RESEARCH ARTICLE

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Anatomical investigation, phytochemical screening and pharmacognostical evaluation of the plant root - *Ixora johnsonii Hook.f*

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Abstract: A preliminary investigation is provided to encouraging results for comprehensive studies on different aspects of the plant root - *Ixora johnsonii Hook.f.* Root of this plant was studied to fix the parameters for pharmacognostical standards. The present study are highlights the pharmacognostical evaluation of root which includes macroscopic and microscopic features. Further, preliminary phytochemical analyses, organoleptic character, fluorescence behavior of different extracts and histochemical localization of phytochemicals. As there is no pharmacognostical work on record of this ethno botanically much valued drug, the present work was taken up with a view to lay down standards, which could be useful to detect the authenticity of this pretty plant.

Keywords: *Ixora johnsonii*, Rubiaceae, Pharmacognosy,

1. Introduction

Ixora johnsonii Hook. f. (Rubiaceae) is a less known, endemic and critically endangered plant present in Southern Western Ghats of Kerala, India [1-2]. This plant is a small shrub, of about 30-50 cm in height with woody erect stem [3]. It is known as kattu coffee and kattu chetti in Malayalam. *Ixora johnsonii* Hook.f. (Figure 1a) is a perennial undershrub, with a single flowering shoot [4]. Roots of *I. johnsonii* are used by the tribe Ullader at Kurumbanmoozhy in Pathanamthitta district of Kerala for curing large and unhealed wounds and sores. For this treatment, they collect the fresh roots and made into a paste in supernatant of rice gruel. This paste is applied around the mouth of wounds and sores until they subside [5].

The plant selected for study is a rare critically endangered one; it is felt that a more thorough examination of the root of *I. johnsonii* would be worth-while, since such a study may enable one to identify the taxon in any fragmentary form. Inspite of the ethnomedicinal use (importance) attributed to this plant; there is no pharmacognostical parameter on the roots of *Ixora johnsonii* Hook.f. The work is devoted to an anatomical investigation of the root of *I. johnsonii*, in order to provide an account of the anatomical features, which can be used for diagnosing and

distinguishing it from other species and its adulterants.

Material and Methods

Plant material

The plant specimen for the present study was collected from Pathanamthitta and Kottayam districts of Kerala, India. It was identified and authenticated by comparison with the specimen by Dr. E. Santhosh kumar, TBGRI, Palode, Thiruvananthapuram, Kerala, India.

Methods

Macroscopic and microscopic analysis

Macroscopic analysis:

The organoleptic characters of the root such as colour, odour, nature, texture was studied for morphological investigation.

Microscopic analysis:

For studying the microscopic characters, root samples were cut and removed from the plant and fixed in FAA (Farmalin-5ml +Acetic acid-5ml +70% Ethyl alcohol-90ml). After 24 hours of fixing, the specimen was dehydrated with graded series of tertiary-Butyl alcohol [6]. 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. The cross sections were prepared and stained [7]. The paraffin embedded specimens were sectioned with the help of Rotary Microtome. Dewaxing of the sections was by customary procedure[7]. The sections were stained with Toluidine blue [8], and wherever necessary sections were also stained with safranin and Fast – green and IKI (for Starch). Powdered material of root was cleared with NaOH and mounted in glycerin medium after staining. Different cell component was studied and measured. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic Unit. Descriptive terms of the anatomical features are as given in the standard Anatomy book [9].

Histochemical localization

Histochemical studies were carried out to localize proteins, starch, phenolics, alkaloids, tannins and flavonoids using standard procedures [10]. The root sections embedded in paraffin were used for histochemical studies. The sections were deparaffinised and hydrated before staining them for the analysis. The secondary metabolites were localized in the root of *I. johnsonii* and the observations were recorded as well as photomicrographs were also taken.

Physico – chemical analysis

The percentage of loss of weight on drying, ash values and extractive values were obtained by employing standard methods [11-13].

Fluorescence analysis

The powdered root 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 (365nm). This powder was also treated with 1 N NaOH (aqueous), 1N NaOH (ethanolic), 1 N HCl, 1:1 H_2SO_4 and 1:1 HNO_3 as per standard procedure [14] and changes in color were recorded.

Preliminary phytochemical screening

Shade dried root was milled into coarse powder by a mechanical grinder, sieved and packed in separate container. Solvents were used for extraction based on their polarity. Hexane, ethyl acetate, acetone, methanol and aqueous were used as solvents.

