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Original Article

Pharmacognostic Studies on the Root of *Anthocephalus cadamba* (Roxb.) Miq.

Suman Acharyya[1*], Ranjan Padhy[2], Santosh Kumar Dash[3]

ABSTRACT

Purpose: To undertake the pharmacognostic studies of *Anthocephalus cadamba* (Roxb.) Miq. Root for the purpose of identification and differentiation from related species. **Methods:** The macroscopic and microscopic features of the root were studied, including the use of powder microscopy with the aid of suitable tools and reagents. Physicochemical parameters such as ash value, extractive value and weight loss on drying were also determined. The root powder was successively extracted with different solvents followed by preliminary phytochemical screening of the extracts. **Results:** Macro- and micro-scopic studies revealed cork i.e. the layer of periderm present above the cortex along with lenticels. The periderm is many layered membranous with irregularly fissured crevices containing phellum and phellogen. Secondary phloem is comparatively massive without lignified tissues i.e. bast fibres and contains sieve tubes, phloem parenchyma, many enriched with starch grains. The secondary xylem lignified mingled with medullary rays, vessels, parenchyma and wood fibers. Preliminary phytochemical screening of different extracts revealed the presence of alkaloids, carbohydrate, protein, gum, steroid, tri-terpenoid, saponin, flavonoid and tannin in the root. **Conclusion:** The findings of this study facilitate pharmacognostic standardization of the plant material and add clues in the preparation of herbal monographs for Phyto pharmacopeia.

Key words: *Anthocephalus cadamba,* Kadamba, Root, Pharmacognostic studies, Macroscopic, Microscopic, Phytochemical.

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INTRODUCTION

Anthocephalus cadamba (Roxb.) Miq. syn. *Neolamarckia cadamba* (Family: Rubiaceae) commonly known as 'Kadamba' in Ayurveda[1] is a large deciduous tree between 37.5 -45 meter height. The stem of younger trees appears greyish-green with smooth bark. As it gets older, the bark gets rough and grey with longitudinally fissured.[2] Leaves glossy, dark green, opposite, simple pulvinus base sub sessile to petiolate, broadly ovate to elliptical-oblong, entire, apex mucronate and venation pinnate. The flowers that appear from August to October are orange to yellow. Inflorescence in clusters, terminal globose heads, subsessile and fragrant. Fruitlets numerous with upper parts containing 4 hollow or solid structures. Seed trigonal or irregularly shaped.[3] The species occurs in wooded grasslands, deciduous woodland and bushland, riverine and groundwater forests in altitudes between sea level to 1500 meter.[4] The trees found in the greater part of India in moist localities in West Bengal, Bihar, Orissa (Odisha), Andhra Pradesh, Karnataka, Kerala and peninsular India.[3,5]

The plant finds its application in several traditional and folklore systems of medicine around the globe. The tribal of India use the leaf paste orally against dyspepsia and locally applied in mouth ulcer in children. Lodhas apply bruised leaves on boils for removing of sub dermal inflammatory deposits.[6] Leaves are nutritious, astringent and bitter; their decoction is reported to be used for gargling in aphthae or stomatitis. Dried powdered leaves used as anthelmintic and the tribal people of India used hot water extract of the leaves as an astringents, stomatitis and for washing wounds in throat.[4,6-8] The flowers are used as vegetable and as gurgle to remove the foul smell from mouth.[4,7] The fruit is cooling and said to destroy the phlegm and impurity of blood when eaten.[4] The ripened fruit are aromatic, acidic with astringents property. Lodhas take ripened fruits as carminative/masticate.[7] Fruit juice is given during fever and gastric disturbance.[4,42] Similarly stem barks reported to possess astringent, febrifuge, antiseptic and acts as diuretics.[9] Juice of the bark given orally against cough, fever and in inflammation of eyes.[4,8] Dried stem bark also used as folk medicine (ethno medicines) in the treatment of various skin diseases, anemia, uterine complaints and for improvement of semen quality.[10-11] Lodhas apply stem bark paste on swelling of legs and juice to cure eye inflammation. In Konkan, the fresh juice of the bark is applied to the heads of infant when the fontanels sunken.[9] Mundas prescribe the bark paste duly suspended in water in reducing blood sugar in the patients with diabetes mellitus.[6]

