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(Research Article)

Pharmacognostical Study and Preliminary Phytochemical Screening of the Leaf of *Ixora johnsonii* Hook.f. - A Rare, Endemic, Critically Endangered Species of Southern Western Ghats of Kerala, India

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ABSTRACT

Ixora johnsonii Hook.f. (Rubiaceae) is a critically endangered, pretty weed found in Western Ghats of South India. Roots of this plant are used by the tribes for curing wounds and sores. The present study highlights the macroscopic and microscopic features of leaf of I. johnsonii along with the physico – chemical and preliminary phytochemical analyses. Physical constants like ash values and extractive values were also studied. The leaf contains many bioactive compounds such as flavonoids, tannins, saponins and alkaloids. The data obtained in the present study will help in the botanical identification and standardization of the drug in the crude form and also to distinguish the drug from its adulterants.

Key Words: *Ixora johnsonii*, Rubiaceae, Kattu coffee, Kattu chetti,Pharmacognostic study.

INTRODUCTION

Ixora are popular ornamental plants grown in the tropics for their beautiful inflorescences which are different shades of white, yellow, orange, pink and red. Five species of *Ixora* are endemic to Kerala. One among them is *Ixora johnsonii* Hook.f. [1] *Ixora johnsonii* Hook.f. (Rubiaceae) is a less known, endemic and critically endangered (IUCN 2010 – Red List) plant present in southern Western Ghats of Kerala, India[2]. This plant is a small shrub, of about 30-50 cm with woody erect stem (Fig.1). It is locally called as kattu coffee or kattu chetti in Malayalam.The present research work deals with pharmacognostical study and preliminary phytochemical evaluation of the leaves of *Ixora johnsonii (I. Johnsonii).* The data obtained in the present study will help in the botanical identification and standardization of the drug in the crude form and also to distinguish the drug from its adulterants.

MATERIALS AND METHODS

Plant Material

The plant specimen for the present study was collected from Kottayam districts of Kerala, India. The plant was identified and authenticated by Dr. E. Santhosh kumar, Tropical Botanical Garden and Research Institute, Palode, Thiruvananthapuram, Kerala, India.

Macroscopic and Microscopic Analysis

Macroscopic analysis

For recording morphological observations, ten matured plants with uniform size and spread were selected randomly. Observations on vegetative characters and their variations were recorded. The macroscopic characters of the plant such as colour, odour, nature, texture were also studied for morphological investigation.

Microscopic analysis

The first step involved in microscopic study is fixing. The selected leaf samples were cut and removed from the plant and fixed in FAA (Formalin-5ml +Glacial Acetic acid-5ml +70% Ethyl alcohol-90ml). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the schedule given by Sass[3]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60^0 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks. This is followed by sectioning. The cross sections were prepared and stained as per the procedure of Johansen. The paraffin embedded specimens were sectioned with the help of Rotary Microtome. Dewaxing of the sections was by customary procedure[4]. The sections were stained with Toluidine blue as per the method published by O'Brien *et al* [5] and wherever necessary sections were also stained with safranin and Fast – green and IKI (for Starch). For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluidwere prepared[3]. Glycerin mounted temporary preparations were made for macerated/cleared materials. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic Unit. For the study of crystals, starch grains and lignified cells, polarized light was employed. Descriptive terms of the anatomical features were followed as given in the standard Anatomy book [6].

Preparation of Plant Extracts

The leaves of *I. johnsonii* were separated and washed with tap water to remove the dirt particles and shade dried. Then the dried leaves were milled into coarse powder by a mechanical grinder, sieved and packed in separate containers. Solvents were used for extraction based on their polarity (Hexane, ethyl acetate, acetone, methanol and aqueous). Leaf powder was mixed with water (aqueous) and organic solvents individually. Extraction was done by soaking one part of powder into four parts of water or liquid solvents (1:4) separately and kept for percolation process for 7 days. The crude extracts were filtered individually and the filtrates were inoculated into solid extracts by allowing them for evaporation of solvents or water completely under room temperature. The powder forms of extracts were stored in a refrigerator individually at 4^{0}C for further studies.

Physico – chemical Analysis

The percentage of loss of weight on drying, total ash, acid – insoluble ash and water soluble ash were obtained by employing standard method of analysis described in Pharmacopoeia of India[7,8].

