Research Article

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PHARMACOGNOSTIC EVALUATION OF *WRIGHTIA ARBOREA* (DENSST.) MABB.

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ABSTRACT

Wrightia arborea (Densst.) Mabb. belonging to the family Apocynaceae, is a small deciduous tree, distributed throughout the warmer parts of India. Different organs (root, bark and leaf) have been used in traditional medicine for many years. The present study was undertaken to investigate the pharmacognostic characters of root, bark and leaf, which were carried out in terms of organoleptic, macroscopic, microscopic, physiochemical analysis and phytochemical testing as per WHO recommended methods for standardization of different organs of the plant. In quantitative microscopy, the average stomatal index was found to be 23.0, vein islet number 25.14 and vein termination number 20.0. Physico-chemically, the moisture content was comparatively more in bark, while it was less in the root; extractive values of chloroform, ethanol and water soluble extractive were 8.8 % w/w, 21.4 % w/w and 23.68 % w/w respectively. The total ash value was less in root, than that of the leaves; whereas acid insoluble ash value was more in leaves than root. The preliminary phytochemical screening revealed the presence of alkaloids, flavonoides, phlobatannin in bark and leaves; phenolics, reducing sugars, saponins, tannins found in all studied organs, while leucoanthocyanins, iridoids, steroids and terpenoid revealed in the leaves only. Of the various phytochemicals and minerals estimated, saponin and tannins were found in larger amounts in leaves than others, whereas the minerals, calcium were more in leaf. The present pharmacognostic parameters reported on this plant can serve as a valuable source of identification and provide a suitable diagnostic tool for standardization besides adulterant identification in related species or weeds.

Keywords: *Wrightia arborea* organs, traditional medicine, pharmacognostical parameters.

INTRODUCTION

Wrightia arborea (Densst.) Mabb. (Syn. *Wrightia tomentosa* Roem and Schult) belongs to the family Apocynaceae, is a small deciduous tree, distributed throughout the warmer parts of India at an altitude of 600 m in the Himalayas and in 1,200 m in the Nilgiris[1]. In India, it is known by its different vernacular names, Kala Indrajav (Marathi), Dudhi, dharuli (Hindi), Dudhkoraiya (Bengali), Atkuri (Asam), Pita karum (Oriya), Dudhlo (Gujrat), Bilikudegidda (Kannada), Tellapaala (Telgu) and Pala (Tamil) Nilampala (Malyalam)[2]. A preparation of the bark of *Wrightia arborea* is being useful in menstrual and renal complaints in traditional medicine for many years[3-5]. The dried bark of *Wrightia arborea* is employed as a substitute and an adulterant for the bark of *Holarrhena antidysenterica*[1]. The stem bark and root bark are found to be useful in snake bite and scorpion stings[2]. The leaves with salt are given for to relief from toothache[1]. The dried leaf powder is believed to be useful as diaphoretic, expectorant and also used in dysentery[6]. The root is used to cure headache, while a root powder is taken with water to retrieve fever[6,7]. In Thailand, the dried bark of this species is used as an antipyretic[8] where as in Tamilnadu state of India, the powdered stem bark is mixed with curd and is taken orally to treat urinary stones[9]. Previous phytochemical studies on this plant have revealed the presence of conessine dihydrate, holarrhine, kurchicine and a very minute quantity of conkurchine alkaloids, flavonoids, phlobatanins, simple phenolics, tannins, β-sitosterol, lupeol, α amyrin and reducing sugars in bark[1,10,11]. Leaves indicated the occurrence of alkaloids, flavonoids, glycoflavones-iso-orientin, isoflavone, wrightiadione, phenolic acids, saponins, steroids, tannins and terpenoids[12-15]. The plant of *W. arborea* is reported to possess various biological activities like the antimicrobial[11,16,17], antimycobacterial[18], antipyretic and anti-inflammatory[19],anti-allodynic[20], antihyperglycemic[21], antioxidant[22], anti-tubercular[23], antitumour[24], cytotoxic[14] and toxicology testing[25]. Some morphological features on the leaf of *W. arborea* have been studied by Divakar *et al*[26]. In the present paper, an attempt was made to provide the detailed pharmacognostic evaluation of *W. arborea.* Various parameters like macroscopic, microscopic, quantitative microscopic values, physicochemical, besides phytochemical profile of the root, bark and leaf have been handled which may serve as pharmacopoeial standards for proper identification of this species.

