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RESEARCH ARTICLE

ESTABLISHMENT OF MONOGRAPH OF *ACORUS CALAMUS* LINN. RHIZOMES

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ABSTRACT

Acorus calamus Linn. (Araceae) is a semi-aquatic, perennial, aromatic herb with creeping rhizomes, arching tapered reed-like leaves and minute yellow-green flowers growing wild in wet areas like edges of streams and around ponds & lakes, in ditches and seeps and also cultivated. It is widely distributed throughout India and Ceylon, ascending the Himalayas up to 6600 feet in Sikkim, plentiful in the marshy tracts of Kashmir, Sirmoor, Manipur and Naga Hills. The rhizomes are considered to possess anti-spasmodic, carminative and anthelmintic, aromatic, expectorant, nervine, sedative, stimulant and nauseating properties. Medicinally used for the treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent fevers and glandular and abdominal tumors. The current study was therefore carried out to identify requisite pharmacognostical details i.e. organoleptic, microscopical, fluorescence analysis, quantitative microscopy and physical constants such as ash value and extractive values of the fruit. The extacts were subjected to preliminary phytochemical screening. The study revealed specific indices for the particular crude drug which will be useful in identification, control on adulterations of the crude drug and preparation of a monograph of the drug.

Keywords: Standardization, Acorus *Calamus* Linn, phytochemical, epilepsy, phytochemical screening.

INTRODUCTION

A system to ensure that every packet of medicine that is being sold has the correct substances in the correct amount and will induce its therapeutic effect this is known as standardization. It is very important that a system of standardization is established for every plant medicine in the market because the scope for variation in different batches of medicine is enormous. Plant material may vary in its phytochemical content and therefore in its therapeutic effect according to different places of collection, with different times in a year for collection, with collection at the same time and places but in different years and with different environmental factors surrounding the cultivation of a particular medicinal plant. Adding to this variability is the fact that in herbal medicine several plants may be used together in the same preparation. This means that there should be a quality control test for the entire preparation to ensure quality of the product[1]. Medicinal plant materials are characterized according sensory microscopic and macroscopic characteristics. Organoleptic evaluations can be done by means of organs of sense, which provides the simplest and quickest means to establish the identity and purity and thereby ensure quality of a particular sample. A number of different bases are used for morphological studies and natural variations in these characteristics play an important role for preliminary evaluation of crude drugs. The basis of analysis by evaluation of microscopic characters is that there are always sufficient differences in the same type or different types of plants as far as the cell characteristics are concerned. Standardization profiles of herbal drugs are not available for most of the drugs. The study revealed specific indices for *Acorus calamus* Linn. rhizomes which will be useful in proper identification, control on adulterations of the crude drug and preparation of a monograph of the drug[2].

Acorus calamus Linn. (Araceae) commonly known as sweet flag or sweet root. Many Native American tribes were familiar with *A.calamus*. It was used as an anesthetic for toothache and headaches. The ancient Chinese used it to lessen swelling and for constipation. In India, Ayurvedic medicinal practice used the magical root to cure fevers, asthma and bronchitis, and as an all around sedative. According to Arabic, Roman and later European folk botany, the plant is also an aphrodisiac. According to Dioscorides the smoke of drug if taken orally through a funnel, relieves a cough. The rhizomes possessing sweet aromatic odor is reported to have strong anticonvulsant effects[3]. Other reported uses associated with *Acorus calamus* are antispasmodic[4], neuroprotective, antiulcerogenenic[5], antimicrobial[6], anti-inflammatory[7], antioxidant and anticholinestrase[8] and hepatoprotective[9]. These activities are due to presence of various chemically active phytoconstituents which is one of the topics of discussion through phytochemical screening of the drug in this research article.

MATERIALS AND METHODS

Procurement of Drug

The plant material (rhizomes) of *Acorus calamus* was purchased from NC Herbs suppliers Barraut, Uttar Pradesh. The drug was identified and taxonomically authenticated by Dr. H.B. Singh, Head, Raw Materials Herbarium & Museum (RHMD) at National Institute of Science Communication and Information Resources, New Delhi (NISCAIR). A voucher specimen of authenticated

plant material with reference no. NISCAIR/RHMD/Consult/-2010-11/1587/185 was preserved in NISCAIR, New Delhi.