Preliminary phytochemicals screening were carried out to assess the qualitative chemical composition of freshly prepared root extracts using selected solvents of increasing polarity by standard procedures [15-18].

Figure 1(a) Habitat

Figure 1(b) Root

Figure 1. Exomorphology of *Ixora johnsonii*

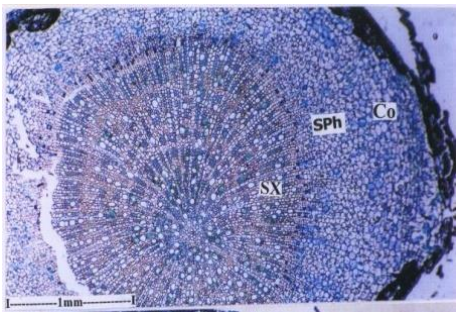


Figure 2(a) T.S of Root-entire view

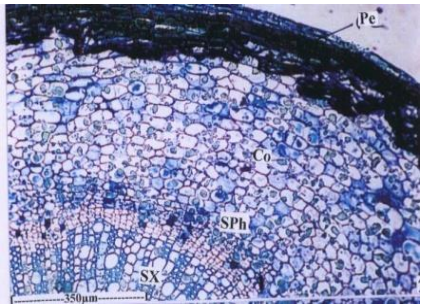


Figure 2(b) A sector enlarged

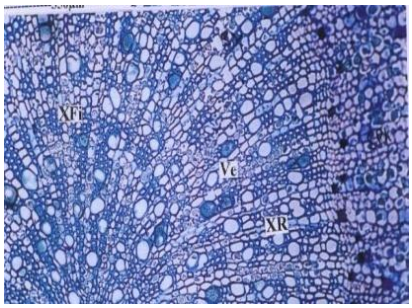


Figure 2(c) Secondary xylem

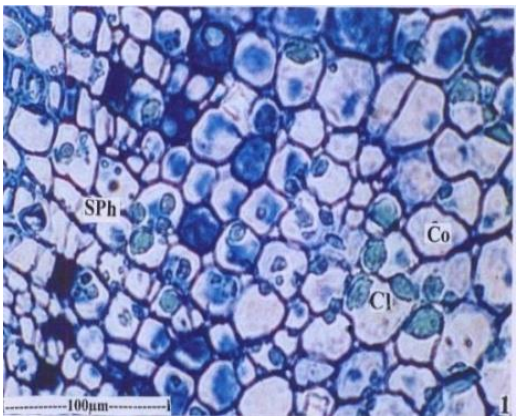


Figure 2(d) Secondary phloem

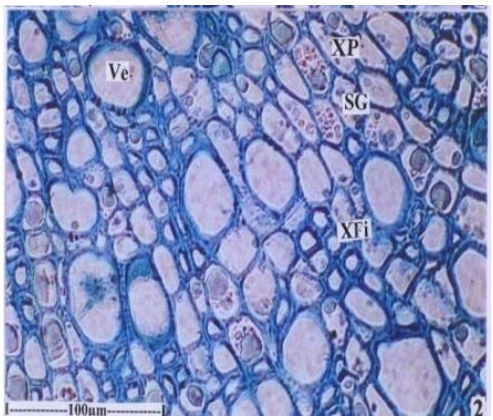


Figure 2(e) Secondary xylem -enlarged

(Co: Cortex; CI: Cell inclusion; Pe: periderm; Sx: Secondary Xylem; Sph: Secondary phloem, SG: Starch grains; Ve: Vessel; XFi: Xylem Fibres; XP: Xylem Parenchyma; XR: Xylem Ray)

