Indian Journal of Natural Products and Resources
Vol. 6 (2), June 2015, pp. 143-152

Pharmacognostical standardization of *Arisaema leschenaultii* Blume

P Annie Sulochana Selvakumari
St. John's College, Palayamkottai, Tamil Nadu- 627002, India

Received 27 March 2013; Accepted 2 May 2015

Arisaema leschenaultii Blume is endemic to South India and Sri Lanka. It grows in shades, especially on shola floor of 1200 – 2000 m altitude, it is rare and threatened in silent valley. The corms and tender leaves are cooked with tamarind paste and eaten as a vegetable by Kanis tribe in Tirunelveli hills. It is employed medicinally by Singhalese as a substitute to *Arisaema jacquemontii* Blume, whose juice is good for ringworm infection and the paste is applied as ointment in skin diseases. The corms are used to cure piles. This paper focuses on the pharmacognosy of *A. leschenaultii* Blume because pharmacognosy is an important link between pharmacology and medicinal chemistry. Morphological and anatomical description of plant, pharmacognostical and analytical standards and physical constants were obtained by employing standard methods of analysis as described in Pharmacopoeia of India. The results of the present investigation provide dependable diagnostic features of the vegetative organs of the plants for the identity of the drug in entire and in fragmentary conditions.

Keywords: *Arisaema leschenaultii*, Pharmacognosy, Morphology, Anatomy, Physico-chemical constants, Phytochemistry.
IPC code; Int. cl. (2014.01)–A61K 36/00

Introduction

There are about 150 species of *Arisaema* Mart. in the world[1]. In India, there are about 42 species including the introduced ones and 12 species in South India[2]. *Arisaema leschenaultii* Blume is found in South India and Sri Lanka[3,4] and rare as well as threatened in silent valley[5]. It grows in semi shaded, moist areas of evergreen forests in hills, especially on Shola floor at 1200-2000 m altitude[6,7]. Flowering and fruiting is in between June and September. The corms and tender leaves are cooked with tamarind paste and eaten as a vegetable by Kanis tribe in Tirunelveli hills, employed medicinally by Singhalese as a substitute to *A. jacquemontii* Blume, whose juice is good for ringworm infection and the paste is applied as ointment in skin diseases[8]. The corms are used to cure piles. There has been no report of the pharmacognostical studies of *A. leschenaultii* to date. Hence an attempt has been made to work on its pharmacognostical studies to help its identification. Pharmacognosy is essential because the men of Ayurveda have mentioned just the name of the drugs. Deforestation leads to the extinction of many drugs, therefore plants of rare occurrence may be identified with the help of pharmacognostical standards.

Correspondent author:
E-mail: anniesspsjc@gmail.com

Materials and Methods

Macroscopic studies

Collection of the plant was undertaken in Kodaikanal, Tamil Nadu. In determining the identity, Hooker's Flora of British India[9], Gamble's Flora of the Presidency of Madras[10], Nicolson's Araceae in Dassanayake's Flora of Ceylon[3], Mohanan and Henry's Flora of Thiruvananthapuram[11], Kerala and more recent revisionary and other critical works were consulted. Voucher specimens of the collection were incorporated at the Herbarium of St. John's college (SJC), Palayamkottai, Tamil Nadu. Identification of the species was confirmed with authentic herbarium specimens. Mature and healthy plants were collected and morphological characters were studied. Plant was examined using a hand lens in the field and a dissection microscope in the laboratory and the characters were noted down. Photographs of the specimens are provided for easy identification (Plate 1).

Microscopic studies

Anatomy

The fresh plant parts (leaf, petiole, underground stem and root) were collected, cleaned, cut into pieces and fixed in 70 % ethanol FAA (Formalin: Acetic acid: Alcohol, 5:5:90). The fixed materials were dehydrated in tertiary butyl alcohol series[12] cleared in xylol and embedded in paraffin wax (m p 58-60 °C). Sections of 10 µm thickness were cut in a rotary