MATERIALS AND METHODS

Chemicals

All the chemicals used were of analytical grade and were obtained from Merck and Molychem, Mumbai, India. Microphotographs of all parts of the plants were taken by using Jenaval Camera affixed to the microscope

Plant Material

A small tree, *Wrightia arborea* (*W. tomentosa*) is growing and available at present in Botanic garden of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (MS), India which was identified by Dr. V N. Naik of Botany department and its description, is given in his catalogue[27]. The specimen of this plant is deposited and kept in a folder of the genus *Wrightia* under the Family Apocynaceae as per Bentham and Hooker system

of classification. Fresh root, bark and leaves were collected during 2006-2007 and used for the study of macroscopic and microscopic characteristics. Collected samples of the plant were washed, shade dried at room temperature for 20 days. The dried plant samples were pulverized into fine powder using a grinder and passed through 40 mesh sizes and stored in an airtight container for further use.

Macroscopic evaluation

Different macroscopic characters such as shape, size, color, odor leaf base, petiole, venations, margin, apex, base, surface, and texture of the leaves are recorded. For macroscopic studies of bark the materials were characterized with respect to shape, the aspect of the internal surface, and types of bark fractures and configuration. The freshly collected roots of the plant were spread on a white paper and examined different organoleptic features by repeated observations using hand magnifying glass and recorded[28,29].

Microscopic Evaluation

For microscopic studies, the leaf, bark and root were removed from the plant and fixed in FAA (Formalin 5 ml + Acetic acid 5 ml + 70 % Ethanol 90 ml). After 24 hours of fixing, the transections of all studied organs were taken by free hand. The sections were stained with safranin (1 %), light green (1 %), aniline sulphate (1 %) and mounted in DPX after the customary dehydration. Some hand sections were also examined in glycerin.

Quantitative Microscopy

The leaf epidermal studies were carried out on fresh specimens. The peels were removed mechanically using some chemicals. They were stained in 1 % safranin mounted in glycerin and made semi-permanent by ringing with DPX solution. Stomatal index (SI) and stomatal frequency were calculated[30,31]. The vein islet number and vein termination number of the leaf were determined according to the reported method[31].

Histological Color Reaction

The Histochemical color reactions of all the plant parts were performed by the standard methods[33,34].

Physiochemical Characters

Different physiochemical parameters such as the moisture content, percentage of total ash, acid insoluble ash and acid soluble ash; extractive values like chloroform-soluble extractives, alcohol-soluble extractives and water-soluble extractives were calculated according to the methods described in the Indian pharmacopoeia[35].

Phytochemical Evaluation

Phytochemical evaluations such as qualitative and quantitative were done from the shade-dried powdered material. For qualitative phytochemicals, standard prescribed methods were used[36-40] and for quantitative phytochemicals; the recommended procedures were followed for determining alkaloid[41], saponin[42], tannin, phenolics[43] and minerals[44].

RESULTS AND DISCUSSION

Macroscopic Evaluation

A small tree reaching, 7-9 m height abounding in yellow milky juice, with opposite, divaricated, scabrous, branches; the pieces of bark appear recurved, the outer surface is yellow to greenish brown, whereas inner is browingly white and strong fibrous. Fracture is tough and fibrous and there is no characteristic taste of bark. Root branched tap root, elongated and cylindrical, whitish brown in color the terminal portions of the roots have thin true rootlets (Figure 1 and 2); Leaves, 7-15 x 3.5 x 6 cm, elliptic-oblong, acuminate, tomentose on both sides. On drying-dark-brown color, base acute: main nerves 8-14 pairs; petioles, 3-6 mm long. Flowers malodorous, 2.5 cm or more across in short, dense, erect, terminal corymbose tomentose cymes, white when on the tree, turning yellow shortly after being gathered. Calyx pubescent outside, glandular inside, segments 3 mm long, broadly ovate, obtuse, with ciliate membranous margins. Corolla tube, 5-6 mm long; lobes, 12-15 mm long, oblong, rounded at apex; corona orange, of 5-10 oblong lacinate scales. Fruits follicles cylindric, 15-25 x 1.2 cm long with a groove on each side at the junction of the carpels, rough with white tubercles. Seeds, 1.2-1.6 cm long, slender attenuated at the apex with deciduous white coma, 2.5-3.7 cm long at the lower end[45,46].

