Pharmacognostic Evaluation of *Acorus calamus* L.

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**ABSTRACT**

**Introduction:** *Acorus calamus* L. is a semi aquatic medicinal plant, commonly used in traditional medicinal systems of Asian and European countries. Rhizomes are used as therapeutic agent for various diseases. The present study focuses on pharmacognostic evaluation of the rhizomes. **Methods:** Pharmacognostic evaluation was carried out by organoleptic evaluation, anatomical studies, powder microscopic analysis and ash value studies. **Results and Conclusions:** Organoleptic studies showed presence of characteristic triangular leaf scars and the anatomical studies showed the presence of aerenchyma and amphivasal vascular bundles. Presence of tannins was also observed.

**Key words:** *Acorus calamus*, Anatomy, Powder Microscopy, Ash value, Phytochemistry, terpenoids.

**INTRODUCTION**

*Acorus calamus* L. (commonly called as ‘Sweet flag’) of family Araceae, is a semi-aquatic, perennial, aromatic herb with creeping rhizomes, sword shaped leaves and spadix inflorescence (Figure 1 a, b). *A. calamus* grows either as wild or cultivated crop throughout India.[1] The rhizomes are used in almost all civilisations of world; Asians have been using the plant for at least 2000 years for many medicinal purposes. Sumerians and Egyptians used calamus as sacred incenses. Aromatic leaves of *A. calamus* were placed on floors of medieval churches and houses as effective air-fresheners and insecticides.

**Pharmacological applications of *A. calamus***

*A. calamus* is used in traditional medical systems of India, Thailand, China and native America for various pharmacological applications such as insomnia, melancholia, neurosis, epilepsy, hysteria, loss of memory, antispasmodic, carminative, anthelmintic, anti-inflammatory, therapeutic against diseases like eczema, rheumatism, diarrhoea, bronchial catarrh, intermittent fevers, abdominal tumours, liver disorders and for diseases of kidney.[2,3] In addition, insecticidal,[4] hypolipidemic,[5] antimicrobial,[6] and antidiabetic[7] properties of *A. calamus* rhizomes are reported. Essential oils of *A. calamus* are used in cosmetic and brewing industries.[8]

Pharmacognostic tools are essential in identifying the medicinal plants. This study covers the entire pharmacognostic study on the rhizomes of *A. calamus*.

**MATERIALS AND METHODS**

**Chemicals**

Chemicals required for pharmacognostical, phytochemical analysis were purchased from SRL Pvt. Ltd., India. Solvents and acids of Analytical grade were purchased from Rankem Ltd, India. Standards like α asarone, β asarone and eugenol were purchased from Sigma (USA). Filter papers were purchased from Whatman Ltd., UK.

**Collection of plant**

Rhizomes were collected from *A. calamus* plants grown in loamy red soil (pH 5.5-8) at moderate climate and optimum temperature (25-30 °C) for a period of 6 months at M/s JK Herbal Farm, Veerakanur, Perambalur District, Tamil Nadu, India (11°14’ 0" North, 78°53’ 0" East). Collected rhizomes were thoroughly washed with tap water once, subsequently with distilled water (4 times) and shade dried (Room temperature 37 °C).

**Identification and Pharmacognostical studies of *A. calamus***

Plant was identified on the basis of organoleptic, macroscopic and microscopic (anatomy) observations as summarised below:

**Organoleptic analysis**

Surface appearance, texture, colour, size and odour of rhizomes were evaluated by physical examinations.
For anatomical studies, fresh rhizome bits were fixed in FAA (5% Formalin, 5% Acetic acid and 90% of 70% Ethanol) for 24 hours and subsequently dehydrated using series of t-Butyl Alcohol.[14] Fixed samples were embedded in paraffin wax blocks, sectioned using rotary microtome (10 µm thickness), stained with toluidine blue, safranin, fast-green, IKI as per protocols of O’Brien et al.[15] viewed under Nikon Labphoto2 polarized microscope (Nikon Inc, USA). For powder microscopic observations, coarse powders of rhizome were spreaded in a glass slide, viewed in bright field and polarised light microscope (Nikon Labphoto2 polarized microscope, USA).

