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Pharmacognostic and preliminary phytochemical studies on shoot of *Calamus leptospadix* Griff.- An ethnomedicinal plant of Assam

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Calamus leptospadix Griff. belonging to family Arecaceae, is an ethnomedicinal plant of Assam used by the folks of state for many medicinal properties like anthelmintic, hepatoprotective, and antidiabetic. Its vernacular names include *Betgaj*, *Lejaibet*, *Bet, Bethas*, *Rattan palm*, *Cane*, *Tangtangma*, *Titipi*, *Jeyying* and *Ayyagoomi*. Since there are no reports of systemic pharmacognostic and phytochemical studies on the shoot, the present work was planned to study the detailed macroscopical, microscopical, quantitative standards, physicochemical and chromatographic characteristics of the shoot. Preliminary phytochemical investigation indicated the presence of alkaloids, carbohydrates, glycosides, flavonoids, steroids, saponins, tannins and phenolic compounds. Fluorescent characteristics were analysed for the powdered crude drug and TLC profile was developed for petroleum ether, chloroform and methanolic extract of the shoot. Thus these conventional parameters would serve as a standard reference for identification, authentication and for distinguishing the plant from its adulterants.

Keywords: *Calamus leptospadix*, Microscopy, Shoot extract, Fluorescent, Authentication.

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Introduction

Calamus leptospadix Griff. is a clump-forming, short, water-loving, dioecious, forest under storey, high climber, but, when growing along damp river plains it tends to form thickets. It has spiny, green stems, 6 m tall, 5 cm diam. with no obvious leaf scars and huge segmented, pinnate (feather) leaves, 4 m long and 1.2 m wide, green above and beneath. This plant is widely distributed in Bangladesh, Bhutan, North eastern India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Sikkim, Tripura, West Bengal), Myanmar (Kachin) and Nepal; mostly on lowland or mountain rainforest along river margins to 1300 m elevation[1-4].

In Assam it is traditionally believed by the folk people that the tender shoots, leaves and seeds of this plant are used as vermicide. In Ayurvedic system *Calamus* species are used in fever, piles, dyspepsia, biliousness; flowers are used as antiseptic, anti-bacterial, externally for cuts, burns, bruises and scalds[5]. Young shoots of the plant are used as vegetable[6]. This plant is ethnomedicinally well recognized however no detailed study or published reports along with scientific evidence are available with this important plant.

Since there are no reports of systemic pharmacognostic and phytochemical studies on the shoot, the present work was planned to study the detailed macroscopical, microscopical, physico-chemical and chromatographic characteristics of the shoot of which, would serve as a standard reference for identification, authentication and for distinguishing the plant from its adulterants.

Materials and Methods

Plant Material

The shoots of *C. leptospadix* were collected from Jokai, Dibrugarh, Assam, India during the month of July, 2012. The plant was identified and authenticated by Dr. N Odyuo, Botanical Survey of India, Eastern Regional Centre, Shillong. A voucher specimen (Plant Specimen no. Du/SB/2012/06, Plant Reference no. BSI/ERC/2013/Tech/Plant identification/636) has been kept in Department of Pharmaceutical Sciences, Dibrugarh University, Assam for future references. Young and tender shoots were cut into pieces, washed thoroughly with water and then dried partially under sunlight and

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partially under the shade for a week. The dried shoot pieces were then pulverized in a mechanical grinder to coarse powder and then stored in airtight containers free from moisture.

Macroscopy

The macroscopy of crude drug includes its visual appearance to the naked eyes and its sensory characteristics i.e. odour, taste and touch. Simple microscope of magnification 10 X was also used for the perception of special structural features[7]. Macroscopic studies of *C. leptospadix* shoot were also done.

Microscopy

For microscopic study, young and tender shoot of the plant were collected and washed with water. Thin sections were cut both transversely and longitudinally, boiled in 10 % potassium hydroxide solution to remove the fatty matters, colouring substances, etc., stained with safranin and observed under light microscope. Photomicrographs were taken with Leica Digi 3 Photomicrographic unit at 40 X magnification[8-11].