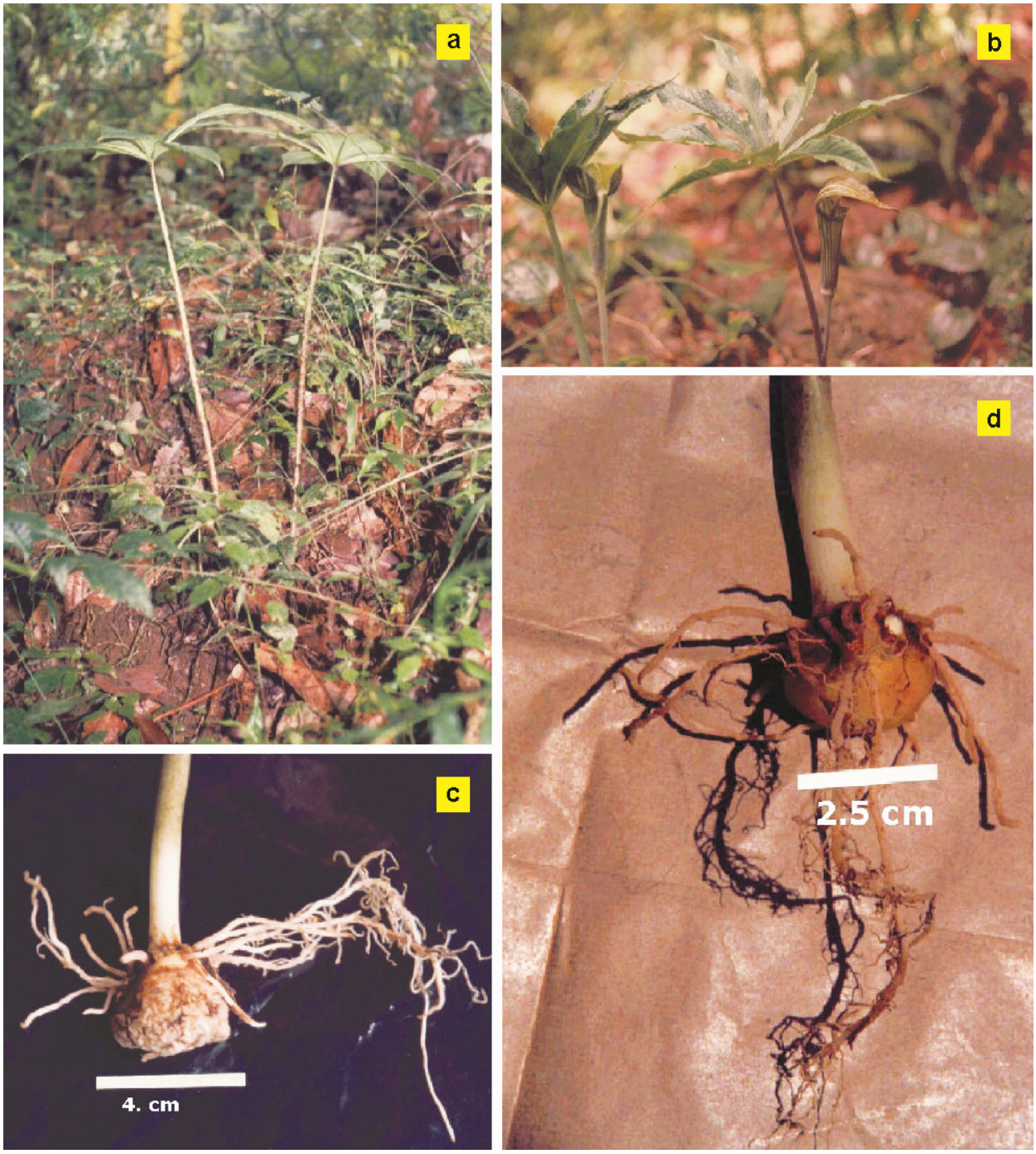

Plate 1–*Arisaema leschenaultii* Blume: a. In its natural habitat; b. Plant with inflorescence; c. Corm; d. Corm (peeled).

microtome (Spencer model), stained with toluidine blue[13] and mounted by following the usual plant microtechnique[14]. The anatomical characters were determined using NIKON-Lab phot 2 – Photographic Trinocular Microscopic Unit, using normal light and polarized light. Measurement of cells was made with micrometer. The permanent slides are kept in the Department of Botany, SJC, Palayamkottai.

Stomatal index

Stomatal index is the percentage, which the number of stomata forms the total number of epidermal cells, each stoma being counted as one cell[15]. Fully developed leaves were cut into pieces of one sq cm and boiled in 5 % potassium hydroxide solution for 10 min. After thorough washings in water, the lower epidermal peels were taken off, stained with 1 %

aqueous safranin solution and mounted in 5 % glycerol. The stomatal index for a species is constant and was calculated by using the equation,

$$I = \frac{S \times 100}{E + S}$$

Where,

I = Stomatal index; S = Number of stomata per unit area; E = Number of epidermal cells in the same unit area

Averages of 10 measurements counting were taken after random sampling.

Starch grains and raphides

Finely powdered corms of the plants were mounted on glycerine as a thin film with Lugol's iodine solution and the characters of the starch grains and raphides were studied under light microscope. The sizes of starch grains and raphides were measured using micrometer and recorded.

Scanning Electron Microscopic study

Finely powdered and sieved samples (corm) were mounted on specimen stubs using Scotch double adhesive tapes and coated with gold to a thickness of 100 A using Hitachi vacuum evaporator model HUS 5GB. Gold-coated plates were observed in a Hitachi Scanning Electron Microscope (SEM) model S-450, operated at 15 KV and photographed.

Fluorescence analysis

The powdered sample and the extract of the powder in various solvents such as petroleum ether (40-60 °C), benzene, chloroform, methanol and water were examined under ordinary light and ultra violet light (365 nm and 255 nm). The powder was also treated with various chemical reagents and the change in colour was recorded. The fluorescent characters were determined.

Physico-chemical characters

The percentage of loss of weight on drying, total ash, acid-insoluble ash, water-soluble ash, sulphated ash and residue on ignition were obtained by employing standard methods of analysis as described in Pharmacopoeia of India[16].

