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PHARMACOGNOSTICAL AND PHYTOCHEMICAL INVESTIGATION OF AERIAL PARTS OF *ARISTOLOCHIA INDICA* L.

Mall Mridula*, Tomar Amita, Sushma, Dohare Bindu, Ghosh AK
College of Pharmacy, Institute of Foreign Trade and Management (IFTM), Lodhipur Rajput, Moradabad, UP, India

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ABSTRACT
Aristolochia indica L. (Family Aristolochiaceace) commonly known as Ishwari, Nakuli or Gandhanakuli. It possesses good medicinal value in traditional system of medicine. The present investigation deals with macroscopic, microscopic and phytochemical investigation which includes leaf constants, physiochemical parameters like ash value, extractive value and moisture content. The powdered leaves were subjected to fluorescence analysis with different chemicals. Phytochemical investigations revealed the presence of carbohydrate mainly reducing sugar, alkaloid, steroid, saponin and tannin. The ash value, acid insoluble ash value, water soluble ash value and sulphated ash were observed to be 15.2%, 5.15%, 1.95% and 10.15% respectively. The moisture content, petroleum ether soluble extractive value, benzene soluble extractive value, chloroform soluble extractive value, alcohol soluble extractive value and water soluble extractive value were found to be 3.66%, 0.424%, 2.79%, 15.53% and 8.80% respectively. The present study of macroscopic, microscopic and phytochemical investigation would help in identifying the drug from substitute and adulterants and can be used to prepare a monograph for the proper identification of the plant.
Keywords: Ishwari, macroscopic, microscopic, chemical investigation, identification.

***Corresponding Author**
Mridula Mall, M.Pharm Student, College of Pharmacy, Institute of Foreign Trade and Management (IFTM), Lodipur Rajput, Delhi Road, Moradabad-244001(UP) India Email: mridulampharm@yahoo.in

INTRODUCTION

Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. Over three-quarters of the world population relies mainly on plant and plant extract for health care. More than 30% of the entire plant species, at one time or other was used for medicinal purposes.AI is a perennial shrubby glabrous twiner with a long woody rootstock. Throughout India, it is distributed especially in tropical and subtropical regions of India from Bengal, Chittagong, Bihar, Orissa, Gaya hills and all districts of South India[1-5,7,8.] Various constituents and pharmacological activities of this plant have been reported. It mainly contains Aristolochic acid, methyl esters of 12- nonacosenoic acid, besides n-heptadecane, n- triacontane, palmitic acid, hexacosannic acid, stigmast-4-en-3-one, friedelin, cycloeucalenes and rutin[1-4]. A cytotoxic lignan, savinin has been isolated from the roots. The plant has been used in skin diseases. It is used as the appetiser, aphrodisiac and anthelmintic[1,3,9]. The fresh juices of the leaves are popular antidote to snake poison[1,3.] The leaves and bark are used in intermittent fever[1,9]. In traditional medicine the underground parts of the plant are rubbed with honey and given to treat leprosy and when macerated with black pepper, it is prescribed in Diarrhoea[1]. It possesses antioestrogenic, antifertility, abortifacient and interceptive activity. It is used ethanomedicinally as an antitumor, anti-inflammatory, antibacterial, antioxidant and antimicrobial[1,3-9].

MATERIALS AND METHODS

Collection of the plant material

The leaves were collected in the month of February 2011 from the Jhansi, Uttar Pradesh, India. The plant was identified and authenticated by Dr. Tariq Husain, National Botanical Research Institute, Lucknow. The specimen was deposited in LWG herbarium with Accession No. 97858 for further reference.

Processing of the plant material

The leaves were collected, shade dried, coarsely powdered and passed through the mesh no. 40. It was stored in an air tight container for further analysis in a cold place.

Morphological studies

The morphological characters were studied by employing a simple microscope[11].

