Research Article

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION OF WHOLE PLANT OF ARISTOLOCHIA INDICA L. - A THREATENED MEDICINAL PLANT

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ABSTRACT

Aristolochia indica L. is threatened medicinal plant, belongs to the family Aristolocaceae. The plant is used in traditional system of medicine for healing various diseases. However, the present study was aimed to evaluate the parameters to determine the quality of the plant. This study comprises morphological, microscopical, phytochemical and pharmacognostic investigations of the plant.

Keywords: *Aristolochia indica* L., Aristolocaceae, Pharmacognosy, Phytochemical screening.

INTRODUCTION

Aristolochia indica L., of Aristolochiaceae known as Ishwar balli (Kannada), Indian Birthwort (English), Isharmul (Hindi), Ishwari (Sanskrit). In Ayurveda the leaves and the roots are used for treatment of fever and insect bites. *A.indica* has been also used for other medicinal purposes[1]. The plant is used to treat cholera, fever, bowel troubles, ulcers, leprosy, poisonous bites[2] and also used as emmenagogue, abortifacient, antineoplastic, antiseptic, anti-inflammatory, antibacterial, antioxidant and phospholipase A_2 inhibitor[3-5]. Because of these important medicinal properties of *Aristolochia indica* L. is under threat due to extensive collection and continuous deforestation. This requires conservation of this traditional medicinal plant for the future generation and for available throughout the year for the use.

Today in the market fake drugs are available to cheat the common people, among one of them *Aristolochia indica* is also sold. Since the root is more potent as an antidote for snakebite adulterations are made. In this context, though there is Pharmacognosy of leaf is available, whole plant Pharmacognosy is not available, hence in the present study, whole plant pharmacognostic studies and phytochemical investigation of *Aristolochia indica* has been undertaken.

The present study scientifically validates the use of plant in traditional medicine and it contributes to the development of standardized parameters of herbal drugs used in Indian system of medicine.

MATERIALS AND METHODS

Macroscopic studies of *Aristolochia indica* L.

Aristolochia indica L., (Indian Birthwort) is a perennial twining climber with greenish, whitish stem. Leaves are glabrous and very variable, usually obvoate-oblong to subpanduriform entire with somewhat undulate margins, cordate, acuminate. Flowers are few, in axillary racemes with a perianth up to 4 cm long having a glabrous pale-green inflated and lobed, base narrowed into a cylindrical tube terminating in a horizontal funnel-shaped purple mouth and a lip clothed with purple-tinged hair. Capsules are oblong or globose-oblong, 3-5 cm long and the seeds are flat, ovate and winged.

Collection of plant material and authentication

Fresh whole plants of *Aristolochia indica* L., were collected from Karnatak University campus Dharwad and were authenticated and voucher specimen has been deposited in the P. G. Department of Botany, Karnataka University, Dharwad for future reference.

Drying of plant material

The whole plant material of *Aristolochia indica* L. was subjected to shade drying for about 10-15 weeks. The shade dried plant material was further crushed to powder and the powder was passed through the mesh 22 and stored in air tight container for further analysis.

Macroscopic and microscopic analysis

The macroscopic and microscopic examinations of plant studied were based on the method[6-8].

Transverse sections of leaf, stem, and root were stained with saffranin and Fast green as per the procedure [9]. Powder microscopy was performed according to the prescribed procedure[10,11] and stomatal index by following standard method.

The photomicrographs were taken by Bright field microscope with digital camera Canon Photo shot G2.

Determination of behaviour of plant powder

Behaviour of plant powder with different chemical reagents was determined under natural light and fluorescent-UV light.

Extraction of powdered plant material

The plant material collected from their natural habitat was cleaned, shade dried at room temperature, coarsely powdered and stored in an air tight glass container. 50

gms of each coarse powder was successively extracted with different solvents *viz*. Petroleum ether, Alcohol and Chloroform (40-60) in Soxhlet extractor for 16-18 hours. Then, the extracts were filtered and concentrated using rotary flash evaporator and residues were dried in desiccators over sodium sulfite below 60°C. Freshly prepared extracts were subjected to phytochemical evaluation for the detection of various constituents using conventional protocol [12].

RESULTS AND DISCUSSION

Pharmacognostic investigations

The detailed and systematic pharmacognostic evaluation would give valuable information for the future studies. The detailed morphology of *Aristolochia indica* L. was carried out to support proper identification of drug.

Stomata and Stomatal index

Aristolochia indica L. has two types of stomata, namely anisocytic and paracytic. The epidermal cells are larger than subsidiary cells. Stomatal index and the percentage of stomata found in unit area of leaf exhibited marked variation in the adaxial and abaxial surface. Abaxial surface has an increased stomatal frequency than adaxial surface (Fig. 1. D). The values are represented in the table 1.