Fluorescence Analysis

The powdered leaf samples and the extract of the powder in various solvents and water were examined under ordinary light and ultra violet light (365nm). These powder were also treated with 1 N NaOH (aqueous), 1N NaOH (ethanolic), 1 N HCl, 1:1 H_2SO_2 and 1:1 NNO_3 as per the standard procedure described by Pratt and Chase[9], further the changes in colour were also recorded.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out to assess the qualitative chemical composition of freshly prepared leaf powder as well as various crude extracts.The phytochemical tests were performed as per procedures described in the standard reference book and literatures.[10-14]

RESULTS

Macroscopic Characters

Leaves opposite, decussate, simple, entire, petiolate; petiole 2.5-10 mm long, green to reddish-brown; lamina 8-25 x 4-13 cm, elliptic obovate, cuneate at the base mucronate at apex, lateral veins 9-12 pairs, prominent on the adaxial side, greyish - white patches radiate from the midrib (Fig 1a); stipules interpetiolar, laterally joined at the base forming a tube, broadly triangular with a central cups with dense golden brownish hairs on the inner side (Fig 1b). Leaves are thick, leathery and dark green in colour.

Microscopic Characters

Leaf

The leaf is unique in having thick and prominent midrib and thick lamina (Fig.2a). The midrib consists of a thick, vertical pillar like adaxial cone (Fig.2e) and a wide, semicircular abaxial part. The midrib is 800µm in height; the adaxial cone is 250 µm high and 250µm wide. The abaxial part is 550µm wide. The epidermal layer of the midrib consists of fairly wide, squarish or oblong cells with prominent cuticle. The epidermis is 25 - 30 µm thick. The ground - tissue within the adaxial cone comprises angular, compact, thick walled cells. The abaxial part consists of outer zone of three or four layers of angular, compact, fairly thick walled cells and inner zone of thin walled ground tissue. The vascular stand is single, wide, closed circle with central core of parenchyma. The vascular

cylinder consists of short, parallel, compact radial lines of xylem elements which are narrow and thick walled. Phloem occurs in thin continuous layer along the outer circumference, external to the phloem is again thin continuous line of sclerenchyma cells. The vascular cylinder is 350µm in diameter (Fig. 2b).Calcium oxalate druses are common in the ground parenchyma cells (Fig. 2b). The druses are solitary in the cells. The crystal bearing cells are not modified either in shape or size. The crystals are 20 - 30 µm thick.

Lamina

The lamina is uniform in thickness. It is distinctly dorsiventral and hypostomatic. It is 150µm thick. The adaxial epidermis is thicker with large, vertically oblong cells and thick cuticle; it is 30 µm in thickness. The abaxial epidermis is comparatively thin, measuring 20µm in thickness. The cells are horizontally rectangular to squarish. The stomata occur at the epidermal level. The guard cells have prominent stomatal ridges (Fig. 2c). The mesophyll tissue consists of short, cylindrical and conical loosely arranged single layer of palisade cells. The spongy parenchyma comprises 5 or 6 layers of lobed cells forming wide air-chambers.

Venation Pattern

Main veins, minor veins and veinlets are thick and straight. The vein - islets are distinct; they are variable in size and shape; the outline is rectangular, squarish or polygonal (Fig.2d). The vein terminations are either simple (unbranched), thick and straight (Fig.2d) or branched forming dendroid outline.

Leaf- Margin (Fig.2f)

The marginal part of the lamina is slightly thin and slightly bent down. It is about 140µm thick. The epidermal cells are slightly reduced in size. The cells along the extreme marginal part are necroses forming a thick mechanical barrier to the internal tissues. The differentiation of the palisade cells and spongy parenchyma cells are obscured in the marginal parts, the mesophyll cells are in 5 or 6 horizontal rows and compactly arranged leaving no intercellular spaces.

Epidermal Tissue

In paradermal (Surface) sections (Fig.2g), the epidermal cells and stomatal morphology were studied. The adaxial epidermal cells are polyhedral in outline with thick straight walls. Thin cuticular striations are seen in dense parallel lines within the boundary of the cells. The adaxial epidermis is apostomatic. The abaxial epidermis comprises fairly wider epidermal cells with thin, straight or slightly wavy anticlinal walls. Cuticular striations are seen even in the abaxial epidermal cells (Fig.2h). The stomata are paracytic type. Two subsidiary cells lie parallel to the guard cells (Fig.2h). The guard cells are oblong elliptic with elongated narrow stomatal aperture (Fig.2h). The guard cells are 15 x 30µm in size.