Microscopical studies

Free hand sectioning was done for fresh leaves and petiole to obtain thin section. Phloroglucinol and hydrochloric acid in the ratio 1:1 was used as a stain and mounted on a glass slide and focused under a microscope. Structures were drawn using camera Lucida and photographs were taken[10,11].

Powder microscopy

Shade dried leaf was finely powered with the help of an electric grinder. The fine powder of the leaves were subjected to microscope as per standard procedure mentioned in Wallis and Trease[10,11].

Determination of leaf constant

The different parameters like stomatal number, stomatal index, vein islet No. and vein termination No. and palisade ratio were determined as per standard procedure mentioned in Khandelwal, WHO Guidelines and Pulok Mukherjee[13-15].

Fluorescence analysis

Powdered leaves were subjected to exposure under Ultra violet after treatment with various chemical and organic reagents.

Physiochemical parameters

The various physiochemical parameters like ash value and extractive values were performed as per the standard procedures mentioned in Khandelwal, WHO Guidelines and Pulok Mukherjee[13-15].

Extraction procedure

The powder of aerial part was defatted with the petroleum ether and marc was successively extracted with benzene, chloroform, alcohol and water with the help of Soxhlet apparatus.

Phytochemical screening

The various extracts obtained after extraction were subjected to preliminary phytochemical screening. A small amount of sample was treated with Molisch reagent, Fehling's reagent, Benedict reagent, Barfoed reagent, Selwinoff's reagent, Tollen's reagent, Cobalt-chloride solution, Tannic acid to identify carbohydrates. For protein and amino acid Biuret test, Millon's test, Ninhydrin test were done[13]. Salkowski test was done to test the presence of sterols. Sample was treated with Mayer's reagent, Hager's reagent, Dragendroff's reagent and Wagner's reagent to detect the presence of alkaloids. Glycosides were tested using legal's test, Baljet's test, Brontrager's test and Keller Killiani test[13]. Saponin was identified by the foam and haemolytic test, phenolic compounds and tannins presence were tested with the help of ferric chloride solution, lead acetate solution and aqueous Bromine solution[13]. The Shinoda test was done for the flavonoids[13].

TLC

The TLC of alcoholic extract was carried out to find the number of constituents present in the plant[16].

RESULT

Macroscopic

The morphological study reveals that the AI contains simple leaf. Shape of the leaf is cordate, apex is mucronate and it has reticulate system of veins. Colour of the leaf is dark green and is bitter in taste without any specific characteristic odour.

Microscopic

AI leaves are dorsiventral leaf. It contains single layer of upper and lower epidermis with a thin cuticle, mesophyll differentiated into one to two layers of cylindrical cells closely packed with their long axis at right angle to epidermis and spongy parenchyma containing oval, rounded cells loosely arranged towards the lower epidermis, vascular bundles consist of Xylem and Phloem (Figure 1).

Powder microscopy

Powder microscopy revealed the presence of anomocytic stomata, oval rounded starch grains, xylem vessels present in bundles and also contained cluster type of calcium oxalate crystals.

Leaf constants

The leaf constant stomatal number, stomatal index, palisade ratio, vein islet number and vein termination number are tabulated in table 1.

Fluorescence analysis

The fluorescence analysis of AI Leaves is tabulated in table 3.

Physiochemical analysis

The loss on drying, ash value (including total ash value, acid insoluble ash value, water soluble ash value, sulphated ash value), extractive value (including petroleum soluble extractive value, benzene soluble extractive value, chloroform soluble extractive value, alcohol soluble extractive value and water soluble extractive value) of leaves are tabulated in table 2.

Phytochemical screening

The extracts and powder drug were subjected to phytochemical screening for analysis of type of phytoconstituents. The extract and powdered drug contain carbohydrate (mainly reducing sugar), alkaloid, oils, steroid, saponin and tannin and result is tabulated in table 4.

TLC

The alcoholic extract produced four spots (R_f value for spot1, 2, 3 & 4 are 0.11, 0.20, 0.25 and 0.31 respectively) in the solvent system Chloroform: Methanol (75:25)

when observed under iodine chamber/ UV chamber/ naked eye.