Microscopy

Microscopy of Root (T.S)

T.S of root (Fig.1.A.) shows single layered epidermis covered by cuticle, cortex is made up of group of parenchymatous cells. Some cells contain calcium oxalate crystals. Endodermis and pericycle is incospiocuous. The primary phloem is found beneath the secondary cortex and next to crushed primary phloem there is secondary phloem. The secondary phloem is composed of sieve tubes, companion cells and phloem parenchyma. In between secondary phloem and secondary xylem there is distinct cambium. Towards the inner side of cambium there is secondary xylem. The secondary xylem has large vessels. The primary xylem strands are easily recognizable possessing protoxylem poles towards periphery and metaxylem towards the centre. There is tetrarch condition with scanty pith in the centre. The medullary rays of parenchyma are present.

Microscopy of Stem (T.S)

T.S of stem (Fig.1.B) shows outer epidermis is made up of single layer consists of compact barrel shaped cells without intercellular spaces. Cortex region consists of collenchyma, chlorenchyma and endodermis. Endodermis is lying immediately outside the sclerenchymatous zone of pericycle. This layer is wavy and contains many starch grains. Just beneath the endodermis there is a multilayered zone of sclerenchymatous pericycle. The cells are lignified and appear polygonal in cross section. The pericycle region consists of a continuous band of sclerenchyma. Seven bundles are arranged in a ring. Each bundle show typical dicot sclerenchyma characteristics. Pith is large and conspicuous. Secondary thickening developing from a conventional cambial ring (but sometimes the pith and primary medullary rays are unusually dilated, deforming the secondarily thickened structure). Xylem with fibre tracheids, Vessel end-walls simple. Primary medullary rays wide. Wood parenchyma apotracheal, or paratracheal.

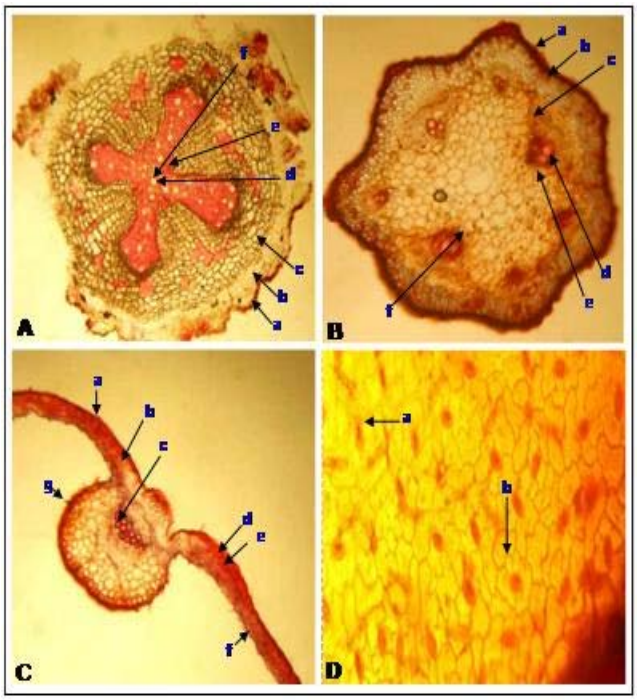

Figure 1: **A**-T.S. of young root, a-epidermis, b-cortex, c-endodermis, d-medulla, e-phloem, f-xylem. **B**-T.S.of stem, a-epidermis, b-hypodermis, c-conjuctive tissue, d-meta xylem, e-proto xylem, f-phloem. **C**-T.S.of leaf, a-upper epidermis, b-cuticle, c-vascular bundles, d-palisade parenchyma, e-spongy parenchyma, f-lower epidermis g-trichome, **D**-Surface view of leaf epidermis showing **a**- paracytic and **b**- anisocytic stomata.

Table 1: Stomatal index on adaxial and abaxial leaf surfaces of *Aristolochia indica* L.

Trials	Adaxial surface of leaf		Abaxial surface of leaf	
	Margin	Middle	Margin	Middle
1.	128	118	136	178
2.	130	122	134	200
3.	135	130	145	218
4.	145	140	160	228
5.	155	152	172	238
Average	138.59	132.40	149.40	212.40
	135.49		**180.90**	

Microscopy of Leaf (T.S)

T.S of leaf (Fig.1.C) shows the leaf across the midrib showed an upper epidermis consisting of long elongated palisade cells with calcium oxalate crystals and lower epidermis consists of spongy parenchyma with thin cuticle. Upper and lower epidermis having uniseriate trichomes. Stomata were paracytic and anisocytic type, whereas in *Aristolochia bracteata* stomata are anomocytic[13].

The midrib bundle was surrounded by a zone of closely packed mesophyll tissue differentiated into 1-2 layers of cylindrical cells closely packed with their long axis at right angle to epidermis. Spongy parenchyma containing oval, rounded cells arranged loosely towards the lower epidermis.