Figure 2. Microscopy Image of Root

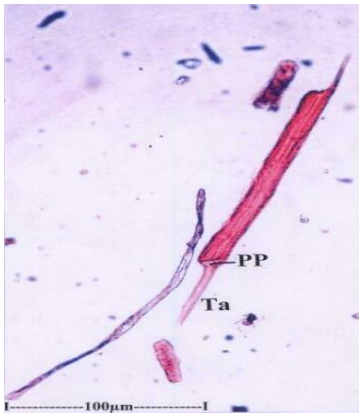


Figure 3(a) A tailed vessel element

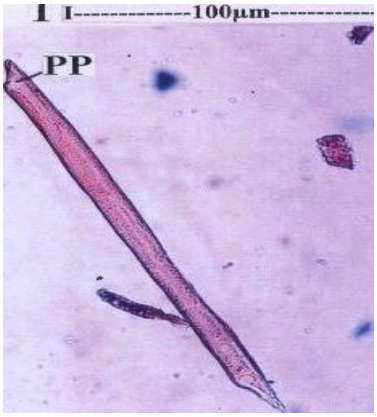


Figure 3(b) A tailless vessel element

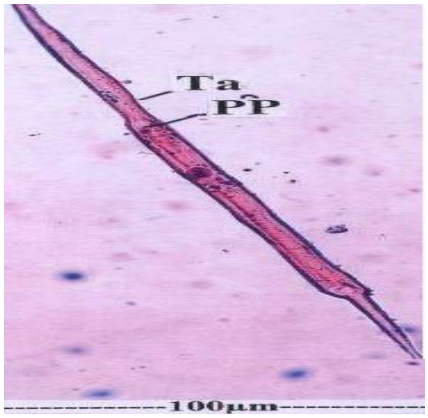


Figure 3(c) A long tailed vessel element

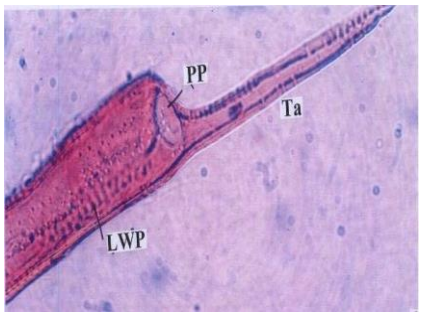


Figure 3(d) A portion of the vessal

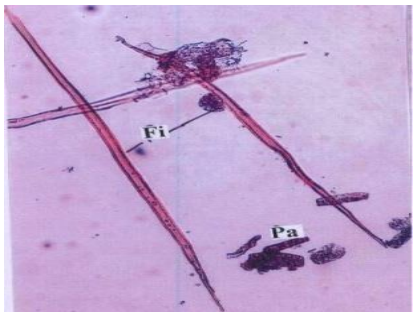


Figure 3(e) Fibres

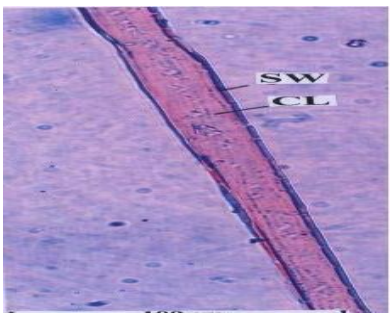


Figure 3(f) Fibre – wall enlarged with perforation plate and long tail

(CL: Cell Lumen; LWP: Lateral Wall Pits; PP: Perforation Plate; SW: Secondary Wall; Ta: Tail; Pa: Parenchyma)

Figure 3. Root Powder Microscopy

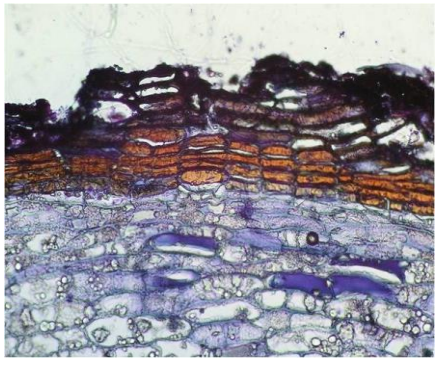
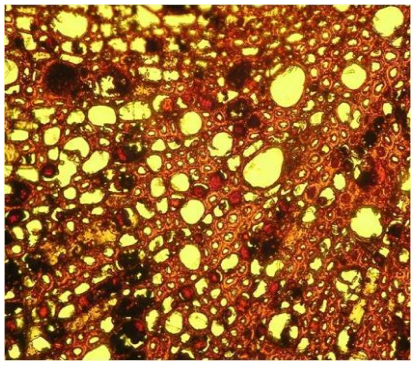


Figure 4(a) Phenolic compound, alkaloids & flavonoids in root bark periderm and vascular tissue.