CONCLUSION

For establishing correct identity and quality of a crude drug standard establishment is an integral part. Majority of the information on identity, purity and Quality of the plant material can be obtained from its macroscopy, microscopy and physiochemical parameters. These physiochemical parameters help in detecting adulteration and mishandling of the crude drug. *A. indica* is widely used for various medicinal purposes from the ancient times so the present pharmacognostical work will help in proper identification of this plant for further studies.

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Table 1: leaf constant of leaves of AI

S. No.	Leaf constants	values
1	Palisade ratio	4.5 - 6.7
2	Stomatal number	80 – 90
3	Stomatal index	12.50 – 15
4	Vein islet number	7 – 9/mm^2
5	Vein termination number	17 – 23/mm^2

Table 2: Physiochemical parameters of leaves of AI

S. No.	Standardization parameters	% W/W
1.	Loss on Drying	11.8
2.	Total ash	15.2
3.	Acid insoluble ash	5.15
4.	Water soluble ash	1.95
5.	Sulphated ash	10.15
6.	Petroleum ether soluble extractive value	3.660
7.	Benzene soluble extractive value	0.424
8.	Chloroform soluble extractive value	2.792
9.	Alcohol soluble extractive value	15.53
10.	Water soluble extractive value	8.800

Table 3: Fluorescence analysis of leaves of AI

S. No	Sample	Colour in light	Colour in UV (365nm)	Colour in UV (254nm)
1.	Drug	Dark green	Black	Dark green
2.	Drug + H_2SO_4	Green	Black	Light green
3.	Drug + H_2SO_4+ Water	Brownish green	Black	Green
4.	Drug + Conc. HCl	Brownish green	Black	Blackish green
5.	Drug + HNO_3 solution	Brown	Black	Green
6.	Drug + Ammonia solution	Light green	Black	Green
7.	Drug + Cobalt Chloride	Blackish green	Black	Black
8.	Drug + $CuSO_4$ solution	Dark green	Black	Green
9.	Drug + $FeCl_3$ solution	Yellowish green	Black	Dark green
10.	Drug + Ammonium thiocynate	Dark green	Black	Dark green
11.	Drug + Ammonium molybdate	Green	Black	Green
12.	Drug + $MgSO_4$ solution	Dark green	Black	Green
13.	Drug + Iodine solution	Dark green	Black	Green
14.	Drug + NaOH solution	Yellowish green	Black	Green
15.	Drug + Lead acetate solution	Green	Purple	Green

Table 4: Summery of Phytochemical screening of Leaves of AI

S. No.	Plant constituents	Petroleum Ether extract	Benzene extract	Chloroform extract	Alcohol extract	Aqueous extract
1.	Carbohydrate	+	+	+	+	+
2.	Proteins	-	-	-	-	-
3.	Amino acid	-	-	-	-	-
4.	Fats and oils	+	+	+	+	+
5.	Steroids	-	-	+	+	+
6.	Glycosides	-	-	-	+	+
7.	Alkaloids	-	-	-	+	-

Where (+) = present and (-) = absent

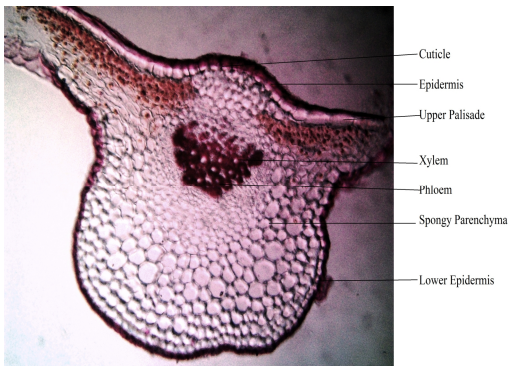

Figure 1: Transverse section of leaf *A. Indica* taken by camera (at magnification 10X*10X)

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