

Preparation of Leaf Extract

The dried leaves powder was extracted with methanol by hot percolation method. The soxhlet apparatus was used in this procedure. After filtration the resultant extract were collected in a clean dry glass beaker. The liquid extract was heated over water bath to evaporate the solvent for 30-40 minutes. The semi-solid extract is taken for TLC analysis and before applying spot the extract was diluted with little amount of methanol.

Stationary Phase

Silica gel G, particle size 10 – 40 μ m applied as a thin layer on a clean glass plate support and activated (110°C for 30 minutes) just before use.

Mobile Phase

Quantity – 50 ml

The mobile phase was – Formalin: Methanol = 8:2

Development Method

One dimensional ascending method by using standard protocol as per IP was followed. [13]

Visualization

After development the TLC plate, initially three spots were visualized in UV chamber (365 nm)..

Documentation

After visualized in UV chamber the spots were yellow, yellowish green and violet in color. The R_f value were measured correctly and carefully and the chromatogram was presented in the tabulated form.

RESULTS

Organoleptic Evaluation

The characters recorded are described below.

Fresh leaves

Shape of leaf: Large and palmate shaped.

Dimensions:

Color: The upper surface of the leaf deep green and the reverse side light green.

Odor: characteristic.

Taste: nasty bitter. An earthy sensation over the tongue lasting for 5-7 minutes.

Rhizomes

Condition: Pricky and hard in type.

Shape: Round and tuberous.

Dimensions:

Color: Earthy brown. The inner surface after cutting showed blackish brown in color.

Taste: bitter in taste.

Fracture: Fibrous.

Physico-Chemical Evaluations

The values of all determinations are summarized in table 1 & 2. In this evaluations the amount of water soluble ash is lesser than acid insoluble ash, where as the amount of total ash was nearly double of their water soluble ash.

Phytochemical screening

The results are shown in table no.3 these results represents the presence the alkaloids, carbohydrates, saponins, glycosides, tannins, flavonoids etc in the leaves of *L. spinosa*. The extractive constituents present in the different solvent extracts are tabulated in the table no 4.

Fluorescence Analysis

The results were summarized in table no 5.

Thin Layer Chromatographic Analysis

The leaves extract showed three distinct spots with different intensities. The colors are yellow, yellowish green and violet in color respectively. The resultant R_f values were summarized in table no 6.

Table 1: It shows loss on drying (LOD) and ash values of powdered leaves of *Lasia spinosa*(L.) Thwaites

Parameters	Values of three replicates (% w/w)	Mean (% w/w) \pm SEM
Loss on drying (LOD)	24.2	24.2 \pm 0.05777
	25	
	23.4	
Ash values: 1) Total ash	16.34	16.90 \pm 0.32334
	17.34	
	17	
2) Acid insoluble ash	9	9.12 \pm 0.4332
	9.67	
	8.67	
3) Water soluble ash	7	7.68 \pm 0.39255
	8.34	
	7.67	

SEM = Standard Error of Mean

Table 2: It shows extractive values of powdered leaves of *Lasia spinosa*(L.) Thwaites:

Method of extraction	Values of three replicates (% w/w)	Mean (% w/w) \pm SEM
Cold maceration: 1) Water soluble	11.2	11.134 \pm 0.038105
	11.6	
	10.6	
2) Alcohol soluble	3	3.4 \pm 0.73029
	3.8	
	3.2	

SEM = Standard Error of Mean

Table 3: It shows phyto-chemical screenings of successive extracts of powdered leaves of *Lasia spinosa*(L.) Thwaites:

Constituents	Pet. Ether extract	Chloroform Extract	Ethyl acetate extract	Methanol Extract	Aqueous extract
Alkaloids	-	-	-	+	+
Carbohydrates	-	+	+	+	+
Fats and oil	+	-	-	-	-
Flavonoids	-	-	+	-	-
Glycosides	+	+	+	-	+
Gum	-	-	-	-	-
Lignins	-	-	-	-	-
Proteins	-	-	+	+	-
Saponins	-	-	-	+	+
Tannins and Phenolic compounds	-	+	+	+	+

+ = Present & - = Absent

Table 4: It shows percent extractives and colors of successive extracts of powdered leaves of *Lasia spinosa*(L.) Thwaites:

Solvents	Extractive values (% w/w)	Colors of extracts
Pet. Ether extract	1.55	Brownish black
Chloroform Extract	2.78	Light black
Ethyl acetate extract	1.6	Black
Methanol Extract	2.9	Green
Aqueous extract	3.1	Dark brown

Table 5: It shows fluorescence analysis of powdered leaves of *Lasia spinosa*(L.) Thwaites

Powdered drug	Visible/Day light	UV 254 nm (short)	UV 365 nm (long)
Powder drug as such	Light brown	Brown	Yellowish brown
Powder + Methanol	Light brown	Yellowish brown	Brownish black
Powder + 1% glacial acetic acid	Brown	Dark brown	Blackish brown
Powder +10% NaOH	Yellowish brown	Dark yellowish brown	Bluish brown
Powder + dil. NH ₃	Yellowish brown	Light brown	Brown
Powder + Conc. HNO ₃	Brown	Blackish brown	Dark brown
Powder+ dil.NH ₃ +Conc.HNO ₃	Yellowish brown	Light brown	Blackish brown
Powder +1M H ₂ SO ₄	Brown	Dark brown	Yellowish balck
Powder +1M HCl	Brownish yellow	Brown	Dark brown
Powder + 10% FeCl ₃	Reddish brown	Light brown	Brownish yellow
Powder +Acetone + Methanol	Light brown	Brown	Black
Powder +10% Iodine	Yellowish brown	Dark brown	Blackish brown

Table 6: It shows Thin Layer Chromatographic Analysis of powdered leaves of *Lasia spinosa*(L.) Thwaites:

Extracts	Mobile phase	No. of spots	Rf. values	Intensity
Leaves	Formalin: Methanol = 8:2	3	0.75 0.55 0.40	+++ + +

+++ = Most intense, + = Least intense

DISCUSSION

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus in recent years there has been an emphasis in standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive means. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken [14]. Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs [1]. The Organoleptic studies shows the important characteristics of the drugs, the structure of the leaves, the hairy surface of the leaves, the typical tongue sensation and the odour may screen the preliminary phytochemical constituents. The percentage of active chemical constituents in crude drugs is mentioned on air-dried basis. Therefore, the loss on drying of plant materials should be determined and the water content should also be controlled. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water. The test for loss on drying determines both water and volatile matter [1, 14].

The residue remaining after incineration of plant material is the ash content or ash value, which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a form of adulteration. The ash value was determined by three different methods, which measured total ash, acid-insoluble ash, and water-soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition. This includes both 'physiological ash' which is derived from the plant tissue itself, and 'non-physiological ash', which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Water-soluble ash is the water soluble portion of the total ash [1, 19]. These ash values are important pharmacognostic tool to standardized the crude drugs. The extracts obtained by exhausting plant materials with specific solvents are indicative of approximate measures of their chemical constituents extracted with those solvents from a specific amount of air-dried plant material. This parameter is employed for materials for which as yet no suitable chemical or biological assay exists [13, 14]. By following the cold maceration method, the yield of water soluble extractive is greater than acid insoluble extractives. The plant material was subjected to preliminary phytochemical screening involving successive solvent extraction by different solvents in

order of increasing polarity to obtain diverse polar and non polar phytoconstituents possessing different solubility pattern, followed by various chemical tests for qualitative detection of various chemical constituents [1, 2]. The phytochemical screening showed that the methanol and aqueous extracts contained alkaloid, the carbohydrates and the phenolic compounds were present in all of the solvent extract except petroleum ether extract. The chloroform, ethyl acetate and the aqueous extract contained glycosides where as the saponins present in methanol and aqueous extract. The ethyl acetate extract contain only the flavonoids.

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation [16, 20].

Thin layer chromatography is an important analytical tool for the separation, identification and estimation of different classes of natural products [2]. In the phytochemical screening (Table no- 3) which showed that the alkaloids, saponins and tannins were present in the methanolic extract.

The three spots which were given after the evaluation of the TLC plate (methanolic extract) may contain the presence of alkaloids, saponins, and tannin derivatives. Quality parameters are carried out on plant samples in order to establish appropriate data that can be used in identifying crude drugs particularly those supplied in powder form [21].

CONCLUSIONS

The present pharmacognostic data emphasize the knowledge of quality and identity of the plant *L. spinosa*. The qualitative and quantitative parameters serve the important information of the plant *L. spinosa*. The plant being a morphologically variable species, these information will also be helpful to differentiate *L. spinosa* from the closely related other species and varieties of *Lasia*.

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