Phytochemistry

Preliminary phytochemical analysis

10 g of the powdered sample was successively extracted with 200 mL of petroleum ether (40-60 °C), benzene, chloroform and methanol in a Soxhlet apparatus. The different extracts were tested for the steroids, sugars, reducing sugars, triterpenoids, alkaloids, phenolic compounds, flavonoids, catechins, saponins, tannins, anthroquinones and amino acids[17].

Thin-Layer Chromatography

The petroleum ether (40-60 °C), benzene, chloroform and methanol extracts of the powdered sample were subjected to Thin Layer Chromatographic analyses.

Paper Chromatography

The water extract of the dry powder was subjected to paper chromatographic studies using Whatman No. 1 filter paper. Identification of amino acids by paper chromatography was also performed[18].

Detection of cyanogenic glycosides

10 g of the fresh corm was cut into pieces and placed in a conical flask with 1 N HCl. The cut pieces were soaked in diluted hydrochloric acid for a few hours. HCN formed. A drop of sodium hydroxide and ferrous sulphate solution was taken in a filter paper. HCN solution was added to the impregnated filter paper. Appearance of Prussian blue colour shows the presence of cyanogenic glycosides[19].

Quantitative estimations

Quantitative estimations of starch, sugars, lipids, amino acids, proteins, nitrogen, phenolic compounds, flavonoids, calcium, sodium, potassium and iron were carried out according to AOAC[20].

Results and Discussion

Morphological features

Cormous herb, corm subglobose, 3-5 x 2-3 cm producing bulbils. Roots contractile. Leaf radiatisect, petiole about 80 cm long, light pinkish to green with small purplish spots, leaflets 9-13, obovate to oblanceolate, cuneate at base, acuminate at apex, pinnately nerved (30 x 8 cm). Peduncle usually shorter than petiole, coloured as in petiole, spathe about 20 cm long, greenish with white and purplish vertical stripes, the basal convolute tube ca. 6.5 cm long; spadix usually dioecious up to 10 cm long. In pistillate spadix basal 3 cm with female flowers, ovary sub-globose, 4-6 ovuled, stigma with tufted papillae on short style. In staminate spadix floriferous portion, 3 cm long, anthers sub-globose with constriction at the tip and appearing reniform, usually in a group of 2-8 on a short stalk or sessile; appendix erect, club shaped as in pistillate flowers. In monoecious spadix pistillate flowers at base followed

by staminate flowers and terminating in a barren appendix. Berries red, 5 x 3 cm, 2-4 seeded. *A. leschenaultii* is monoecious and dioecious species; the male plants are small, female plants are tall and monoecious ones are of intermediate height and rare in occurrence. Spathe may be with white and green or white and purple stripes[21-23]. The female plants having spathe with white and purple stripes were collected for investigation. Due to the paucity of this corm, much care was undertaken not to disturb its population in the natural habitat, during collection.

Anatomical features

Lamina

Dorsiventral. Epidermis is single layered; cells are mainly isodiametric, more or less barrel shaped. Cuticle is thin and smooth. Stomata are confined to abaxial surface and are paracytic. Epidermal cells show undulation on both the surfaces. Mesophyll includes palisade and spongy parenchyma. Palisade cells are in one row, transcurrent and confined to adaxial surface. The second row is rather poorly developed. Spongy parenchyma is 2-3 layered. At the region of thick veins, below the epidermis, a few layered thick-walled hypodermis is present. Vascular bundles are many, consisting of tracheids and thin-walled parenchyma. Xylem occupies adaxial and phloem abaxial positions. Raphide sacs containing bundles of calcium oxalate needles are common in mesophyll.

Petiole

Outline is circular in cross section. Epidermis is single layered with thick cuticle. Sclerenchymatous strand below the epidermis is discontinuous. There is no distinction between the cortex and the central cylinder. Ground tissue is parenchymatous and the cells are of varied sizes arranged with intercellular spaces. Vascular bundles are many and arranged irregularly. Each vascular bundle is with one file of wide metaxylem elements and a phloem strand; some include extended protoxylem.

Corm

The transverse section of the peeled corm of *A. leschenaultii* shows epidermis replaced by an incipient corky layer. Cork is many layered with thin-walled cells in radial rows. Small, condensed, amphivasal vascular bundles are scattered in the parenchymatous ground tissue. Xylem consists of a few tracheids associated with parenchyma. Fibres are absent. Ground tissue is parenchymatous and the cells contain starch. Tannin cells are present. Tannins containing drugs limit fluid losses and enhance tissue regeneration in case of superficial wound or burn. Internally, they are antidiarrhoeals. Commercially tannins find extensive application in leather industry. Bundles of calcium oxalate needles are present in raphide sacs. There is no sclerenchyma. Internal periderm is observed.

Root

Epidermis is single layered. Cortex is parenchymatous and lacunate. Endodermis is distinct. Xylem strands alternate with phloem. Xylem constitutes vessels, tracheids and associated parenchyma. Xylem is exarch; tannin cells are present and pith is parenchymatous.