Powder characteristics

The dried bark was subjected to size reduction to get coarse powder which was further reduced to uniform powder of 40 mesh size. The sieved powder was mounted on a glass slide and seen under microscope at 15 x 10X magnification of a trinocular microscope. Preliminary tests, fluorescence analysis and reaction of the powder towards various chemicals were examined.

Quantitative standards

Percentage of Total Ash, Acid insoluble Ash, Water soluble Ash, Extractive values, Loss on Drying and Foaming Index were examined[11]. The Acid values, Saponification values and Ester values of the powdered drug were calculated as per the standard methods as mentioned in the Indian Pharmacopoeia, 2007[(Ref 12)].

Fluorescence analysis

The behaviour of the powdered shoots with different chemical reagents and their fluorescent characteristics were observed under ultraviolet (254 and 366 nm) and visible light[13-14]. Quantitative fluorescence analysis using the fluorescence produced by a compound in UV light for quantitative evaluation is possible with the help of an instrument Fluorimeter or spectrofluorimeter[9]. The instrument used for detecting fluorescence was UV-Viewer Ultraviolet Fluorescence analysis cabinet, MAC® Macro Scientific Work.

Preliminary phytochemical studies

For preliminary phytochemical studies; the dried powdered bark was defatted by extraction with petroleum ether (40-60°C). The marc was dried and extracted successively with chloroform, methanol, hydroalcoholic (ethanol: water=70: 30) using a hot Soxhlet extractor. The extracts were concentrated under vacuum in rota evaporator at 50°C, dried and stored in desiccators for further use. The different extracts obtained by successive solvent extraction were tested separately for the presence of various phytoconstituents, viz. alkaloids, amino acids, carbohydrates, fats and fixed oils, flavonoids, glycosides, saponins, gums, lignins, proteins, steroids, triterpenoids, tannins and phenolic compounds[9-11]. The TLC of the extracts was done using Silica Gel G as adsorbent. Glass plates of 5 x 20 cm size were coated with silica gel G (Himedia Laboratory, Mumbai, India) with the help of spreader to a layer thickness of 0.25 mm. After spreading the plates were first air dried and then activated at 110ºC for 30 min. After cooling, the plates were kept in desiccators until required for further use[15-17]. Mobile phases selected were as follows:

i. Chloroform : Methanol : Glacial acetic acid (2.5: 1.5 : 0.5)
ii. Toluene : Ethanol : Diethyl amine (3:1:1)
iii. n- Butanol : Glacial acetic acid (4 : 1)
iv. Chloroform : Ethyl acetate (4.5 : 0.5)
v. Chloroform : Ethyl acetate (4 : 1)
vi. Toluene : Ethyl acetate (4.5 : 0.5)

Results and Discussion

Macroscopy

The shoot is greenish white in colour in fresh form and brownish in dried form. It has a characteristic odour, bitter in taste. The shoots are long rod shaped with 45-50 cm in length and 1.2-2 cm in thickness with sheath and 1-1.2 cm without sheath. It is smooth in texture without the sheath. A photograph of the edible shoot part of *C. leptospadix* that was collected is shown in Plate 1.

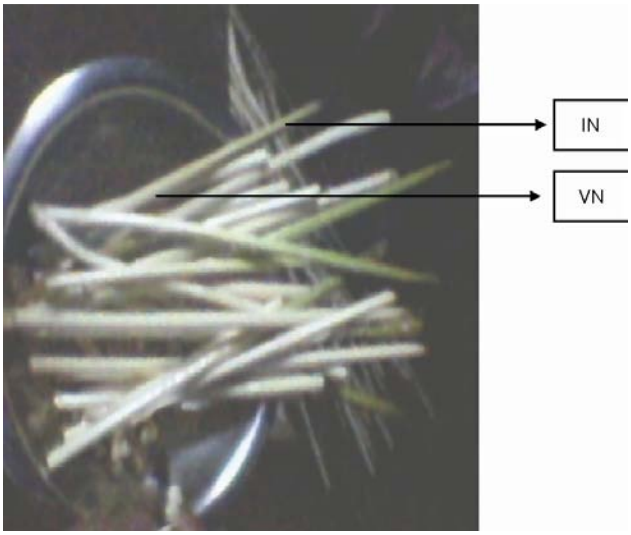

Plate 1-Edible shoot part of *Calamus leptospadix* Griff.- IN- Internode, VN- Vegetative node.