Deposition of voucher samples
A voucher sample of identified plant was deposited in National Institute of Herbal science-Plant Anatomy Research Centre, Chennai for further authentification.

Physico-Chemical analysis
All physico-chemical analyses were carried as triplicates on three different days.

Moisture content
Moisture content of *A. calamus* rhizomes was estimated by drying one gram of rhizome powder in hot air oven at 105 °C for 1 hour, subsequently cooled in desiccators and the loss in weight was recorded.

Ash valve
Ash value was calculated as per the protocols of Kokate et al[16] and WHO report (1998).[17] To estimate total ash value, four grams of rhizome powder was evenly spreaded in a pre-ignited silica crucible, heated to 600°C in a muffle furnace for a period of 6 hours, eventually the residue was cooled and weighed.

Water-soluble ash content was estimated by adding 25 ml of water to crucible containing total ash, boiled for 5 minutes and filtered through Whatman no.1 filter paper. Remains in the filter paper was transferred to crucible and ignited for 15 minutes. Water-soluble ash content was calculated as: difference in weight between total ash and the residue obtained after treatment.

Acid-insoluble ash was quantified by adding 25 ml of dilute hydrochloric acid to crucible containing total ash, boiled for 5 minutes, subsequently filtered through whatman no.1 filter paper, washed with hot water until solution becomes neutral and the residue was transferred to a crucible, ignited for 30 minutes and weighted.

Energy Dispersive X-ray analysis (EDAX)
Energy Dispersive X-ray analysis was carried out by spreading the ash evenly on aluminium stub using double sided adhesive tape, sputter coated with gold, analysed in a Hitachi VP-SEM S-3400N scanning electron microscope with EDS detector system. Data was analysed qualitatively as well as quantitatively using EDS software.

RESULTS
Humankind knows usage of medicinal plants and their preparations from time immemorial. Herbal medicines are still mainstay for about 75-80% of world population for primary health care, due to better cultural acceptability, compatibility and lesser side effects. Complimentary medicinal systems are now in lime light in developed countries like Germany, France, European Unions, and United States America.[18] Medicinal plants are source of raw materials for both traditional and modern medicine. Rationalisation of new multidrug and multitarget concept of therapy in classical medicine will have great implication on future basic research in phytomedicine and on evidence based phytotherapy.[19]

Quality control in phytomedicine
In general, there are three main factors in quality control of phytomedicine namely: a) identity of the plant, b) purity of the compounds and c) content and processing.
Pharmacognosy

Organoleptic evaluation
Pharmacognostical studies are pivotal in herbal sciences, as it ensures plant identity and prevents adulteration. *Acorus calamus*, a perennial herb grows up to 2 meters with sword shaped leaves and spadix inflorescence (Figure 1a). Figure 1b illustrated the morphology of rhizomes. Rhizomes were brown in colour, tortuous, cylindrical, curved, and shortly noded. Adventitious fibrous roots and erect aerial shoots were produced along nodes. Upper surface consists of triangular leaf scars, while under surface bears tuberled, irregular, circular root-scars. Rhizomes were highly aromatic with sweet odour and bitter taste.