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Several pharmacological and biological tests have been reported on this plant are evident from literatures. Alcoholic extracts of dried leaf possess anthelmintic activity.[12] Various extract of the whole plant is also reported to have antimicrobial, wound healing, antioxidant and anti-malarial activity.[11,13-14] The alcoholic and aqueous extract of this plant showed significant antibacterial and antifungal activities.[15] Chlorogenic acid isolated from the leaves reported to possess hepatoprotective activity *in vitro* and lipid peroxidation in liver microsomes *in vivo.*[16] Methanol and aqueous extract (100, 200, 400 mg/kg p.o.) of dried root was reported to have hypoglycemic activity.[17] Methanol extract (200, 400 and 600 mg/kg p.o.) of dried barks of the plant was reported to have analgesic, anti-inflammatory, antipyretic activity and various extracts *viz* petroleum ether, chloroform, methanol, aqueous (300 mg/kg p.o.) revealed diuretic and laxative.[18-19] Methanolic root extract evaluated for its anti-hyperglycemic and antilipidemic activity in alloxan induced diabetic rats.[20]

However, only a few phytochemicals have been reported on this plant in the literature viz types of sapogenins such as cadambagenic acid, quinovic acid and ß-sitosterol was isolated from the bark.[4] Few alkaloids are also reported from the bark and leaf like cadambin, 3α-dihydrocadambine and isohydrocadambine. Leaves also yield ß-sitosterol.[21]

In the light of all the above and keeping the medicinal overview of *A. cadamba* , the present investigation was being carried out to study some pharmacognostic features of the root as a whole including its intact and powdered form not available in the literature. The studies were carried out in accordance with WHO General Guidelines for Herbal Drug Standardization methodologies.[22] The findings from this study would be useful as standards for the species as well as a source of reference for further scientific investigation of the species.

MATERIALS AND METHODS

Collection, authentication and preparation of plant material

Intact root pieces were collected during April 2014 carefully from experimental plants inhabiting in forests of Ganjam district of Odisha, India and authenticated by Dr. M.S. Mondal, Taxonomist, Botanical Survey of India, Howrah. A voucher specimen (vide no. BUB 0193) was deposited in the herbarium museum P.G. Dept. of Botany of Berhampur University, Berhampur for future reference. After authentication, fresh roots were collected in bulk, washed with potable water to remove adhering dirt followed by rinsing with distilled water, and then shade dried and powdered.

Macroscopy

The following macroscopic characters for the fresh and dried roots were noted: surfaces, size and shape, fracture, texture, colour, odour and taste.

Microscopy

Anatomical characteristics of the root

Fresh root pieces were subjected to dehydration procedure from aqueous to alcohol, in xylol and finally embedded into paraffin blocks and sectioned with the help of rotary microtome. Thin sections with thickness varying from 10 - 15 μm were collected. Dewaxing of the sections was done by customary procedure.[23] The sections were stained with phloroglucinol and hydrochloric acid in the ratio 1:1 and mounted with Canada balsam/ DPX (Diphenyle xylene) on micro slides. Microphotographs were taken using a Leica DMLS microscope attached with Leitz MPS-32 camera. Photo micrographs of different magnifications were taken to study the anatomical features.

Powdered Microscopic Characteristics

The shade dried powdered root screened through sieve no. 40 was used for the powdered drug analysis. The specimens were separately treated with glycerin, N/20 iodine solution, 10 % w/v alcoholic ferric chloride (for detection of phenolic compounds), phloroglucinol-hydrochloric acid (1:1) for detecting lignin and ruthenium red solution (for detection of mucilage). After staining, the samples through temporary microslide preparation taking the mount ant glycerin and were observed under a compound microscope.[24]

Preliminary phytochemical studies

The dried and powdered root (50 g) was successively extracted with petroleum ether (60 – 80°C), chloroform, methanol and water by reflux for 2 h. Following extraction, the liquid extracts were concentrated under reduced pressure using rotary evaporator to yield dry residues. The extracts were subjected to preliminary phytochemical screening using standard procedures to determine the nature of phytoconstituents content.[25-27]

Physicochemical analysis

The physicochemical parameters including ash values (total ash, acid insoluble ash, water soluble ash and sulphated ash), extractive values (ethanol, ether and water soluble) and loss on drying were performed according to the standard treatises.[28-29]

RESULTS

Macroscopic characteristics of the root

The matured root even if is uneven in thickness but cylindrical, whitish grey with fissures and surface rough due to presence of longitudinal striations and root scars. Taproot branched with true kinds of rootlets (ultimate branches-secondary and tertiary) found to be present. Rootlets penetratingly scattered profuse in the soil around the tap root towards ventricle. The powdered root is odorless and tasteless. In fresh condition the cut surface of the root is smooth, demarcated with white border (extrastelar) and yellow middle (i.e. stellar) region. In matured trees, root extended from 5 to 7 meters.