Pharmacognostic parameters

Macroscopic Characters

The macroscopy of a drug includes its visual appearance to the naked eye. Macroscopic identity of a medicinal plant material is based on shape, size, colour, taste, surface characteristic, texture, fracture characteristic and appearance of cut surface[10].

Microscopic Characters

The dried whole fruit was subjected to size reduction. The powdered drug passing through sieve of 60 mesh size was used for microscopical observations. Microphotography on different magnifications was carried out with the help of digital camera at magnifications depending upon the anatomical details[10].

Foreign Matter Analysis

The 100 gm sample was weighed then spread in a thin layer and foreign matter was sorted into groups either by visual inspection, using a magnifying lens or with the help of a suitable sieve according to the requirements. The remainder of the sample was sifted through a sieve no. 250; dust regarded as mineral admixture. The portions of sorted foreign matter were weighed[10].

Physicochemical Parameters

The determination of various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, swelling index, foaming index, foreign matter and moisture content were analyzed by the methods given in the WHO guidelines for standardization of herbal drugs.

Cold Extraction

The air dried, powdered drug materials was extracted with petroleum ether, chloroform, acetone, methanol, hydro-methanol and water separately in a conical flask at a room temperature for 24 hours with occasional shaking. The extracts are evaporated to dryness and their constant extractive values are recorded[10].

Hot Extraction

The air dried powdered plant materials were extracted with Petroleum ether, chloroform, acetone, methanol, hydro-methanol and water separately in round bottomed flask and a reflux condenser was attached to it. The whole assembly was set up on a waterbath and was subjected for extraction for 1 hour. The extracts are evaporated to dryness and their constant extractive values are recorded[10].

Successive Extraction

The dried and coarsely powdered material (10g) is subjected to successive extraction in a Soxhlet apparatus with different solvents like petroleum ether, chloroform, acetone, methanol, hydro- methanol and water. The extracts are evaporated to dryness and their constant extractive values are recorded[10].

Phytochemical Screening

The phytochemical evaluation of drug was carried out as per the method described in WHO guidelines for standardization of herbal drugs. Previously powdered drug (4 gm) was extracted using reflux condenser with petroleum ether, chloroform, acetone, methanol and water successively. The extracts were evaporated to dryness under vacuum. These extract were used for the analysis of different phyto-constituents *viz*. alkaloids, carbohydrates, phenolics,

flavonoids, proteins, amino acids, saponins, mucilage and resins etc[11].

Fluorescence Analysis

Fluorescence analysis of the drug was conducted on the powder to observe different types of fluorescent characteristics. Subsequently powdered sample was treated with 1N Sodium hydroxide in Methanol, 1N Sodium hydroxide in water, 50% Sulphuric acid, 50% nitric acid, etc.

Different solvents and observed under day light, short wavelength UV and long wavelength UV Light[12].

RESULTS AND DISCUSSION

Macroscopic and organoleptic characteristic like colour, odour, taste, touch, size, shape and fracture were examined. The rhizomes were found to possess sweet aroma with pungent and bitter taste. The results are presented in (**Table 1**.).

Table 1: Macroscopical and organoleptic characters of *Acorus calamus* Linn.

S. No.	Parameters	Leaf	Rhizome
1	**Color**	Green	Externally- Light brown Internally- Buff
2	**Odor**	Aromatic	Sweet aromatic
3	**Taste**	Pungent	Pungent & bitter
4	**Size**	0.2-1.0 m long; 0.8--2.6 cm wide	5.0-12.0 cm long ; 0.8-2.0 cm thick
5	**Shape**	Erect	Creeping
6	**Touch**	Smooth	Rough
7	**Fracture**	-	Short

Microscopic characters were examined in the powdered drug sample and photographs of their special characters and T.S. were taken during the study. The powdered drug analysis showed the presence of parenchymatous cells containing oil globules (**FIG.1**), tabular form calcium oxalate crystals (**FIG.2**), simple spherical starch grains (**FIG.3**) and reticulate annular xylem vessels (**FIG.4**). The T.S. of rhizome and root hair shows smaller cells towards

periphery, somewhat collenchymatous, more or less closely arranged cells towards inner side, rounded and forms a network of chains of single row of cells, enclosing large air spaces (**FIG.5**). Fibro-vascular bundles and secretory cells having light yellowish-brown contents are present in this region (**FIG.5**). Endodermis is distinct, parenchymatous cells enclosing large air spaces similar to those of cortex and several concentric vascular bundles are arranged in a ring towards endodermis. Few vascular bundles scattered in ground tissues (**FIG.6**).