Microscopic Evaluation

The sectional view of *W. arborea* root is circular in outline and showed periderm and ground tissue (Figure 3). Periderm is 5-6 layers, thick and is not clearly distinguished into phellem, phellogen and phelloderm. Followed by periderm is a homogenous cortex comprising of many layers of thin walled large parenchymatous cells. The cortical cells are devoid of any cellular inclusions. A distinct, single layer of endodermis separates the cortical region from vascular region. Ground tissue has phloem fibers, stone cells, oxalate crystals and starch grains. Secondary growth is more. Xylem developed in large amount. Medullary rays of 1-2 rows of cells (Figure 3). The pieces of bark appear recurved, the outer surface is yellow to greenish brown, whereas inner is browingly white and strong fibrous. Fracture is tough and fibrous and there is no characteristic taste of bark. In sectional view, the bark has many layered cork cells, which are arranged in radial rows and are suberised followed by phelloderm and ground tissue. Stone cells occur throughout the section. Calcium oxalate crystals and starch grains are observed. Phloem fibers are present. Medullary rays uniseriate, rarely bi to triseriate rays occur (Figure 4). Transverse section of the petiole shows circular in outline. Epidermis consists of small, thick walled and compactly arranged cells with a thick cuticle outside. Trichomes are 3-4 celled occurs on the epidermis. Collenchymatous hypodermis is 2-3 layered. More collenchyma is noted at the abaxial and adaxial side. The ground tissue is of parenchymatous cells. The vascular tissue comprises of 2-cortical bundles and central arc shaped strand (Figure 5). The leaf is dorsiventral and amphistomatic, upper epidermis is of large polygonal cells, thick walled and with cuticular striations. Lower epidermis is of unequal sized cells. Stomata are generally

confined to lower surface only. Very few stomata are seen near veins on the upper surface and are paracytic. Trichomes are 3-5 celled, uniseriate and unbranched (Figure 6). Mesophyll is differentiated into palisade and spongy tissue. Palisade is 2-layered and spongy tissue is of loosely arranged cells. Vascular tissue has numerous veins, bicollateral, conjoint extending through mesophyll. Midrib has hypodermal many layered collenchyma followed by parenchymatous cortex. An arc shaped, distinct vascular strand occur in the centre (Figure 5).

Quantitative Microscopy

The leaf microscopic characters like of stomatal frequency, stomatal index, vein islet number, and vein termination were determined and furnished in Table 1. The average stomatal index was 23.0, vein islet number 25.14 and vein termination number 20.0.

Histochemical Color Reactions

The histochemical color reactions on the root bark and leaf were performed for the identification of major phytoconstituents. For color tests, transverse sections of different fresh organs were treated with different chemical reagents. The changes in the histochemical zones were observed under the microscope and the results are shown in Table 2. The results indicated that the presence of lignin, starch, fats, saponins and tannins in all organs, while alkaloids and calcium oxalate crystals and flavonoids in leaf.

Physicochemical Parameters

Physiochemical constants like percentage of moisture content, total ash, acid insoluble ash, water soluble ash, chloroform soluble extractive, ethanol soluble extractives and water soluble extractive were determined and depicted in Figure 7. The moisture content was comparatively more in bark (15.35 % w/w), while less in root (7.4 % w/w); extractive values (Chloroform, ethanol and water soluble extractive values; 8.8 % w/w, 21.4 % w/w and 23.68 % w/w respectively). Total ash value less in root (8 % w/w), whereas more in leaves (17.65 % w/w); acid insoluble ash in leaves (2.2 % w/w) and root (1.85 % w/w) (Figure 7).