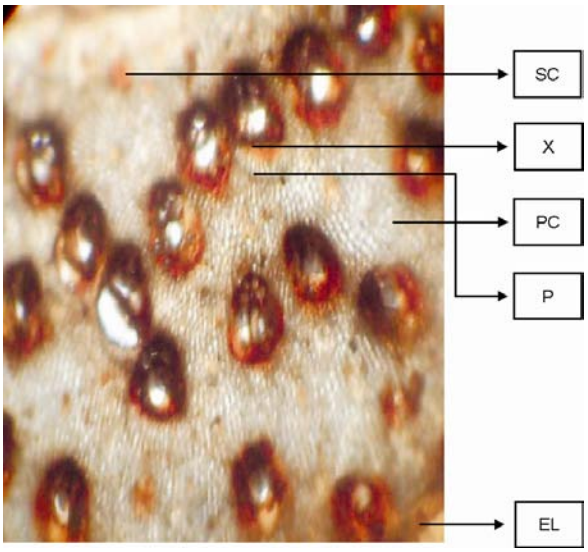

Plate 2-Transverse section of the shoot of *Calamus leptospadix* Griff.-SC- Stone cells; X- Xylem; PC- Parenchymatous cells; P-Phloem; EL-Epidermal Layer.

Microscopy

The transverse section of the shoot showed the presence of stone cells, xylem, phloem, epidermal layers, parenchymatous cells and longitudinal section showed the presence of longitudinal sclereids and pericyclic fibres as shown in Plates 2 & 3.

Determination of quantitative standards

The purpose of standardization of medicinal plant product was to ensure therapeutic efficacy. Standardisation is essentially a measure to ensure the quality control of herbal drugs[9].

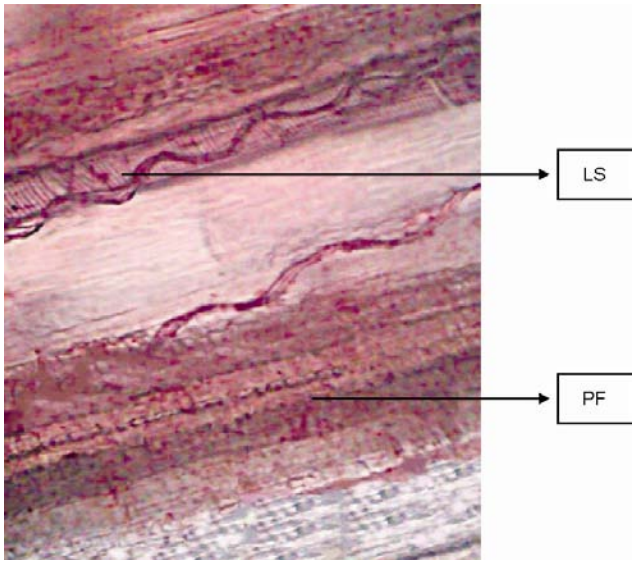

Plate 3-Longitudinal section of the shoot of *Calamus leptospadix* Griff. LS- Longitudinal sclereids; PF- Pericyclic fibres.

Table 1 - Ash values, extractive values and loss on drying of powdered shoot drug of *Calamus leptospadix* Griff. expressed as Mean ± SEM

S. No.	Parameters	Mean ± SEM of shoot of *.C leptospadix*
1	Total ash	12.87 ± 0.233
2	Acid insoluble ash	6.9 ± 0.3055
3	Water soluble ash	4.53 ± 0.5812
4	Water soluble extractive	4.9± 0.458
5	Alcohol soluble extractive	5.8± 0.3434
6	Loss on drying	11.8 ± 0.200

It was found that the total ash was 12.87 % w/w, acid insoluble ash and water soluble ash were 6.9 % w/w and 4.53 % w/w, respectively. Other parameters such as loss on drying, water and acid extractive values were studied in the young and tender shoot of *C. leptospadix* Griff. and their results are shown in Table 1. Ash value is an important parameter as it helps to determine the percentage of inorganic substance present in raw materials. Quantitative standards are a number of standards numerical in nature which can be applied for the evaluation of crude drug either in the whole or powdered form.