Physico – chemical Analysis

The results of the ash and extractive values of *I. johnsonii* leaves are presented in Table-1. Since the ash values are constant for a given drug/extract, these values are also one of the diagnostic parameters of the drug. The drug sample has more water soluble ash than acid insoluble ash.

Fluorescence Analysis

The findings of fluorescence analysis of *I. johnsonii* are presented in Table-2.The fluorescence analysis of drug extract helps to identify the drug with specific fluorescence colour and also to find out the fluorescent impurities.

Preliminary Phytochemical Screening

Preliminary studies were carried out to determine the phytochemical characters of the leaf of *Ixora johnsonii*. Screening of plant extract using five solvents of increasing polarity from hexane to water indicates the presence of all major phytochemical constituents like alkaloids, terpenoids, flavonoids, phenolics and saponins (Table-3). Methanol extract showed more efficiency in the recovery of phytochemicals than all other extracts. Methanolic extracts of leaves showed the presence of flavonoids, alkaloids, phenolic compounds, tannins, resins, saponins, glycosides and terpenoids.. Except methanolic extract of leaf of the selected plant, tannin is absent in all other solvent extracts. Like methanolic extract, saponin was present in acetone extracts of leaf of the plant. Glycosides was absent in methanolic extract of leaf, instead it was in ethyl acetate extract of leaf. Gum/mucilage was completely absent in all the solvent extracts. Flavonoids are present in mid and high polar solvent extracts of leaf.

DISCUSSION

In the leaf of *I. johnsonii* a single peristomatal rim was found parallel to the stomata and the outer stomatal ledge was not conspicuous. This is an exception as in all the other species of *Ixora* peristomial rims, if present surrounds the stomata and are in rows of 2 or 3 and the outer stomatal ledge is quite conspicuous.

Important Identifying Characters

1. Presence of greyish - white patches radiate from the midrib of leaf.
2. Stipules are intra and interpetiolar, laterally joined at the base forming a tube, broadly triangular, brown colored with a central cups with dense golden brownish hairs.
3. Presence of a thick, vertical pillar like adaxial cone having angular, compact, collenchyma cells and a wide, semicircular abaxial part in the midrib region of the leaf.
4. Stomata are hypostomatic and paracytic type.
5. Presence of prominent stomatal ridges in the guard cells.
6. Cuticular striations are seen in dense parallel lines both in the adaxial and abaxial epidermal cells
7. Calcium oxalate crystals of druses or sphaero-crystals are fairly abundant in the ground parenchyma cells of the midrib.

CONCLUSION

Information available for botanical diagnosis of *I. johnsonii* is not only meager, but also too elementary. A detailed description and photograph of this taxon are provided for facilitate easy identification. In the present study, anatomical features of leaf of mature plant is presented, which may offer easy guidelines for botanical identity of the species. Thus, the anatomical characters coupled with preliminary phytochemical results are specific for the pretty plant *I. johnsonii*.

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Table-1: Physico – chemical analysis of leaf of *I. johnsonii*

Sr. No	Parameters	Leaf
1	Loss of weight on drying	83.20
2	Total ash	3.46
3	Acid – insoluble ash	0.31
4	Water-soluble ash	1.28
5	Extractive values	-
a)	Petroleum ether ($40 – 60^0$C)	2.32
b)	Benzene	2.87
c)	Chloroform	2.92
d)	Ethanol	2.97
e)	Water	3.24

Table-3: Qualitative Phytochemical constituents present in various extracts of leaf of *I. johnsonii*

S. No	Plant constituents	Leaf Extract				
		H	EA	A	M	Aq
1	Alkaloids	+	+	+	+	+
2	Flavonoids	-	-	+	+	+
3	Phenolic compounds	-	-	-	+	+
4	Tannins	-	-	-	+	-
5	Gums/Mucilage	-	-	-	-	-
6	Saponins	-	-	+	+	-
7	Steroids	-	-	-	-	-
8	Glycosides	-	+	+	-	-
9	Terpenoids	+	+	+	+	+
10	Resins	-	-	-	+	-