Microscopic characteristics

Matured root measuring 5mm thick has wide periderm and solid vascular cylinder [Figure 1]. Cork layer is present above the cortex amidst lenticels. The periderm is scaly flacks with shallow irregular fissured crevices containing phellum, phellogen and phelloderms. Phellum constitutes cells of rectangular shape *i.e.* brick shaped, thin walled arranged radially. Inner to Phellum narrow strip of Phellogen (cork cambium) followed by phelloderms [Figure 2]. Secondary phloem comparatively massive unlignified, containing sieve tubes, phloem parenchyma and starch grains [Figure 3]. Vascular cambium Ring, found to be present between secondary phloem and secondary xylem. Parenchymatous conjunctive tissues (rays) present below endodermis towards medulla region. The secondary xylem is lignified, in which medullary rays, xylem vessels, xylem parenchyma and fibers are found. Medullary rays run radially from the pericyclic region to the medullary zone. Rays cells observed in 2 to 4 cells wide (*i.e.* biseriate, triseriate or tetraseriate) with a few uniseriate pattern. Xylem vessels are found between the medullary rays [Figure 4]. Protoxylem occurs centrifugal while the Metaxylem centripetally located signifying endarch condition. Trachides have small simple pits, seen profusely on tangential wall. Primary xylem core elements found towards the centre on which the secondary xylem is prevalent. Pith almost fused.

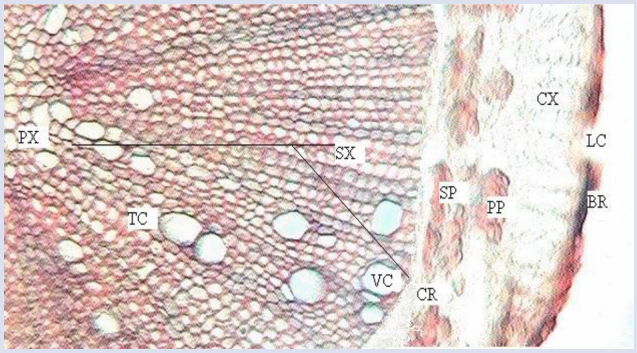

Figure1: Sectorial T.S of root of *Anthocephalus cadamba (X 1000)*.
(CX- Cortex; LC- Lenticel; BR- Bark; PP- Primary Phloem; SP- Secondary Phloem; CR- Cambium Ring; VC- Vessels; TC-Tracheids; SX- Secondary Xylem; PX- PrimaryXylem).

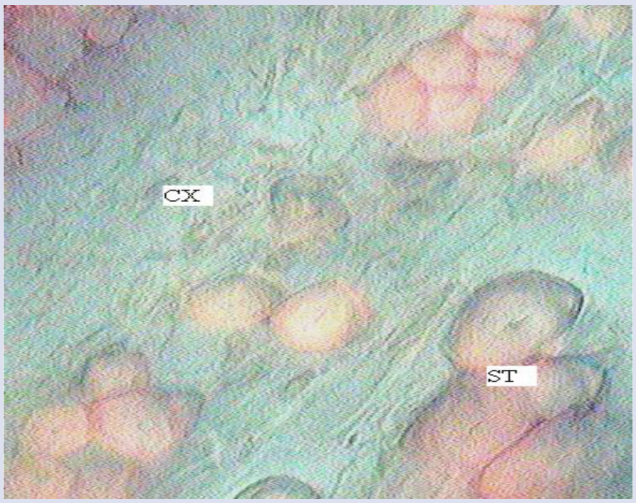

Figure 3: Sectorial T.S. of root Cortex of *Anthocephalus cadamba.*
(CX- Cortex; ST- Sieve tube)

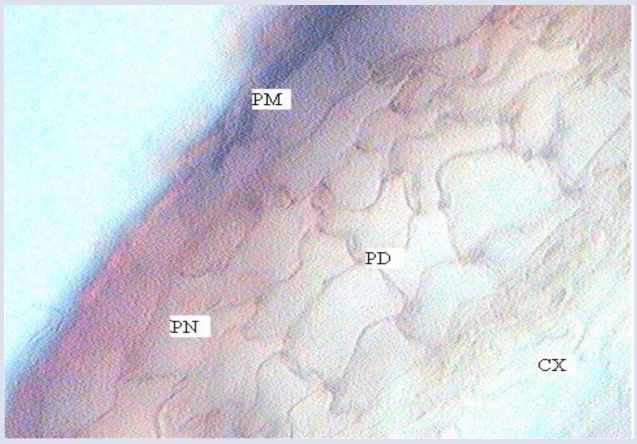

Figure 2: Sectorial T.S of root Periderm of *Anthocephalus cadamba (X 1000).*
(ED-Exoderm; CX- Cortex; PD-Phelloderm; PM- Phellum; PN- Phellogen; P-Periderm).