Stomatal index

Stomatal index is constant for any species and has been proved useful for distinguishing leaflets of Indian from those of Alexandrian Senna and leaves of *Atropa belladonna* from those of *A. acuminata*. Stomatal index in *A. leschenaultii* is 11.4.

Starch grains and calcium oxalate crystals

When examined by polarized light, using crossed nicols, starch granules appeared as luminous objects on a black background. When the nicols were rotated through a right angle, the field became bright while the granules were dark with a bright cross representing the position of the hilum. Bundles of acicular raphides are present in sacs. The length of the calcium oxalate needles are 70-120 µ, mostly over 90 µ. A few needles with blunt ends, short and oblique rhomboid were also observed. When the powdered sample was observed under the light microscope, phenolic compounds in the form of yellow and pink patches were observed along with the remnant of the cells, starch grains, and calcium oxalate crystals.

Scanning Electron Microscopic study of starch grains

Starch grains in the corm of *A. leschenaultii*, consists of granules that are fairly uniform in size, measuring 5-35 µ, mostly 15-25 µ in diam. They are polyhedral with blunt angles or more or less rounded. In the centre, there is often a cleft, representing the position of the hilum. The granules are smooth, simple or compound (2-5).

The gross morphology gives definite information about the drug. Morphological characters for the identification of the taxa has been described and photographs are displayed (Plate 1). Microphotographs

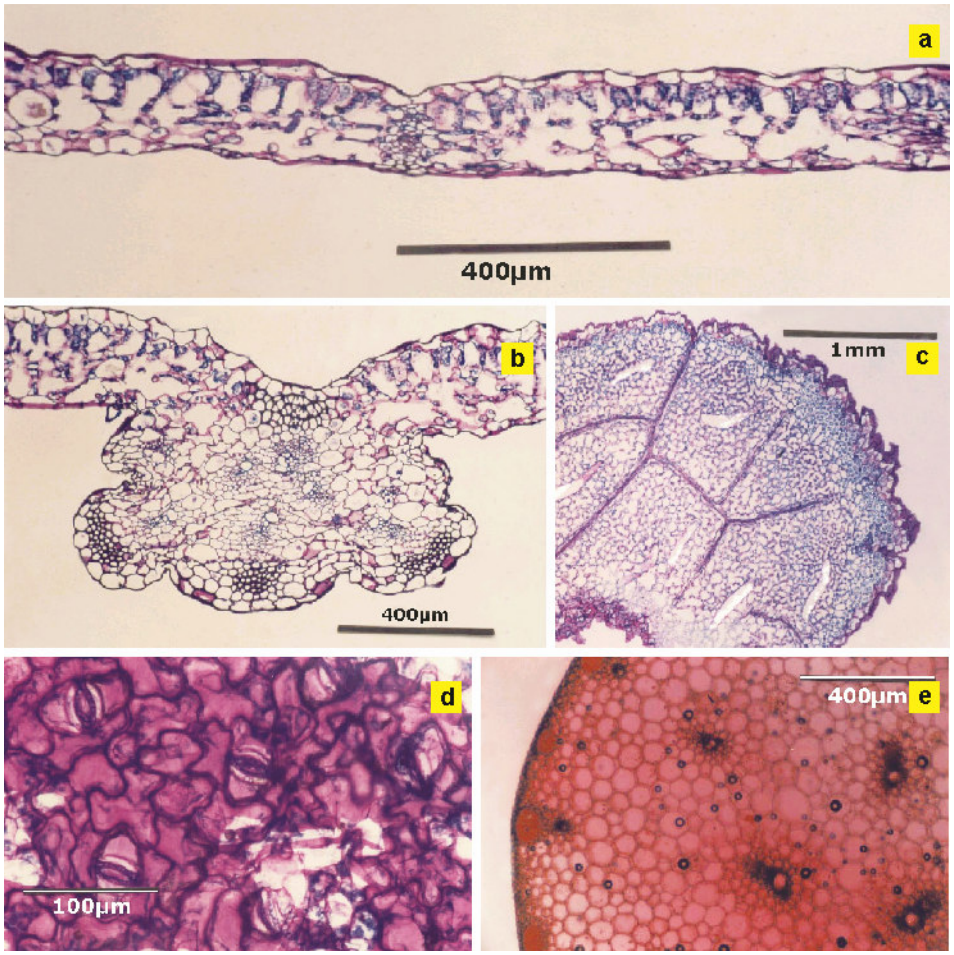

Plate 2–Microphotographs of *Arisaema leschenaultii* Blume leaf showing anatomical characters; a. Leaf lamina (T. S.); b. Mid-rib (T. S.); c. Leaf paradermal section-raphides (Polarised light); d. Leaf epidermal peeling paracyclic stomata; e. Petiole (T. S.)

showing the anatomical characters of the plants are displayed in Plates 2 & 3. The epidermal cell wall of *A. leschenaultii* shows undulation. Most undulations of the sidewalls of the epidermal cells appeared in leaves grown in shade[24]. *A. leschenaultii* grows under shade in evergreen forests. In Aroideae, the tuberous genera have a highly condensed vascular system[25]. The polarized light was very much useful to detect the lignified elements, crystals and starch grains. Vessels are restricted to metaxylem of the roots in the species. The roots show the primary structure with epidermis, aerenchymatous cortex and distinct endodermis enclosing the stele. Histological study is valuable for the identification of the drugs.

