ORIGINAL ARTICLE

Pharmacognostical evaluation of *Arisaema murrayi* (J. Graham) Hook. leaves and tubers for quality control assessment

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Abstract

Introduction: *Arisaema murrayi* (J. Graham) Hook. is one of the plants belonging to the family *Araceae*, commonly called Murray's Cobra Lily or Snake Lily which is known for its medicinal benefits. However, no published work is available to date on pharmacognostical evaluation. **Materials and Methods:** Pharmacognostic evaluation of leaf sand tubers was carried as per the standard guidelines which are based on morphological, microscopical, and physicochemical analysis. **Results:** The morphological evaluation of tubers revealed that tubers were hemispheric in shape, up to 5 cm radius, leaves were found to be coming up shortly after the peduncle, with peltate shape. The layers of the epidermis in the leaf are closely packed. Below the epidermis, there is 2–3 layer of collenchyma cells present. Microscopy study of powder showed the presence of a few layers of fibrous tissue. Cluster crystals of calcium oxalate and starch grains were also present in the powder. Preliminary phytochemical analysis showed the presence of carbohydrates, proteins and amino acids as primary metabolites, and alkaloids as secondary metabolites. **Conclusion:** Pharmacognostic characters studied will be helpful in morphological and microscopical standardization of *A. murrayi.*

Key words: Pharmacognostical evaluation, *Arisaema murrayi,* quality control, evaluation

INTRODUCTION

Since ancient times humans have been dependent on nature for multiple supplies and this dependency persists to date. Due to the utilization of herbs for survival, the human race comes to know about various health beneficial properties of herbs. Soon after initial discoveries, medicine becomes an indispensable part of human life. However, the quality and therapeutic properties are the two key points that were most discussed during the evolution of almost all systems of medicine. In the modern era, although the utilization of herbs and herbal products as medicine is gaining importance, at the same time, the urge for quality control methods for herbal drugs arises. Pharmacognostical evaluation is considered the first step of quality control and utilization of any herb to be used as medicine.[1]

The newly discovered herbs are been identified and evaluated as per certain recommended methods under pharmacognostical evaluation. Once the herbal material is evaluated for specific parameters, then the results obtained are considered as standards for that drug. These set standards are further used for purpose of standardization of the same drug in future. Whenever the herb is collected from the wild or cultivated source, the task of standardization is rudimentary practice.[2] Once the standards are set for any crude drug, then it is easy to carry out an evaluation of the same drug sample in the future.

Arisaema is a genus of flowering plants belonging to the family *Araceae*. Genus Arisaema covers more than 160 plants.[3] These plants are widely distributed throughout the globe and found native to eastern Africa, central Africa, Asia, and eastern North America. Asiatic species of Arisaema are often called Cobra lilies. *Arisaema murrayi* (J. Graham) Hook. is also one of the plants belonging to the family *Araceae*, commonly called Murray's Cobra Lily or Snake Lily. The plant gets its name due to its beautiful flower which often resembles with cobra snake's hood. The plant is believed to be

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a deadly poisonous plant, but local people still use its leaves to treat inflammation.[4] In the Indian subcontinent, Murray's Cobra Lily is mainly found in Himalayan regions as well as in the Western Ghats in the hilly forest as undergrowth as well as gentle open slopes among bushes more prominently seen in Mahabaleshwar, Altitude ranges between 600 and 1200 m above sea level.[5] The plant prefers shady, moist, and well-drained fertile soil. It is a tuberous herb, 30–50 cm high. A single leaf, with 5–7 leaflets, comes out after the flowers. Tubers hemispheric, up to 5 cm in diameter.

Several species are reported in the genus Arisaema;[6] hence, it was decided to carry out pharmacognostical standardization of accurate plant species. Assurance of the quality of herbal drugs ensures their efficacy too.[7] Therefore, an attempt has been made to evaluate the pharmacognostical parameters of *A. murrayi* (J. Graham) Hook., as the literature survey and scientific data revealed that no pharmacognostic studies had been carried out on the aerial parts of the plant. Hence, it was thought worthwhile to evaluate the plant by macroscopical, microscopical, physicochemical, and phytochemical parameters.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The whole plant of *A. murrayi* (J. Graham) Hook. was collected from Kolhapur district (Maharashtra) during the rainy season. The plant material was authenticated by Prof. M. D. Wadmare (Head, Department of Botany, Smt. Kasturbai Walchand College, Sangli). Authenticated washed plant material was cut into small pieces and dried under hot air oven at 40°C for 72 h and pulverized in a mechanical grinder. The coarse powder was passed through sieve no 40 and taken for further studies. The powdered material was stored in an airtight container away from light in a cool place.[8]