Anatomy of rhizome
Anatomical studies of *A. calamus* rhizomes illustrate the following characteristic features: Ground plan view (5X) showed the presence of central stele surrounded by narrow cortex and thin epidermis. Epidermal cells were circular or squarish, thin walled and thickly cuticularised (300 µm width) (Figure 2a), followed by aerenchymatous cortex (600 µm width) with shapeless air chambers; Stele wide and circular (Figure 2a). Scattered in the cortical zone there were diffused fibre–strands as well as vascular strands. The vascular strands were 200µm wide with wide, thick walled angular xylem elements, surrounded by phloem and ensheathed by sclerenchymatous cells. The sclerenchyma strands were about 50µm thick with wider, thick walled lignified fibres. The vascular system consists of a peripheral ring of vascular bundles abutting the endodermis and central scattered vascular bundles (Figure 2a1). Vascular strands were amphivasal in nature i.e. Phloem surrounded by a layer of xylem elements (Figure 2c). The central bundles were large, amphivasal with massive phloem tissue surrounded by two or more layers of xylem elements. While the peripheral vascular bundles attached to the endodermis has a central mass of phloem surrounded by a layer of xylem elements. Figure 2b illustrated, polarised light picture of lignified fibre sheath of vascular bundles. Central ground tissue was parenchymatous; the cells were wide, thin walled and compact; no air chambers were evidenced. Scattered in the cortical and central ground parenchyma, the dark masses of cell contents were may be of tannins (Figure 2a1).

Powder microscopy
The images of powder microscopy analysis display the following components; a) Tracheids- were quite long with blunt end and the lateral wall thickenings are scalariform or close helicals; b) Fibres were abundant in the powder and are short, wide in the middle and tapering towards the centre, the walls were thick and lignified and the lumen was wide, the lateral wall pits were not evidenced. With regard to the size, the fibres were 300 -250 µm in length and 20 µm in wide in the middle (Figure 2 d,e,f,g).

Figure 2: Anatomical studies of *A. calamus* rhizome
a & a1) Transverse section (T.S) of Acorus calamus rhizome (5X magnification); b) T.S. of Acorus calamus rhizome (20X magnification) c) Entire view of vascular bundles of *A. calamus* rhizome (40X magnification) d & e) Powder microscopy of *A. calamus* rhizome; f & g) Powder microscopy of *A. calamus* rhizome- tracheids
Ash value
The total ash value was 6.12% of which 99% was acid soluble and 66% was water soluble (Table 1). EDAX study revealed the presence of sodium, Magnesium, silicon, phosphorous, sulphur, chlorine, potassium and calcium. Of which, potassium content was more followed by chlorine, calcium, sulphur, phosphorous, sodium, magnesius, and silicon. (Figure 3; Table 1)

Results on anatomical studies of rhizomes were well coincided with the descriptions summarized in manuals of *Acorus calamus* L.[20] Based on organoleptic and anatomic features, the plant was identified as *Acorus calamus* and the same was authenticated by Plant Anatomy Research Centre with registration number PARC/2008/203.

**Figure 3:** EDAX analysis of *A. calamus* rhizome ash

**Table 1:** Ash value of rhizomes of *A. calamus* L. and Elemental analysis of Ash

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<th>S No</th>
<th>Ash Analysis</th>
<th>Weight (%)</th>
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<tr>
<td>1</td>
<td>Total ash value</td>
<td>6.12</td>
</tr>
<tr>
<td>2</td>
<td>Water soluble ash</td>
<td>66%</td>
</tr>
<tr>
<td>3</td>
<td>Acid soluble ash</td>
<td>99.09%</td>
</tr>
<tr>
<td>4</td>
<td>Acid insoluble ash</td>
<td>0.01%</td>
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<table>
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<tr>
<th>S No</th>
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<tr>
<td>5</td>
<td>Carbon</td>
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</tr>
<tr>
<td>6</td>
<td>Oxygen</td>
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</tr>
<tr>
<td>7</td>
<td>Sodium</td>
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<tr>
<td>8</td>
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<td>9</td>
<td>Silicon</td>
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<td>14</td>
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**CONCLUSION**
A golden triangle of traditional medicine, modern medicine and science may result in discovery of newer, safer and cost-effective therapies. Globally there is a positive trend towards holistic health, integrative sciences, systems biology approaches in drug discovery and therapeutics. Quality control is pivotal in phytomedicine and quality assurance begins with the identity of the raw material i.e. the plants and plant parts. Thus, pharmacognostical studies were carried out to ensure the identity of the plant. Upon identifying the plant, the purity was monitored for contaminations of heavy metals and pesticides.

**REFERENCES**

7. www.himalayahealthcare.com/herbfinder/h_acorus.htm