Phenolic compounds in the form of pink and yellow patches have been observed in the dry powder of corm of the taxa. Presence of colouring matter also may be of assistance for the identification of the drugs and the detection of adulterants. The shape of the starch granule, its size and position of its hilum vary with the species and therefore are important elements for microscopical identification[26]. Properties of starch from *A. negishii* have been studied by Makino *et al*[27]. The diameter of the starch granules assists in distinguishing varieties of Ipecacuanha and in distinguishing Cassia bark from Cinnamon and in detecting Senna stalk in powdered Senna leaf[28]. Hence, the SEM studies on the starch grains of the species will be of great use in confirming the identity of the crude drug.

The results of fluorescent analysis of the dry powder of the taxa is presented in Table 1. Many alkaloids, in the solid-state show distinct colours, when placed under the UV lamp. The reaction of the various chemical compounds present in the dry powder of the plant drug with different acid and alkali shows the colour change and the fluorescence can be determined under UV light. Drugs such as Hydrastis, Calumba, Viburnum and wild Cherry bark show brilliant effect in ultraviolet light and these may be

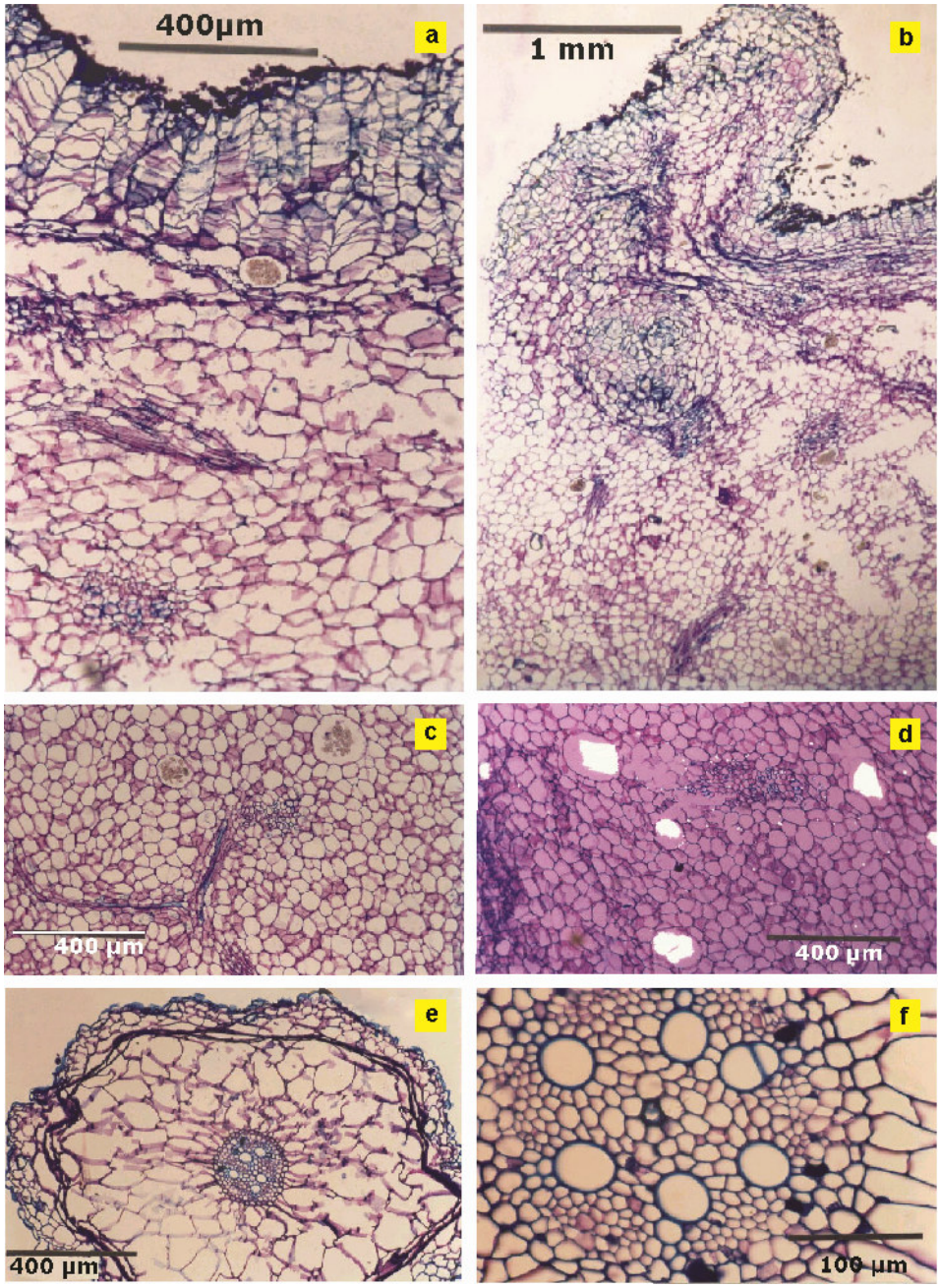

Plate 3–Microphotographs of *Arisaema leschenaultii* Blume corm showing anatomical characters; a. Corm T. S. (Periderm and outer portion); b. Corm T. S. (Periderm tube); c. Corm T. S. (Inner Section); d. Corm T. S. – Raphides and vascular bundles (polarised light); e. Root T.S.; f. Root T. S. – Stele enlarged

used to aid in identification and to detect certain adulterants, which do not exhibit a similar fluorescence[29].

Table 2 shows the physico-chemical characters of the drug. The physico-chemical characters such as percentage of loss of weight on drying, total ash, acid-insoluble ash, water-soluble ash, sulphated-ash, residue on ignition of the sample was determined. These values are rarely constant for drugs, but may be within a range. Loss of water in the samples is principally due to water; small amounts of other volatile materials also contribute to the weight loss. The moisture content of a drug should be minimized in order to prevent the decomposition of crude drugs due to either chemical change or microbial contamination. The total ash usually consists mainly

Table 1 — Fluorescent character of the powder of *Arisaema leschenaultii* (Corm)

S. No.	Treatments	Under ordinary light	Under UV light	
			365 nm	255 nm
1.	Powder as such	Pale yellow	Pale yellow	White
2.	Powder + 1N NaOH (ethanolic)	Yellowish brown	Yellow	Brown
3.	Powder + 1N NaOH (aqueous)	Yellowish brown	Yellow	Brown
4.	Powder + HCl (1:1)	Brown	Brown	Brown
5.	Powder + H_2SO_4 (1:1)	Pale yellow	Yellow	Yellow
6.	Powder + HNO_3 (1:1)	Yellowish brown	Yellow	Brown
7.	Extracts			
	a) Petroleum ether (40-60 °C)	Colourless	Colourless	White
	b) Benzene	Pale yellow	Pale yellow	Brilliant white
	c) Chloroform	Yellow	Yellowish brown	Brilliant white
	d) Methanol	Yellow	Yellowish brown	Brilliant white