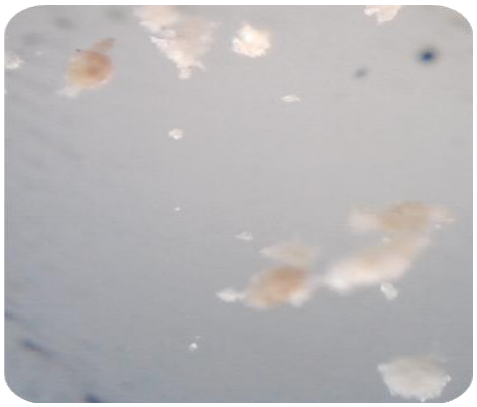

Figure 1: Oil globules

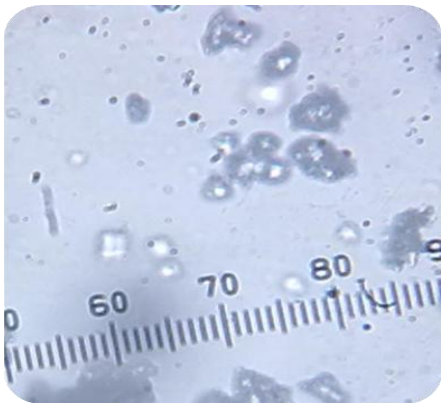

Figure 2: Calcium oxalate

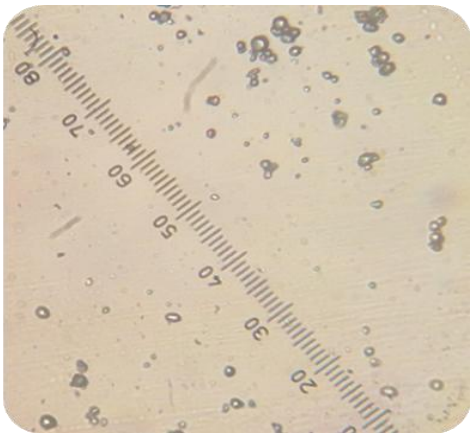

Figure 3: Starch grain

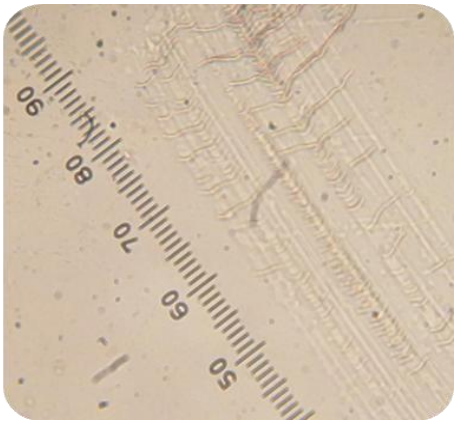

Figure 4: Xylem vessels

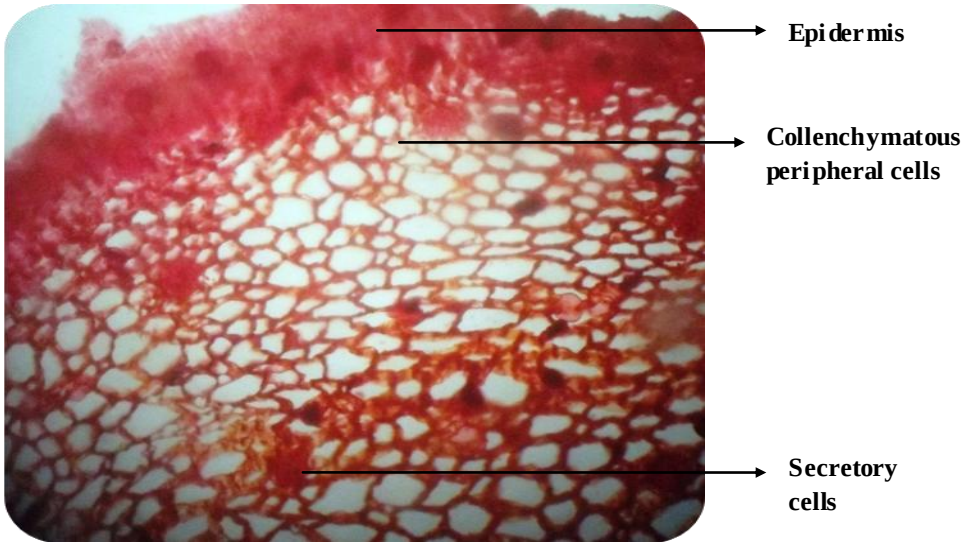

Figure 5: T.S. of rhizome

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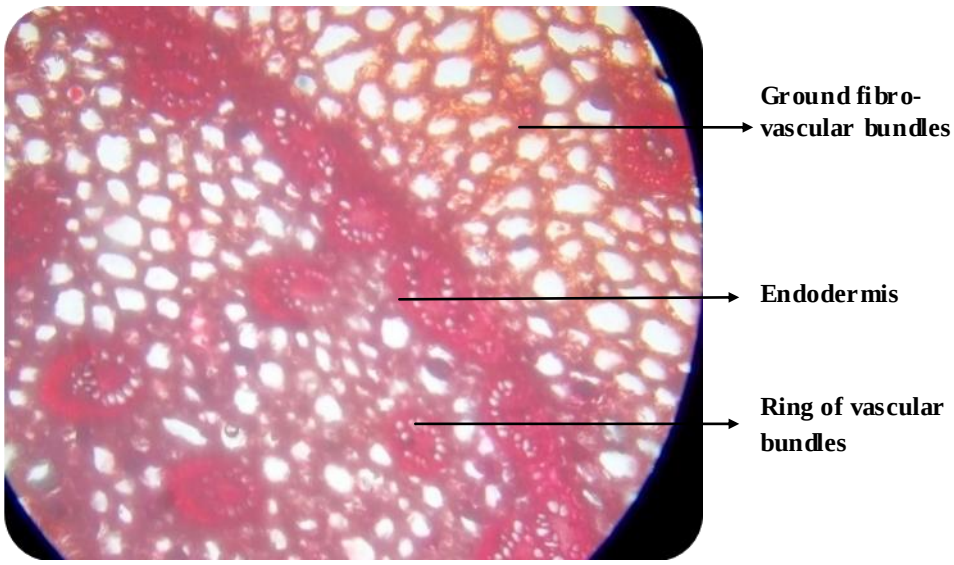

Figure 6: T.S. of rhizome

Physicochemical analysis of powder

TABLE 2: Percentage of foreign matter analysis, loss on drying, swelling index, foaming index, ash values and extractive values of *Acorus calamus* Linn.

PARAMETERS	RESULTS (%)
Foreign Matter	2.33
Loss on Drying	8.12
Swelling Index	8.667
Foaming Index	Less than 1
Ash Values	
Total Ash	6.015
Acid Insoluble Ash	0.515
Water Soluble Ash	3.5
Extractive Values	
Cold Extract	
Pet Ether	6.6
Chloroform	7.8
Acetone	8.4
Methanol	15.1
Hydromethanol	19.0
Aqueous	16.8
Hot Extract	
Pet Ether	18.6
Chloroform	6.83
Acetone	7.1
Methanol	15.8
Hydromethanol	25.9
Aqueous	15.1
Successive Extract	
Pet Ether	19.4
Chloroform	7.1
Acetone	3.4
Methanol	16.4
Hydromethanol	16.5
Aqueous	18.0

Extractive Values

Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with solvent. It is employed for that material for which no chemical and biological assay method exit. The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents.