Morphological Evaluation

The morphological evaluation of the plant parts was carried out by observing their size, shape, color, odor, taste, and texture by naked eyes and using simple microscope as well. The shape of leaves, venation pattern, apex, and margins were also observed. Further, the shape and size of tubers were noted. The fresh and rhizomes were subjected to morphological evaluation and documented by direct observation method (naked eye observation) of the sample.[9]

Microscopical Evaluation

The microscopical evaluation was carried out for leaves and tubers also. The fresh leaves were selected and the transverse sections were taken using a razor-sharp steel blade. The sections were initially soaked in water and then cleared with help of a clearing solution by gentle warming. Further, the clarified sections were washed with double distilled water and then stained with phloroglucinol-concentrated hydrochloric acid (1:1). The thin most section was mounted on a slide with the help of glycerin and observed through a compound microscope. The photographs of the section were taken using the digital camera which was attached to the microscope.

The powder of leaves and tubers was also evaluated separately for their microscopic characteristics. The powder was treated with a mixture of phloroglucinol-hydrochloric acid (1:1), acetic acid, and iodine solution to recognize the presence of fibers, starch grains, cellular structures, calcium oxalate crystals, etc.[10]

Physicochemical Evaluation

Physicochemical parameters were evaluated for the powdered leaves as well as tubers. The Indian pharmacopoeia as well as the World Health Organization suggested physicochemical parameters for the evaluation of crude drugs.[9] Based on those guidelines, the Arisaema powder was evaluated for loss on drying, ash values (total ash, acid-insoluble ash, and water-soluble ash), and extractive values. All the evaluation was done in triplicate and observation was recorded.

Preliminary Phytochemical Analysis

For preliminary phytochemical studies, hydroalcoholic extract of the dried powdered drug was prepared using 40% alcohol v/v. The powder sample was refluxed using a reflux condenser with 40% ethyl alcohol for 1 h. Exhausted extraction with solvent was ensured. The extract was concentrated under reduced pressure. Further, the extract was evaluated for presence/absence of different metabolites.[2,11]

RESULTS

Morphological Evaluation

The morphological evaluation of tubers revealed that tubers were hemispheric in shape, up to 5 cm radius. Tubers were fleshy, white along with some covering sheaths [Figure 1]. Leaf was found to be coming up shortly after the peduncle, with peltate shape [Figure 2], glossy, dark green in the upper surface, purple, and shining on the lower side. The leaf base was found to be divided into 5–11 segments. Leaf length and breadth were measured and it was found to be 6–15 cm and 4–6 cm, respectively.

Microscopical Evaluation

Leaf microscopy

The transverse section of the leaf passing the midrib convexly projects on the lower side and is slightly grooved with a

shallow central elevation on the upper side. The small, thick-walled upper, and lower epidermis are present. The epidermis layer consists of the single layer of living cells, thickened, and covered with a layer of the cuticle [Figures 3 and 4]. The layers of the epidermis are closely packed. Just below the epidermis, there are 2–3 layers of collenchyma cells present. Below the collenchyma cells are a few layers of parenchyma cells which are present with intracellular space. The vascular bundle is situated in a ring on the inside of the plant. The xylem is found in the center part of the vascular bundle and the phloem is found in the outside of the vascular bundle. Smaller vessels are constituting the nearby vascular bundle. The simple and compound starch grains and occasionally a few lignified fibers were present in the pericyclic region.

Transverse section of tuber

The transverse section of *A. murrayi* tuber when examined under the microscope shows the presence of outer or inner cork cells, a narrow zone of rectangular and tangentially elongated cork cells. It is followed by wide ground tissue consisting of thin-walled circular to parenchymatous cells with small intercellular spaces. A large number of fibrovascular bundles surrounded by bundle sheath and xylem vessels are scattered in the ground tissue. Most of the cells of inner ground tissue were found to be loaded with simple as well as compound starch grains [Figure 5].

Powder microscopy

The powder of aerial parts of *A. murrayi* plant was greenish in color, slightly bitter, and characteristic in taste. Microscopy study of powder showed the presence of the few layers of fibrous tissue [Figure 6]. Cluster crystal of calcium oxalate and starch grains were also present in the powder.

Physicochemical Evaluation

Results of physicochemical evaluation of powdered aerial parts and tubers are presented in Table 1.

Preliminary Phytochemical Analysis

Preliminary phytochemical screening of hydro alcoholic extracts of *A. murrayi* was carried out and the extract showed the presence of phytoconstituents such as carbohydrates, flavonoids, steroids, and alkaloids. The results of test are summarized in Table 2.