	e) Water	Yellow	Yellowish brown	Dull white

Table 2—Physico-chemical characters of *Arisaema leschenaultii* (Corm)

S. No.	Characters	Percentage
1.	Loss of weight on drying	75.80
2.	Total ash	6.63
3.	Water- soluble ash	2.08
4.	Acid- insoluble ash	1.29
5.	Residue on ignition	5.54
6.	Sulphated ash	7.61

Table 3 — Solvent soluble extractive values of *Arisaema leschenaultii* (Corm)

Solvents	Percentage of extractive values
Petroleum ether (40-60 C)	3.84
Benezene	5.94
Chloroform	12.34
Methanol	16.24
Water	20.12

of carbonates, phosphates, silicates and silica. The ash value is a criterion to judge the identity or purity of the crude drugs. In sulphated-ash determination, all oxides and carbonates are converted to sulphates. The sulphated-ash content is more than the total ash content. The water-soluble ash is used to detect the presence of material exhausted by water. Exhausted ginger and tea leaves are determined by water-soluble ash[28]. Acid insoluble-ash, which is a part of total ash, insoluble in diluted hydrochloric acid is also recommended as standard for certain drugs. Adhering dirt and sand may be determined by acid-insoluble ash content. Excessive earthy matter is likely to occur with roots and rhizomes.

The percentages of solvent soluble extractives with reference to the powdered sample are given in Table 3. The extractive values of the methanol and aqueous extracts are generally high when compared to the other extractive values of the less polar solvents. Ether soluble extractive represents fat, volatile oil, resin, fixed oil or colouring matter present in the drug. Methanol is an ideal solvent for the extraction of various chemicals like tannins, flavonoids, amino acids and phenolic compounds. Water-soluble active constituents chiefly include tannins, sugars, plant acids, mucilage and glycosides.

The determination of different solvent soluble extractive values is used as means of evaluating drugs, the constituents of which are not estimated by other means. Nevertheless, as suitable assays become available the extractive tests are no longer required as pharmacopoeial standards[17].

The results of the preliminary phytochemical screening, thin layer chromatographic studies of the various extracts, paper chromatographic studies of the water extract and paper chromatographic analysis of amino acids of the corm are presented in Tables 4-7. The elemental analysis of the corm revealed the presence of calcium-2.86, sodium-0.001, potassium-0.39 and iron-0.039 mg/gdw.

Rf values obtained by Thin Layer Chromatography (TLC) patterns are useful to establish their identity and purity of the herbs. Stahl has discussed in detail, the importance of TLC as a legally binding method for characterization of drugs[30]. Colchicine was not found in this species. Cyanogenesis, the ability to produce hydrocyanic acid (HCN) is common among Araceae. HCN does not occur free in higher plants but is released from cyanogenic precursors as the result of enzymatic action. Presence of triglochinin glycoside has been reported in *Alocasia, Anthurium*, *Arum, Dieffenbachia, Lasia* and *Pinellia* of Araceae[26].