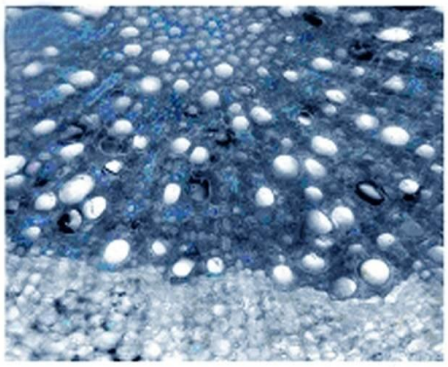


Figure 4(b) Tannin in root secondary xylem

Figure 4. Histochemical localization of phytochemicals in root

Results and Discussions

Macroscopical Characters

Plant has deep tap root system with highly branched lateral roots (Figure 1b). Root is very hard and has a mild smell. Root bark is thick and brown in colour.

Microscopical Characters

Thick root with well-developed secondary vascular tissues was studied. The root consists of a superficial darkly stained, tannin-filled periderm of 100µm thickness (Figure 2a, 2b). The cortical zone is wide and homogeneous comprising tangentially oblong parenchyma cells. Fairly large spherical bodies of unknown nature are seen in almost all cells of the cortex (Figure 2b). Secondary phloem zone narrow and continuous around the xylem. It consists of short radial rows of phloem rays and small polyhedral sieve elements (Figure 2b; 2d).

Secondary Xylem is dense, solid cylinder comprising vessels, fibers, parenchyma and rays (Figure 2c, 2e). The vessels are solitary or in multiples of two or three. They are thin walled and angular in outline (Figure 2e). Diameter of the vessels is 40µm.

The xylem fibers are libriform type. They have thick lignified walls. They are distributed in successing rings, cleaved radially by xylem rays (Figure 2c). Alternating with the fiber cylinders are parenchyma cylinders, rendering the xylem cylinder as though having growth rings (Figure 2a). Xylem rays are narrow, 2-cells wide and run deviating from the centre towards periphery. Similarly, Sudhaharan Nair [19] (2001) reported the anatomy of the root of *I.coccinea*. He revealed that in *I. coccinia* medullary ray is uniseriate and phloem fibres are distributed either isolated or in small groups of 2 or 3. Starch grains are abundant in the xylem parenchyma (Figure 2e) and ray cells.

Cell inclusions

Two major types of cell inclusions are found in the plant. In the root, crystals are less abundant and are of prismatic type. The druses occur in ordinary, unmodified cells. In the root, starch grains are abundant. They occur in the cortical parenchyma cells as well as xylem fibres (Figure 2e). The starch grains are simple concentric type. The starch grains are 15µm in diameter.

Powder Microscopic analysis

The root powder consists of vessel elements (Figure 3a-d) and fibers (Figure 3e& 3f). The parenchyma cells are also seen in the powder (Figure 3e).

The Vessel

The vessel elements are unique in being very long, much narrow and extremely long tailed (Figure 3a, 3c, 3d). Some of the elements are tailless (Figure 3b). They have simple, oblique perforation plates. The tail is up to 300µm long. The lateral wall pits are minute, circular and multiseriate. The vessel elements are 650 - 850 µm long and 40µm wide.

Fibres

The fibers are libriform type with wide lumen and thick walls (Figure 3e & 3f). Pits are not evident. Some cell inclusions are seen in the lumen of the fiber. The fibers are 950 µm long and 20 µm wide.

4. Histochemical localization

In the present study some of the compounds such as starch, alkaloids, tannin, protein, phenolics and flavonoids were histochemically identified and highlighted in the root of *I. johnsonii*. The results are given in Table 1.

(i) **Alkaloids:** In the root, alkaloids were localized in the cortex, secondary xylem parenchyma and secondary phloem (Figure 4a).

(ii) **Flavonoids:** In the root, flavonoids were localized in the secondary xylem and secondary phloem (Figure 4.a).

(iii) **Tannins:** Tannin was localized in the periderm (Figure 4b) and secondary xylem parenchyma cells of the root.

(iv) **Phenolics:** Phenolic compounds were found in the periderm of the root (Figure 4a).

(v) **Protein:** Protein was another compound predominant in the cortical cells and secondary phloem region of the root.

(vi) **Starch:** It was also present in the ray cells, parenchyma cells in the cortex, secondary xylem and secondary phloem of the root.