H-Hexane, EA-Ethyl Acetate, A-Acetone, M-Methanol, Aq-Aqueous

Table-2: Fluorescence characters of powder and different extracts of leaf of *I. johnsonii*

Leaf			
S.No	**Particulars of Treatment**	**Under ordinary light**	**Under UV light (365 nm)**
1	Powder as such(drug powder)	Grey	Black
2	Powder + 1N NaOH (aqueous)	Yellowish green	Brown
3	Powder + 1N NaOH (ethanolic)	Yellowish green	Brown
4	Powder + 1N HCl	Dark green	Brown
5	Powder + H2SO4 (1:1)	Green	Brown
6	Powder + HNO3 (1:1)	Brown at the centre and yellow at the edge	Dark Brown
Extracts			
S.No	**Type of Extract**	**Under ordinary light**	**Under UV light (365 nm)**
1	Petroleum ether	Yellowish green	Pinkish orange
2	Benzene	Brownish green	Pinkish orange
3	Chloroform	Brownish green	Pinkish orange
4	Ethanol	Green	Pinkish orange
5	Water	Dark brown	Dark green

1a. Habitat

1b. Leaf

1c. Stipule

Fig. 1: Exomorphology of *Ixora johnsonii* (Hook).f.

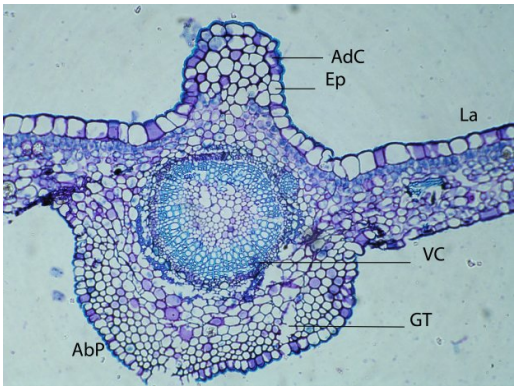

Fig. 2a : T.S of leaf, through Midrib

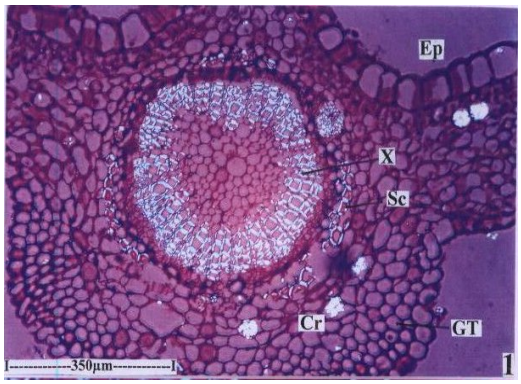

Fig. 2b : T.S of Midrib showing crystal distribution