The compositions of these phytoconstituents depend upon the nature of the drug and solvent use. The use of a single solvent can be the means of providing preliminary information on the quality of particular drug. Extractive value also give the information regarding the quality of the drug (whether drug is exhausted or not)[13]. The extractive values of various extracts are shown in (**TABLE 2**.) and (**FIG. 7-9**).

Ash Values

The ash value of any organic material is composed of their non volatile inorganic components. Control incineration of crude drugs result in ash residue consisting of an inorganic material (metallic salt and silica). This value varies within fairly wide limits and is therefore an important parameter for the purpose for evaluation of crude drugs. In certain drug, the percentage variation of ash from sample to sample is very small and any marked difference indicates the change in quality. Unwanted parts of drug, some time possess a character that will raise the ash value. A high value is indicative of contamination, substitution, adulterations or carelessness in preparing the crude drug for marketing[13,14]. The total ash value**,** acid insoluble ash value, water soluble ash values were determined as per WHO guide lines. The results and observation are presented in (**TABLE 2**).

Phytochemical Screening

Phytochemical evaluation of the plant extracts may provide the information regarding various types of phytoconstituents present. Presence or absence of particular types of phytoconstituents in the plant of the interest may be helpful, partly in the development of analytical profile and in the differentiation of contravention plants. (**TABLE 3**) showed the presence and absence of various phytoconstitutents in different extracts.

TABLE 3: Preliminary phytochemical investigation of different extracts of *Acorus calamus*

S.No.	Phytochemicals	I	II	III	IV	V	VI	VII
1.	Carbohydrate	-	-	-	+	+	+	+
2.	Tannin	+	+	+	+	+	+	+
3.	Amino Acid	-	-	+	+	+	-	+
4.	Alkaloid	+	-	+	+	+	-	+
5.	Terpenoids	+	+	-	+	+	+	-
6.	Steroid	+	-	-	-	-	-	-
7.	Glycoside	+	+	+	+	+	+	+
8.	Saponin	-	-	-	+	+	+	-
9.	Flavonoid	+	-	+	+	+	+	+

(I) Petroleum Ether; (II) Chloroform; (III) Acetone; (IV) Methanol ;(V) Hydroalcohol; (VI) Water; Ethanol (VII); (+) Present; (–) Absent

pH value of 1 and 10% solution of drug

The pH of 1 and 10% solutions of the finely grounded rhizomes of *Acorus calamus* Linn. was measured using pH meter and recorded in the (**TABLE 4**).

TABLE 4: pH of drug solutions

S. No.	% of Plant Extract	pH Value
1.	1 %	6.98
2.	10 %	8.36

Fluorescence Analysis

The air dried plant materials were subjected to different chemicals and lights. (**TABLE 5**) showed a detail fluorescence behavior of crude drug powder.

TABLE 5: Fluorescent analysis of rhizome powder in different reagents

S. No.	Reagent	Color Observed		
		Day Light	254 nm	366 nm
1.	None	Light brown	Light brown	Brown
2.	Distilled Water	Light brown	Cream green	Greenish brown
3.	1 N NaOH in Water	Light brown	Greenish brown	Black
4.	1 N NaOH in Methanol	Yellowish brown	Greenish brown	Brown
5.	50% Nitric Acid	Yellow	Dark green	Black
6.	50% HCl	Light brown	Black	Light brown
7.	conc. H_2SO_4	Reddish brown	Yellowish brown	Greenish brown
8.	Acetone	Light brown	Light green	Black
9.	conc. HCl	Light brown	Greenish brown	Dark brown
10.	Chloroform	Light brown	Light brown	Brown

CONCLUSION

The results obtained in the present investigation are encouraging and will be used as reference data for the standardization of *Acorus calamus* Linn. rhizomes. The drug is collected from wild sources and varies in constituents and pharmacological efficacy due to geographical diversity. Improper collection and storage conditions and adulteration lead to contamination by microbes and heavy metals which indirectly responsible for poor efficacy of the drug. Standardization is the prime need of the time because standardization establishes quality and identity profile that can be used for the purpose of safety monitoring and overall quality assurance of herbal medicines. The list of herbal preparations being exported to foreign countries can be extended if proper standardization is performed to prepare formulations of high quality and efficacy.

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