Determination of Foaming index

The height of foam of 1 cm was measured in the 9th tube; the volume of the plant material decoction in this tube (a) was used to determine the index which was found to be 111.11. The drug contains saponins and has the capability to form froth which depends on the quantity of saponins present. Apart

from this, the saponification, acid and ester values were determined, the results of which have been mentioned in Table 2.

Fluorescence analysis

Powdered drug was treated with different reagents and examined under daylight and UV light (254 & 366 nm) and the results are shown in Table 3.

Preliminary phytochemical tests

The different extracts obtained by successive solvent extraction were petroleum ether, chloroform, methanolic and hydroalcoholic as shown in Plate 4. These are semi-solid in nature with a characteristic odour and dark-brown in colour except the methanolic extract which is brownish orange. These extracts were tested separately for the presence of various phytoconstituents viz., alkaloids, amino acids, carbohydrates, fats and fixed oils, flavonoids, glycosides, saponins, gums, lignins, proteins, steroids, triterpenoids, tannins and phenolic compounds. The procedures for the tests followed were as per guidelines[9, 11]. The results of the different

Table 2- Acid values, saponification values and ester values of powdered shoot drug of *Calamus leptospadix* Griff. expressed as Mean ± SEM

S. No.	Parameters	Mean ± SEM of shoot of castor oil	Mean ± S.E.M of shoot of *C. leptospadix*
1.	Acid value	3.296 ± 0.662	2.54±0.326
2.	Saponification value	125 ± 2.887	92.3±1.453
3.	Ester value	121.7± 3.548	90.15±1.319

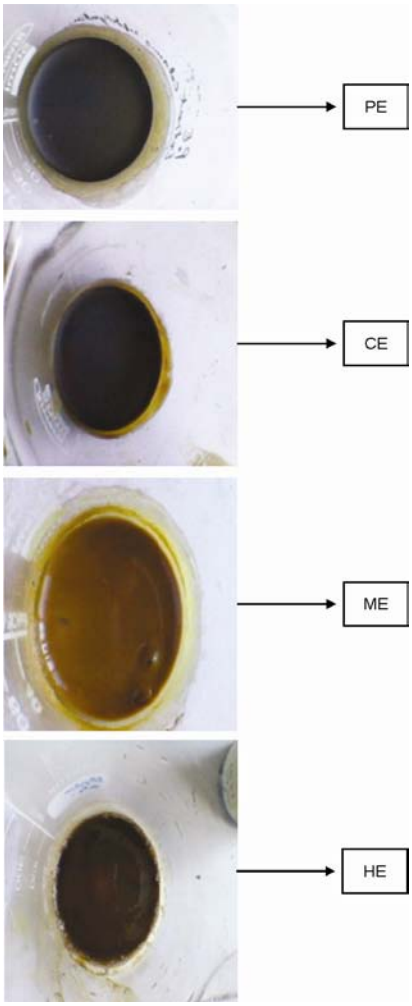

Plate 4- Physical characteristics of shoot extracts of *Calamus leptospadix* Griff. (a) PE- Petroleum ether; (b) CE- Chloroform extract; (c) ME- Methanolic extract; (d) HE- Hydroalcoholic extract.

Table 3- Fluorescence analysis of powdered shoot drug of *Calamus leptospadix* Griff.

S. No.	Treatment	Daylight	UV Light	
			254 nm	366 nm
1	Powder as such	Light Brown	Light Green	Greenish Brown
2	Powder + Acetic acid	Whitish Brown	Creamish	Creamish Brown
3	Powder + Ferric chloride (5%)	Blackish Brown	Brown	Black
4	Powder + Conc. Hydrochloric acid HCL (5N)	Creamish Brown	Brown	Creamish
5	Powder + Conc. Nitric acid (HNO_3)	Reddish Brown	Brown	Black
6	Powder + Conc. Sulphuric acid (H_2SO_4)	Reddish Black	Black	Bluish
7	Powder + Iodine Solution (1%)	Brownish Black	Brown	Brownish Black
8	Powder + Methanol	Brown	Brown	Black
9	Powder + Picric acid	Yellowish Brown	Deep Greenish Yellow	Black
10	Powder + NaOH Solution(1N)	Reddish Yellow	Yellow	Blackish Yellow
11	Powder + Distilled water	Deep brown	Brown	Greenish
12	Powder +Liquid Ammonia (NH_3)	Blackish Yellow	Yellow	Black
13	Powder + Conc. HNO_3+ NH_3	Yellow	Reddish	Yellowish Black
14	Powder + Dil. HNO_3	Reddish Brown	Brown	Blackish
15	Powder + 10% Potassium dichromate solution	Deep Brown	Brown	Black Brown
16	Powder + Benedict's Reagent	Yellowish Blue	Yellowish	Greenish Black
17	Powder + Acetone	Brown	Light Brown	Blue