Table 4 — Preliminary phytochemical screening of *Arisaema leschenaultii* (Corm)

S.No.	Phytochemicals	Petroleum ether (40-60 °C) extract	Benzene extract	Chloroform extract	Methanol extract
1.	Steroids	+	+	+	+
2.	Triterpenoids	-	-	-	+
3.	Reducing sugars	+	+	+	+
4.	Sugars	+	+	+	+
5.	Alkaloids	-	-	-	-
6.	Phenolic compounds	-	-	-	+
7.	Flavonoids	-	-	-	+
8.	Catechins	-	-	-	+
9.	Saponins	-	-	-	+
10.	Tannins	-	-	+	+
11.	Anthroquinones	-	-	-	-
12.	Amino acids	-	-	-	+

- Absent; += Present

Table 5 — Thin-Layer Chromatographic studies of the various extracts

Extract	Solvent system used	Rf values of spots obtained by viewing under UV light (365 nm)	Rf values of spots obtained by keeping plates in an Iodine chamber
Petroleum ether (40–60 °C)	Petroleum ether (40–60 °C): Benzene (5:1)	Yellow 0.03	0.03•
Benzene	Benzene: Chloroform (4:1)	Yellow 0.06	0.06•
Chloroform	Chloroform: Methanol (9:1)	Yellow 0.13	0.13θ
Methanol	Chloroform: Methanol (6:1)	Brown 0.93	0.13θ 0.34○ 0.93•

•= Intense ; θ= Moderately intense; ○= Faint

Table 6 — Paper chromatographic studies of the water extract

Solvent system used	Rf values of the spots obtained by viewing under UV light	Rf values of the spots obtained by keeping the paper in an Iodine chamber
n-butanol : acetic acid : water (1:1:2)	Brown 0.85	0.85°
Methanol: Water (1:1)	Dark brown 0.68	0.68•
	Dark brown 0.85	0.85°

• = Intense;°= Faint

Table 7 — Paper chromatographic analysis of amino acids of *Arisaema leschenaultii* corm

S No	Aminoacids	Rf value	Result
1.	Arginine	0.21	+
2.	DL-aspartic acid	0.25	+
3.	DL-calamine	0.38	+
4.	DL-serine	0.28	+
5.	DL-Threonine	0.37	-
6.	DL-Tryptophan	0.63	+
7.	DL-valine	0.54	+
8.	Glycine	0.30	+
9.	Histidine	0.20	-
10.	L-cystine	0.12	+
11.	L-glutamicacid	0.34	+
12.	L-Leucine	0.58	+
13.	L-Proline	0.41	+
14.	L-Tyrosine	0.56	+
15.	Lysine	0.15	+

- = Absent; + = Present ; Solvent system – n-butanol: acetic acid: water (4:1:5)

In the present investigation also, the corm of *A. leschenaultii* was found to possess cyanogenic glycosides. Usually in the underground stems, the amount of starch is higher than the amount of sugar, because starch is the reserve food material in the corms of the plants. Phenolic compounds are important constituents of some medicinal plants. In food industry, they are utilized as colouring agents, flavourings, aromatizers and antioxidants. Phenolic classes of pharmaceutical interest are tannins, coumarins, anthroquinones, napthoquinones, flavones and related flavonoid glycosides, anthocyanidins, lignans and other simple phenolic compounds. In plants, phenolic compounds play an important role in disease resistance. Phenols are also involved in the protection of herbs from

browsing animals. Flavonoids are generally present in high amount in the plants growing in high altitude, because they absorb UV radiation. Flavonoids include the colouring agents of plants and they are essentially used to treat capillary and venous disorders, alone or in combination with other drugs, they are the common ingredients of vascular protective agents and venous tonic[29]. The high calcium content is probably due to the presence of calcium oxalate crystals in the corms and free calcium. Inorganic elements play an important role in various physiological processes. There are evidences for using plants as indicators for mineralization.

It should be remembered, however, biosynthesis of secondary metabolites although genetically controlled, is affected by environmental influences. The soil, the season and the gathering time are some of the important variable factors with plants and it can hardly be expected that the amount of constituents would be constant under all conditions. These results could be used as a diagnostic tool for the identification of the species. The amino acid profile is a consistent character because they are genetical.

Conclusion

Pharmacognostical studies on the medicinal plant *A. leschenaultii* have been reported for the first time. The macroscopical or morphological description helps in identification of the plant. Microscopical study in entire and powdered form of the drug is one of the aspects of histological evaluation. The size, shape and structure of the starch granules and length of calcium oxalate crystals from any particular plant only vary within definite limits, so that it is possible to distinguish the starches derived from different species. Hence, the study of starch grains and calcium oxalate crystals is useful in confirming the identity and purity of the drug. The physico-chemical constants, extractive values, fluorescent analysis, phytochemical analysis of the dry powder of the corms have been determined by employing standard methods of analysis as described in Pharmacopoeia of India. Based on the pharmacognostical studies it is possible to fit standards for the drug. Thus, the results of the present investigation provide dependable diagnostic features of the vegetative organs of the plant for the identity of the drug in entire and in fragmentary conditions.