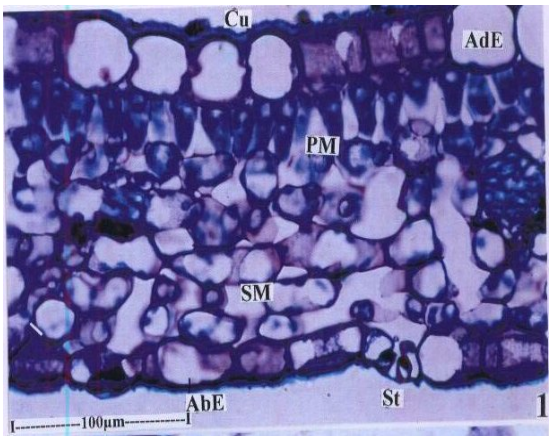

Fig.2c :T.S of Lamina

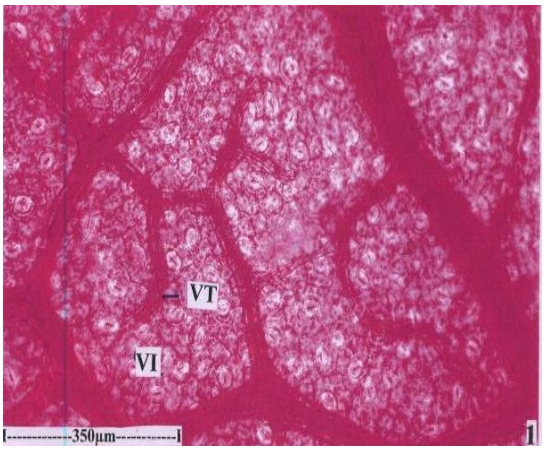

Fig. 2d: Venation of the lamina showing vein islets and vein termination

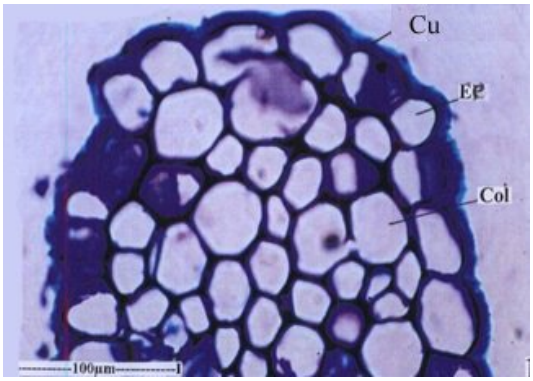

Fig.2e : Adaxial cone – enlarged

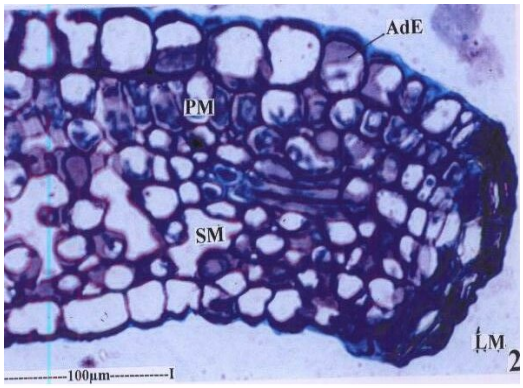

Fig. 2f : T.S of lamina margin

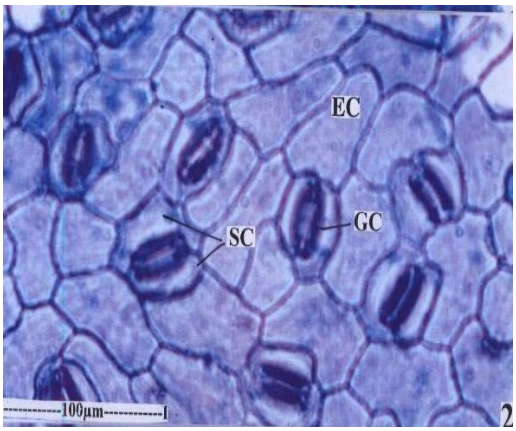

Fig 2.g : Paradermal section of abaxial epidermis

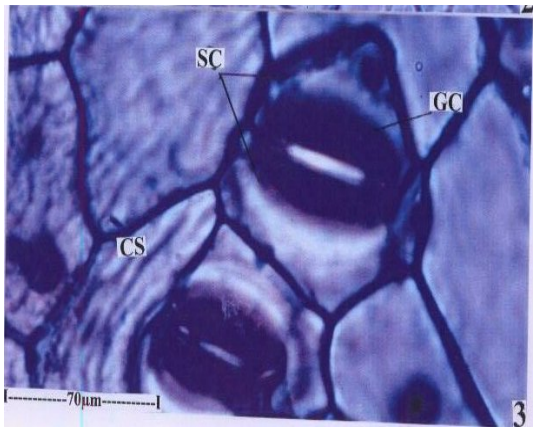

Fig. 2.h: Paracytic stomata enlarged

Figure 2. Microscopy of the leaf of *Ixora johnsonii* Hook.f.
(viewed under Polarized light)

AbE:AbaxialEpidermis; AbP: Abaxial Part; AdC: Adaxial Cone; AdE: Adaxial Epidermis; Cu:Cuticle; Col: Collenchyma; CS: Cuticular Striations; Cr:Crystals; Ep: Epidermis; EC: Epidermal Cells; GC: Guard Cells; GT: GroundTissue; La: Lamina; LM: Leaf Margin; PM: Palisade Mesophyll; Sc: Sclerenchyma; SC: Subsidiary Cells; SM: Spongy Mesophyll; St: Stomata; VC: Vascular Cylinder; VI:VeinIslet; VT: Vein Termination; X: Xylem;

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