Figure 1: Leaves of *Arisaema murrayi*

Figure 2: Different shapes of tubers of *Arisaema murrayi*

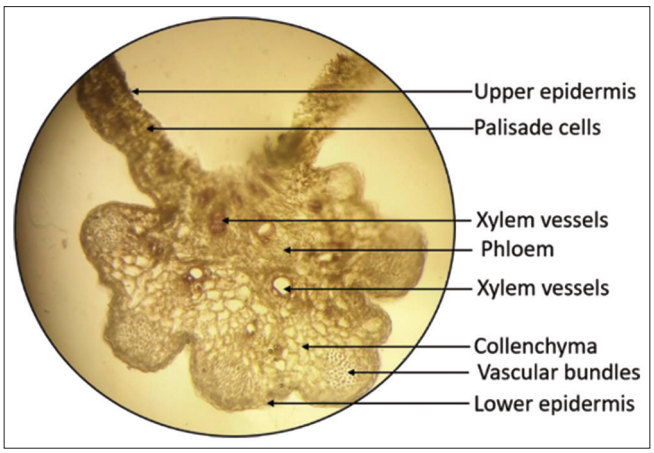

Figure 3: T. S. of leaf of *Arisaema murrayi*

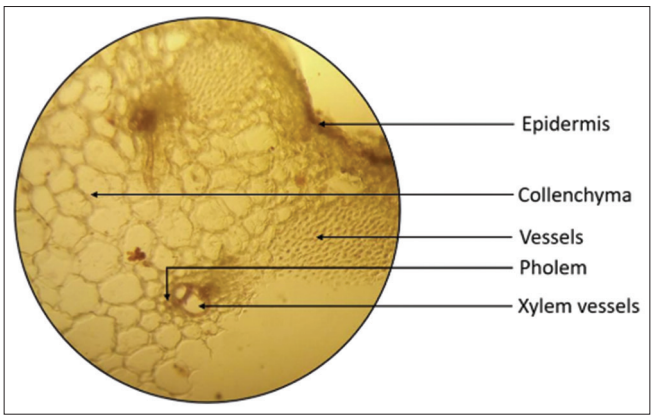

Figure 4: T. S. of Leaf of *Arisaema murrayi* (enlarged)

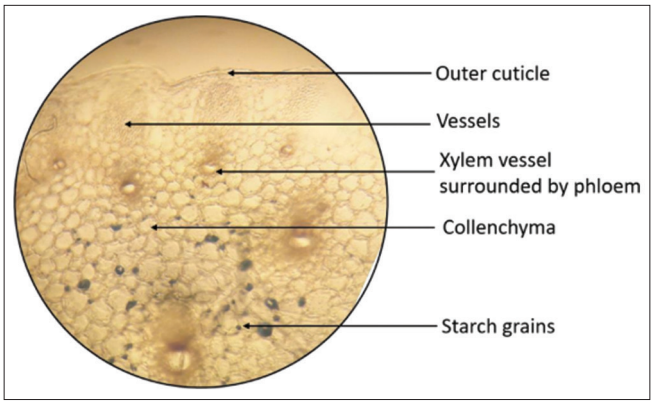

Figure 5: T. S. of tubers of *A. murrayi*

Table 1: Evaluation of physicochemical parameters

S. No.	Physical parameter	Leaf (% w/w)	Tubers (% w/w)
1.	Foreign organic matter	0.61	0.93
2.	Loss on drying	07	12
3.	Water extractive value	14.28	8.4
4.	Ethanol extractive value	14	7.8
5.	Hexane extractive value	5.2	3.6
6.	Chloroform extractive value	7.4	3.7
7.	Total Ash	4.3	6.7
8.	Acid-insoluble ash	0.78	1.2
9.	Water-soluble ash	2.80	3.6

Table 2: Preliminary phytochemical screening of *Arisaema murrayi*

Parameters	Leaf	Tubers
Alkaloids	+	+
Saponins	–	–
Amino acid	+	+
Sterols	+	–
Tannins	–	–
Proteins	+	+
Carbohydrates	+	+
Flavonoids	+	–

DISCUSSION

In the case of medicine quality, safety and efficacy are important aspects. However, in the case of crude drugs or the drugs obtained from natural resources, their identification and pharmacognostic evaluation become a necessary step for their further utilization. The aspect of pharmacognostic evaluation or standardization of crude drugs is related to assurance of quality, purity, and efficacy as well. For evaluation of crude drugs, their morphological, microscopical, physical, chemical, and biological evaluation is carried out. As per the WHO, the macroscopic and microscopic analysis of a drug is necessary to set the standards for identity and purity. Hence, the present research work was performed to establish the quality parameters of *A. murrayi*. This is the first study to report the pharmacognostical evaluation for leaves and tubers of *A. murrayi*.