Phytochemical Screening

The preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phlobatannins in bark and leaves; phenolics, reducing sugars, saponins, tannins found in all studied organs, while leucoanthocyanins, iridoids, steroids and terpenoid revealed in the leaves only (Table 3). Of the various phytochemicals and minerals estimated, saponin and tannins were found in larger amounts in leaves than others, whereas the minerals, calcium was more in leaf (2.92 %) (Figure 8).

Table 1: Quantitative Microscopy

Parameters	Range	Mean
Palisade ratio	6-12	9.0
Stomatal frequency	21-34	29.0
Stomatal index	22-24	23.0
Vein islet number	23-28	25.14
Vein termination	19-21	20.0

Table 2: Histological color reactions

Reagents	Constituents	Colour	Histological zone		
			Leaf	Bark	Root
Anilline SO_4 + H_2SO_4	Lignin	Yellow	Xy.	Fib.	Xy.
Weak Iodine solution	Starch	Blue	Meso., M. cor.	Co.	Cor.
Sudan III / IV	Fats	Red / Pink	Meso., M. cor.	Co.	Cor.
Dragendroffs reagent	Alkaloids	Turbidly Brown	Meso., M. cor.	Co.	--
Ba $(OH)_2$ + $K_2Cr_2O_7$ + $CaCl_2$	Saponins	Yellow	M. col., M.cor.	Ck., Co.	Cor.
$Fecl_3$	Tannins	Blue green	M. cor.	Ck., Co.	Cor.
Vanillin + HCl	Flavonoids	Yellow	Meso., M. cor.	--	--
$AgNO_3$ + H_2O_2	Ca. crystals	Black	M. cor.	Co.	--

$= Ck.-Cork; Co.-Cortex; M. cor. - Midrib cortex; M.col- Midrib collenchymas; Meso.-Mesophyll; Scl. - Sclerenchyma; Xy. – Xylem

Table 3: Phytochemical constituents

Phytochemicals	Inference		
	Leaf	Bark	Root
Acubins / Iridoids	+	-	-
Alkaloids	+	+	-
Anthraquinone	-	-	-
Cardiac glycoside	-	-	-
Coumarins	-	-	-
Flavonoids	+	+	-
Leucoanthocyanins	+	-	-
Phlobatannin	++	+	-
Reducing sugars	+	+	+
Simple phenolics	+	+	+
Steriods	+	-	-
Saponins	+	+	+
Tannins	+	+	++
Terpenoid	+	-	-

Figure 1: A flowering twig

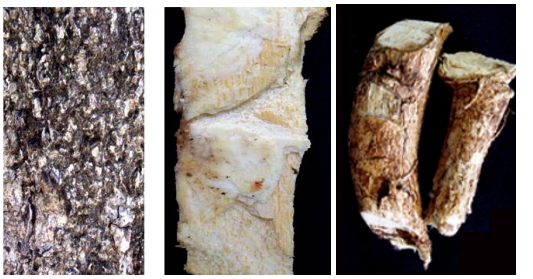

Figure 2: Photograph of fresh bark (outer and inner surface) with root

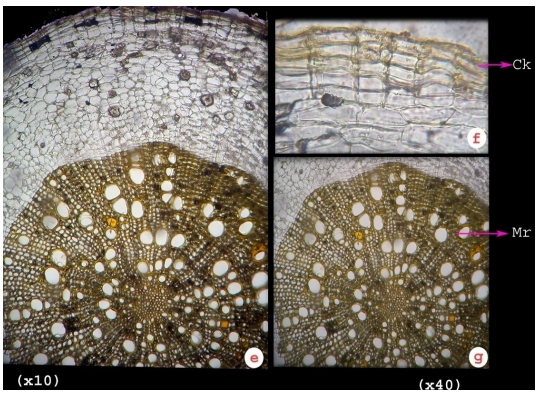

Figure 3: Transverse section of root with enlarged portion (Ck-cork; Mr- Medullary rays)