Table- 1. Histochemical localization in the root of *I. johnsonii.*

SI. No.	Phyto chemicals	Localization in tissues
1	Alkaloids	Root - cortex, Secondary xylem parenchyma and secondary phloem.
2	Flavonoids	Root bark - Periderm, Secondary phloem.
3	Tannin	Root - periderm, Secondary xylem parenchyma.
4	Starch	Root - Secondary phloem, cortical parenchyma, secondary xylem parenchyma and ray cells.
5	Phenolics	Root - Periderm, Secondary phloem.
6	Protein	Root - cortex, Secondary phloem.

Table-2. Physico – chemical analysis of root of *I. johnsonii*

S. No	Particulars (Parameters)	Root (%)
1.	Loss of weight on drying	89.70
2.	Total ash	3.96
3.	Acid – insoluble ash	0.71
4.	Water-soluble ash	2.28
5.	**Extractive values**	
	a) Petroleum ether (40 – 60^{0}C)	3.22
	b) Benzene	3.67
	c) Chloroform	3.76
	d) Methanol	3.84
	e) Water	3.94

Table 3. Fluorescence characters of root powder of *I. johnsonii* and its extracts in different solvents

S.No	Particulars of treatment	Root	
		Under ordinary light	Under UV light (365 nm)
1	Powder as such(drug powder)	Brownish black	Black
2	Powder + 1N NaOH (aqueous)	Yellowish green	Brown
3	Powder + 1N NaOH (ethanolic)	Dark brown	Brown
4	Powder + 1N HCl	Dark brown	Brown
5	Powder + H2SO4 (1:1)	Green	Brown
6	Powder + HNO3 (1:1)	Dark brown	Dark Brown
7	Extracts		
	a.Petroleum ether	Brown	Greenish brown
	b.Benzene	Colourless	Light yellow
	c.Chloroform	Brownish green	Pinkish orange
	d.Methanol	Light brown	Bluish brown
	e.Water	Light brown	Brown

Table 4. Qualitative phytochemical constituents present in various extracts of *I. johnsonii*

SI. No	Plant constituents	Root extracts				
		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 + = positive activity; - = Negative activity.

Physico – chemical analysis

The results of the ash and extractive values of *I. johnsonii* root are depicted in Table 2. The drug sample has more water soluble ash than acid insoluble ash. The extractive value of methanol is more than in other solvents investigated. Proximate analysis showed total ash 3.96%, acid insoluble ash 0.71% and water soluble ash of about 2.28% w/w are present in root. The loss on drying revealed the percentage of moisture present in the drug and its value is 89.70% in root.

Fluorescence analysis

The findings of fluorescence analysis of the root powder of *I. johnsonii* and its extracts in various solvents are presented in Table 3. Root powder gave greenish brown in petroleum ether extract, light yellow in benzene extract, pinkish orange in chloroform extract, bluish brown in methanol extract and brown colour in aqueous extract under UV light (365 nm). At the same time, under ordinary light, the high polar solvent extracts (water and methanol) showed light brown colour, petroleum ether extract brown colour, benzene extract colourless and chloroform extract brownish green colour.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

The present study is a first attempt of phytochemical screening and identification of different chemical constituents present in the root of *Ixora johnsonii*. Screening of root extracts 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. The phytochemicals present in different solvent extracts of root of *I.johnsonii* Hook.f. were presented in Table 4. Methanolic extract of root showed more efficiency in the recovery of phytochemicals than all other extracts. Methanolic extracts of root showed the presence of flavonoids, alkaloids, phenolic compound, tannins, saponins, glycosides and terpenoids. Except aqueous extract, all the other extracts contain alkaloids. Terpenoids were also present in all the extracts but resins are present only in aqueous extract. Gums/Mucilage is completely absent.

Conclusion

The *Ixora johnsonii Hook.f* plant root was studied to fix the parameters for pharmacognostical standards. The anatomical characters coupled with preliminary phytochemical results are specific for the plant *I. johnsonii.* The macroscopic and microscopic feature and preliminary phytochemical analysis were carryout. The different type of solvent extract gave the fluorescence behavior. It may be analyzed for the medicinal activities. Physical constants like ash values and extractive values were also studied. In the root, calcium oxalate crystals are less abundant and are of prismatic type. In the root, simple concentric types of starch grains are abundant in the cortical parenchyma cells, xylem fibres, xylem parenchyma as well as in the ray cells. Methanol extract is showed more efficiency in the recovery of phytochemicals than all other extracts. The root 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.

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Conflict of interest

None of the authors have any conflicts of interest to declare.