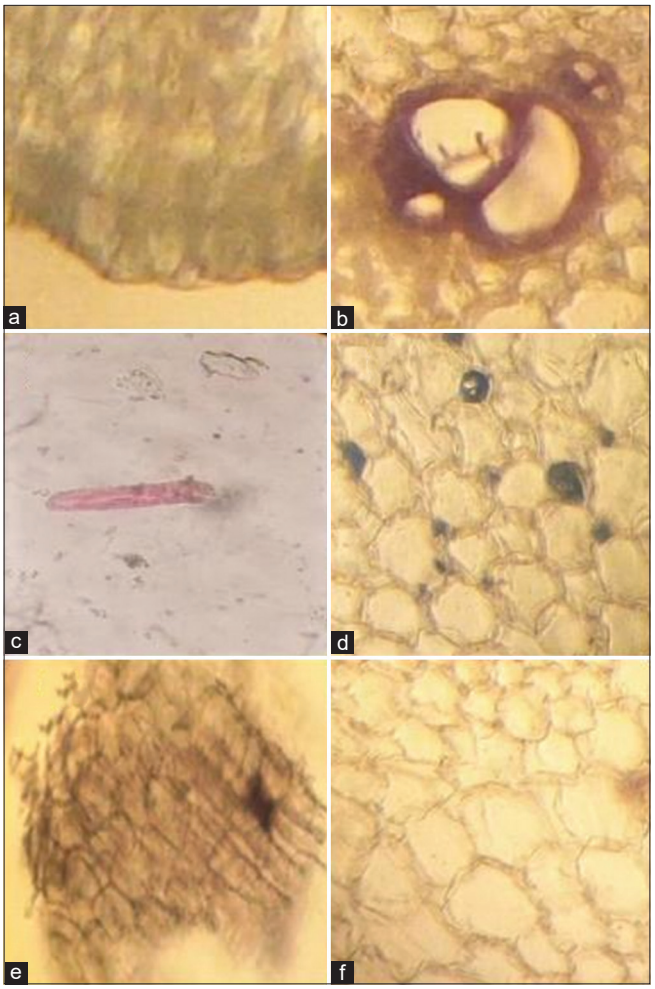

Figure 6: Powder characteristics of *A. murrayi* (J. Graham) Hook. leaf and tubers (a) parenchyma cells, (b) Xylem vessels, (c) Lignified fibers, (d) cortex cells with starch grains, (e) cork cells, and (f) collenchyma cells

Morphological evaluation of *A. murrayi* revealed that the leaf is having a palmately lobed shape with very long petiole or which can be called as leaf stalks. The leaves usually appear before the flowers. The inflorescence of this plant is characteristic, the inflorescence consists of a spathe surrounding a rodlike spadix, which bears small unisexual flowers. The microscopic evaluation revealed the presence of epidermal cells, palisade cells, as well as xylem vessels. The presence of multiple vascular bundles in each lobe of the leaf was a unique feature. The tubers microscopy revealed the presence of the outer cuticle and the collenchyma cells which were filled with starch grains. The intermittent presence of xylem vessels was also observed in it.

As the tubers were having much amount of soil adhered to them; hence, the total ash value was observed higher than the leaves. Tubers were also having huge moister in them which

makes them very difficult to dry easily. Ultimately, powder of tuber was found to be absorbing much amount of moisture, leads to a higher amount of loss on drying even after the initial drying of the drug. Standards set for extractive values implies about quality of the crude drug. If the drug is already extracted in its intact form and later on sold, then the extractive value of such drugs does not meet the standards. Alcohol and water extractive values of leaves were found to be higher. Tubers were not having many constituents to get solubilized in organic solvents. Preliminary phytochemical analysis showed the presence of carbohydrates, proteins and amino acids as primary metabolites, and alkaloids as secondary metabolites.

This is the first report on pharmacongostical evaluation of but few reports related botanical and phytochemical aspects of genus Arisaema revealed that it contains alkaloids, phenols, terpenes, flavonoids, glycosides, as well as triterpenoids.[12] The present report also supports the findings of the previous reports related to phytochemical investigation.

CONCLUSION

Herbal drugs are pharmacognostical evaluation is the first step in quality assessment of any crude drug. The pharmacognostic features examined in the present study, which may serve as a tool for the identification of plant raw material. Pharmacognostic characters studied will be helpful in the morphological and microscopical standardization of A. murrayi (J. Graham) Hook. The phytochemical studies reported in the present study need further scientific investigation to discover chemical constituents present in the crude drug. The biological evaluation and discovery of therapeutic effects will also be useful.

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