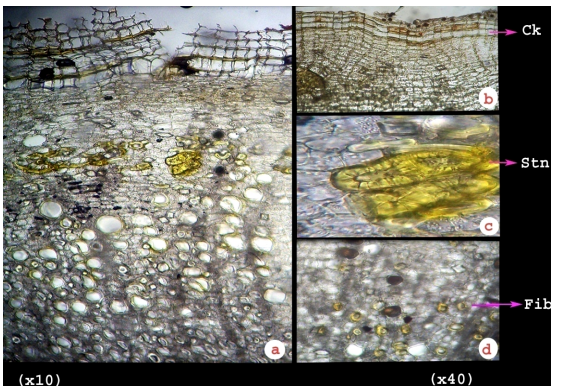

Figure 4: Transverse section of bark-entire view with enlarged portion (Ck-cork; Stn-stone cell; Fib-fiber)

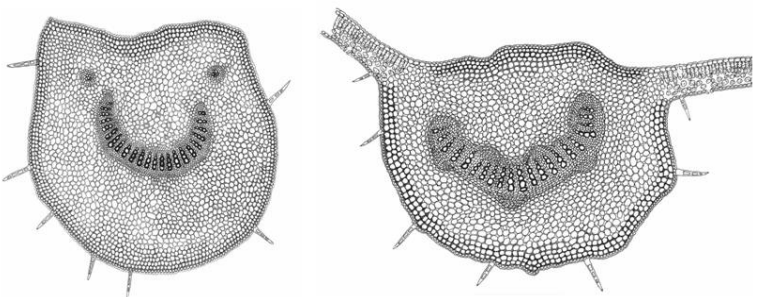

Figure 5: Transverse section of Leaf (petiole and lamina)

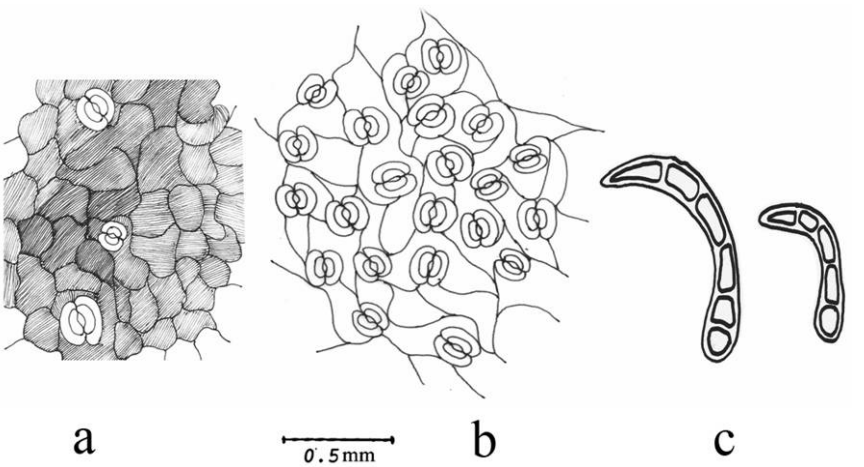

Figure 6: Epidermal structures [a- adaxial epidermis' b- abaxial epidermis; C- trichomes]