Table 4- Preliminary phytochemical tests of shoot extracts of *Calamus leptospadix* Griff.

Plant constituents	Petroleum-ether extract	Chloroform extract	Methanolic extract	Hydro- alcoholic extract
Alkaloids	-	+	+	-
Amino acids	-	-	-	+
Carbohydrates	-	+	+	+
Fats and Oils	+	+	+	-
Flavonoids	-	+	+	+
Glycosides	-	+	+	+
Gums	-	-	-	-
Lignin	-	-	+	+
Proteins	-	-	-	+
Steroids	-	+	+	+
Triterpenoids	-	-	+	+
Saponins	-	+	+	+
Tannins and Phenolic Compounds	-	-	+	-

(+) means present and (-) means absent.

Table 5- Thin layer chromatography (TLC) of shoot extracts of *Calamus leptospadix* Griff.

S. No.	Chromatography Solvents	Extracts	Number of spots detected	R_f Values	Visualising Agents used
1	Chloroform : Methanol : Glacial Acetic Acid (2.5 : 1.5 : 0.5)	Petroleum ether	2	0.32, 0.45	Iodine
2	Toluene : Ethanol : Diethylamine (3 : 1 : 1)	Petroleum ether	2	0.25, 0.63	Iodine
		Methanol	3	0.28, 0.76,0.24	Iodine
3	n- Butanol : Glacial acetic acid (4 : 1)	Petroleum ether	2	0.56, 0.68	Iodine
		Chloroform	4	0.18, 0.34,0.46,0.72	Iodine
4	Chloroform : Ethyl Acetate (4.5 : 0.5)	Petroleum ether	1	0.39	Iodine
		Methanol	4	0.23,0.57,0.79,0.88	Iodine
5	Chloroform : Ethyl acetate (4 : 1)	Petroleum ether	1	0.32	Iodine
		Methanol	4	0.45,0.52,0.63,0.68,0.84	Iodine
6	Toluene : Ethyl acetate (4.5 : 0.5)	Chloroform	1	0.33, 0.37, 0.46,0.65	Iodine
		Methanol	5	0.13, 0.34,0.47,0.51,0.79	Iodine

phytochemical tests and the presence or absence of different phytochemicals are presented in Table 4.

TLC finger print profile of the *C. leptospadix* shoot extracts

The TLC study of the petroleum ether, chloroform and methanolic extracts of the shoot of *C. leptospadix* was done by selection of six different solvent systems via a series of trial and error methods. The different retention factors for the different extracts are presented in Table 5.

Conclusion

The conventional pharmacognostic and phytochemical parameters would serve as a standard reference for identification, authentication and for distinguishing the plant from its adulterants. The histological features of the shoot of *C. leptospadix* Griff., have been established for better identification of the drug with distinct taste and characteristic odour. Preliminary phytochemical investigation indicated the presence of alkaloids, carbohydrates, glycosides, flavonoids, steroids, saponins, tannins and phenolic compounds. The powder drug exhibited different fluorescence character due to the presence of different functional groups in drug chemical constituents. The chemicals such as H_2SO_4, HCl, HNO_3, NH_3, etc. in different proportions may change the configuration of the functional groups present in the powdered drug and changes in the colour also occured. So, fluorescence analysis of crude drug played a vital role in the determination of quality and purity of drug. TLC helped to get different R_f values indicating the presence of various phytochemicals which can be isolated in further studies. The R_f value indicates the position at which a substance is located in a chromatogram. For the purpose of identification it is necessary to relate the R_f values of investigated substances.

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