Reference

1 Airy Shaw H K and J C Willis's, A Dictionary of flowering plants and ferns, Revised 8th Edn, 1973, Cambridge.

2 Karthikeyan S, Jain S K, Nayar M P and Sanjappa M, Florae Indicae Enumeratio-Monocotyledonae, 1989, BSI, Calcutta, 7-16.

3 Nicolson D H, Araceae *In*: Dassanayake, A Revised Handbook to the Flora of Ceylon, Vol VI, New Delhi, 1987, 64-85.

4 Mohanan N and Sivadasan M, Flora of Agasthyamalai, Bishen Singh Mahendra Pal Singh, Dehra Dun, 2002, p. 755.

5 Venkatasubramanian N, Sasidharan K R, Gurudev Singh B and Mahadevan N P, *In*: Manoharan T M, Biju S D, Nayar T S and Easa P S, Silent Valley Whispers of Reason, Kerala Forest Department, 1999, 225-227.

6 Fyson P F, Flora of South Indian Hill Stations, Govt Press, Madras, 1932, Vol. 1, 622-623.

7 Mathew K M, The Flora of the Palni Hills, (Monocotyledons), Rapinat Herbarium, Tiruchirappali, India, 1999, Part III, 1368-1371.

8 Agarwal V S, Drug Plants of India, Kalyani Publishers, New Delhi, 1997, 194.

9 Hooker J D, Flora of British India, 1894, Vol. VI, L. Reeve & Co., London, 495-510.

10 Fischer C E C, *In*: Gamble's Flora of Presidency of Madras, Adllard & Son Ltd., London, 1928, Vol 3, 1571-1592.

11 Mohanan M and Henry A N, Flora of Thiruvananthapuram, Kerala, Botanical Survey of India, 1994.

12 Sass J E, Elements of botanical microtechnique, McGraw-Hill, Inc., New York, 1940,

13 O' Brien T P, Fedar N and Mc Cull M E, Polychromatic staining of plant cell walls by toluidine blue, *O Protoplasma*, 1964, **59**, 364-373.

14 Johansen Donald Alexander, Plant Microtechnique, McGraw-Hill, Inc., New York, 1940, 126-154.

15 Salisbury E J, *In*: Wallis T E, Pharmacognosy, CBS Publishers, New Delhi, 5th Edn, 1927, 113.

16 Anonymous, Pharmacopoeia of India, Govt. of India, Manager of Publications, New Delhi, 2nd Edn, 1996, 947-948.

17 Evans W C, Trease and Evans Pharmacognosy, Harcourt Brace Company, Asia, 14th Edn, 1996, 191-218.

18 Sadasivam S and Manickam A, Biochemical Methods for Agricultural Sciences, Wiley Eastern Ltd., 1996, New Delhi.

19 Sim S K, Medicinal Plants Glycosides, University of Toronto Publication, 1988, 123-135.

20 AOAC, 2001, Official Methods of Analysis, Association of Official Analytical Chemists, Washington D.C.

21 Pullaiah, Flora of Andhra Pradesh, Scientific Publishers, Jodhpur, India, 1997, Vol. 3, 1019-1021.

22 Pallithanam J M and Mathew K M, A pocket flora of the Sirumalai Hills, South India, The Rapinat Herbarium, Tiruchirappali, India, 1999, 262-263.

23 Mohanan and Sivadasan M, Araceae of Silent Valley and Neighbourhood, *In*: Manoharan T M, Biju S D, Nayar T S and Easa P S, Silent Valley Whispers of Reason, Kerala Forest Department, 1999, 227-249.

24 Webber E, Observations on the epidermal structure and stomatal apparatus of some members of Araceae, *Rhodora*, 1960, **62** (741), 251-258.

25 French J C and Tomlinson, Vascular patterns in stems of Araceae, Subfamilies Colocosoideae, Aroideae, Pistioideae, *Amer J Bot,* 1983, **70** (5), 756-771.

26 Jean Bruneton, Pharmacognosy, Lavoisier Publishers, Inc. USA, 2nd Edn, 1999, 175-182 & 310-325.

27 Makino, Tanakia and Yusuo, Properties of starch from *Arisaema negishii, Nippon Shokulin Kagaku Kaishi*, 1996, **43**(9), 1049-1053; *Chem Abstr,*1996, **125**, 326723t.

28 Wallis T E, Pharmacognosy, CBS Publishers, New Delhi, 5th Edn, 1997, 555-560.

29 Kokate C K, Purohit A P and Gokhale S B, Pharmacognosy, Nirali Prakashan, Pune, 1997, 105-137.

30 Stahl E, Thin Layer Chromatography, Springer-Verlag, Berlin, 1969, 720-724.