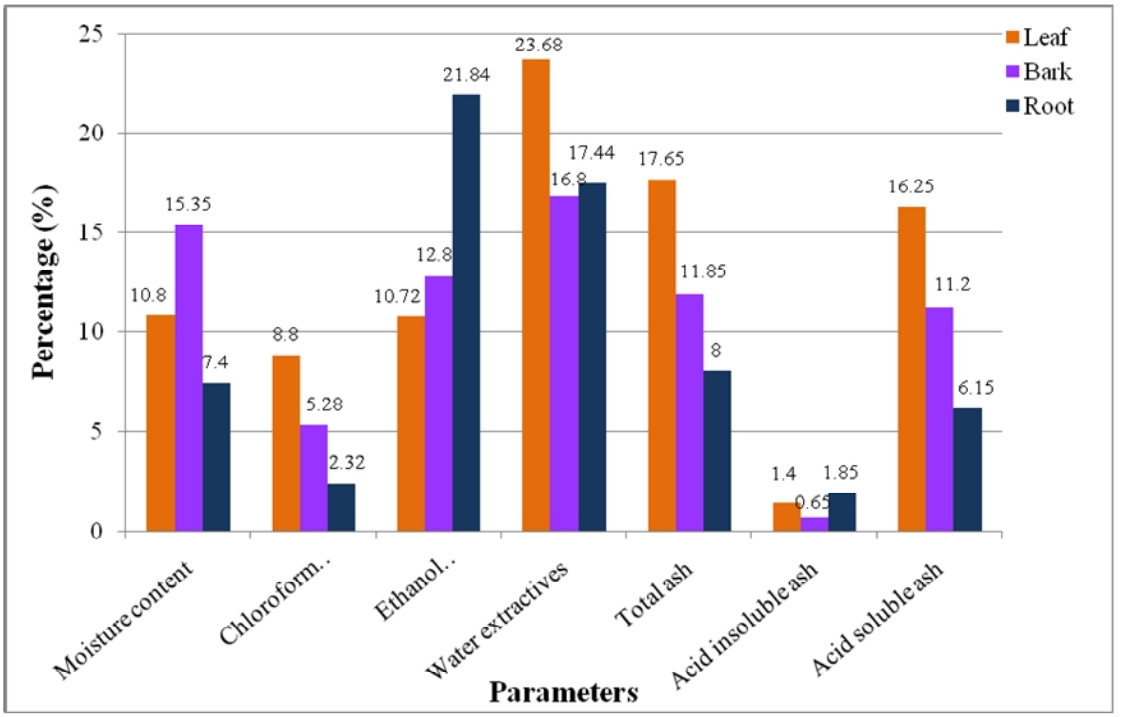

Figure 7: Physiochemical Evaluation

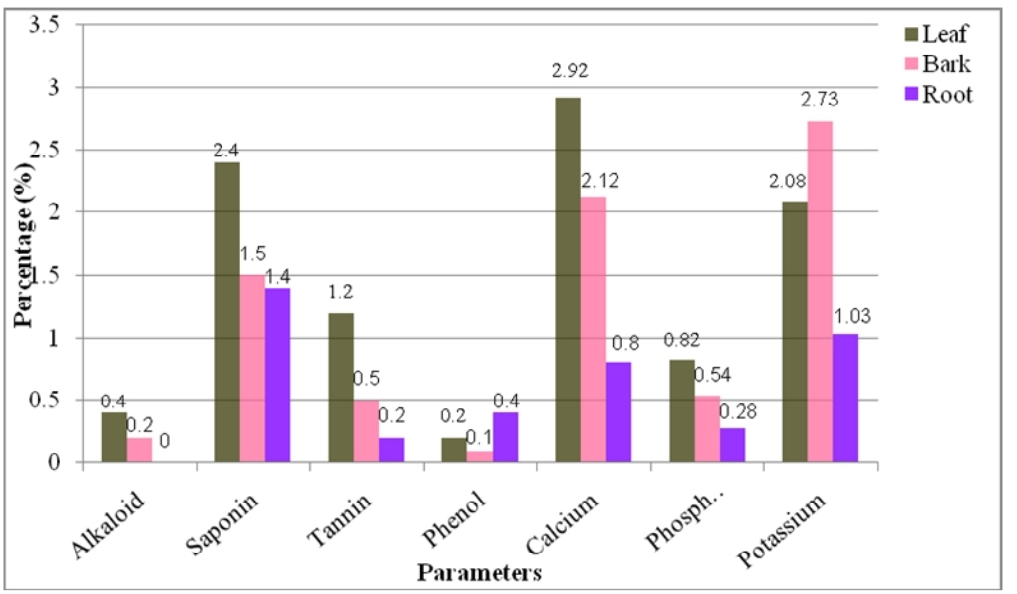

Figure 8: Quantitative phytochemicals

CONCLUSION

India is rich in ethnic diversity and indigenous knowledge of plants. The literature and field surveys have documented the detailed, specific use of plants organs. Although the use of herbal medicine has a long history, it lacked adequate identification knowledge of plant parts based on modern scientific information. The pharmacognostic parameters reported on this plant, *W. arborea* here can serve as a valuable source of identification and provide a suitable diagnostic tool for standardization besides adulterant identification in related species or weeds. Furthermore, various biological activities exhibited by *W. arborea* as well as different chemical compounds owned by this plant species, it is inclined to say that such studies may lead further research towards the field of drug development.

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