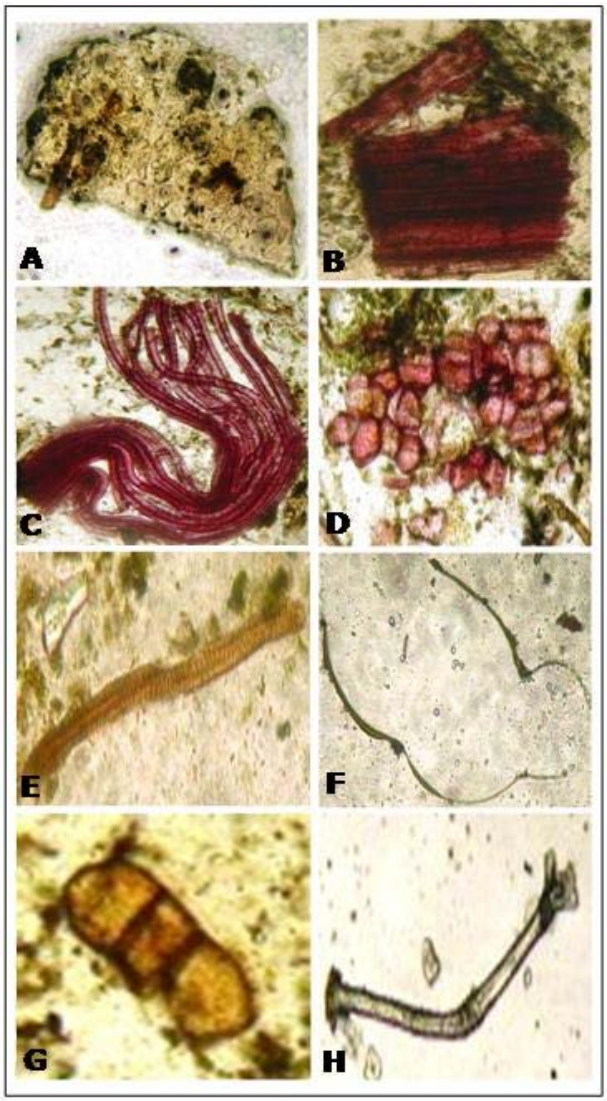

Figure 2: A- Cortex, **B**- Epidermal cells, **C**- Vessels. **D**- Calcium oxalates, **E**- Fragments of fibre, **F**-Uniseriate curved trichome, **G**-Glandular trichome, **H**- Trichome.

Preliminary Phytochemical Analysis

Preliminary phytochemical screening of the *Aristolochia indica* L. plant powder is done following standard methods 11 (Kokate 2000) and results are presented in the table 2.

Behaviour of whole plant powder with different chemical reagents

Behaviour of powder of *Aristolochia indica* L. with different chemical reagents is detected. The colour changes, when observed under day light and fluorescence UV-light by method[14] and results are presented in the table 3.

Table 2: Phytochemical analysis of whole plant extract of *Aristolochia indica* L.

Phytochemicals	Petroleum ether extracts	Alcohol Extracts	Chloroform Extracts
Steroids	+	+	+
Glycosides	-	+	-
Fatty acids	-	-	-
Alkaloids	+	+	-
Flavanoids	+	-	+
Saponins	+	-	+
Tannins	+	-	-
Triterpenoids	+	+	+
Cardiac glycoside	-	-	-
Volatile Oils	+	+	+
Anthocyanidins	+	_	_
Anthracene glycosides	+	+	-

+ = Present, - = Absent.

Table 3: Behaviour analysis of whole plant powder of *Aristolochia indica* L. with different chemical reagents.

Treatment	Colour of powder	
	Day light	UV light (254 nm)
Powder as such	Light green	Light green
Powder + Picric acid	Yellowish green	Light green
Powder + HNO_3	Faint brown	Light green
Powder + HCL	Dark green	Dark green
Powder + H_2SO_4	Light green	Dark green
Powder + $FeCl_3$	Yellowish green	Dark green
Powder + NaOH	Yellowish green	Light green
Powder + Glacial acetic acid	Light green	Light green
Powder + Iodine solution	Dark green	Dark green
Powder + Aqueous solution	Light green	Dark green
Powder + Aq. Mercuric chloride	Light green	Dark green
Powder + HNO_3 + Ammonium solution	Light green	Dark green

Table 4: Organoleptic evaluation of various parts of *Aristolochia indica* L

	Flower	Fruit	Seed	Leaves	Stem	Root
Colour	Pale-green	Dark brown	Brown	Light green	Light brown	Dark brown
Odour	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless
Taste	Bitter	Bitter	Pungent	Bitter	Slight bitter	Slight bitter

CONCLUSION

In the present investigation, various standardized parameters such as macroscopic, microscopic, pharmacognostic and phytochemical studies were carried out and they could be helpful in authentification of crude extract of *Aristolochia indica* L. The results of the present study will also serve as reference material in the preparation of monograph. It is present need to conserve this plant for medicinal usage. Tissue culture techniques may be more useful in the conservation point of view and to make the drug available throughout the year.

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