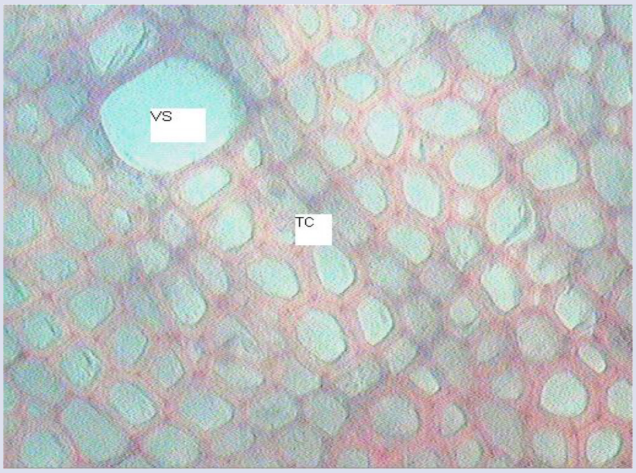

Figure 4: Sectorial T.S. of root secondary xylem of *Anthocephalus cadamba.*
(VC- Vessels; TC- Tracheids)

Powder characters of the root

The root powder consists of vessel elements, fibers, parenchyma and few cork cells. The vessels elements are long and cylindrical. They have lignified simple and horizontal perforations. The lateral pits are circular to elliptic and dense. The fibers are abundant in the powders which are narrow and long with thick lignified secondary walls. Parenchyma cells are found clearly [Figure 5 and 6].

Preliminary phytochemical studies

The result of the preliminary phytochemical screening of different extracts (Table 1) showed presence of alkaloids (in chloroform and methanol extracts), carbohydrates, proteins, gum (in aqueous extract), steroids (in petroleum ether, chloroform and methanol extracts), triterpenoids (in petroleum ether and chloroform extracts), saponin (in chloroform, methanol and aqueous extracts) and flavonoids and tannin (in methanol and aqueous extracts).

Physicochemical characteristics

The values obtained for physicochemical parameters are reported in Table 2.

DISCUSSION

A plant may be considered as a biosynthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man, but also for a multitude of compounds like alkaloids, glycosides , steroids and sterols, saponins, flavonoids, phenolic compounds and volatile oils that exert a physiological effect. The compounds that are responsible for therapeutic effects are usually the

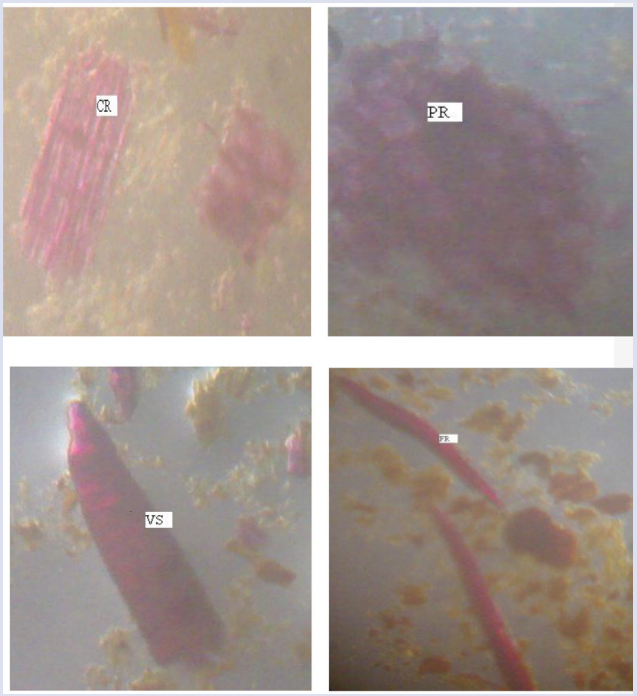

Figure 5: Powder characters of root of *Anthocephalus cadamba*.
(CR- Cork cells; PR- Parenchyma; VC- Vessels; FR- Fibers)

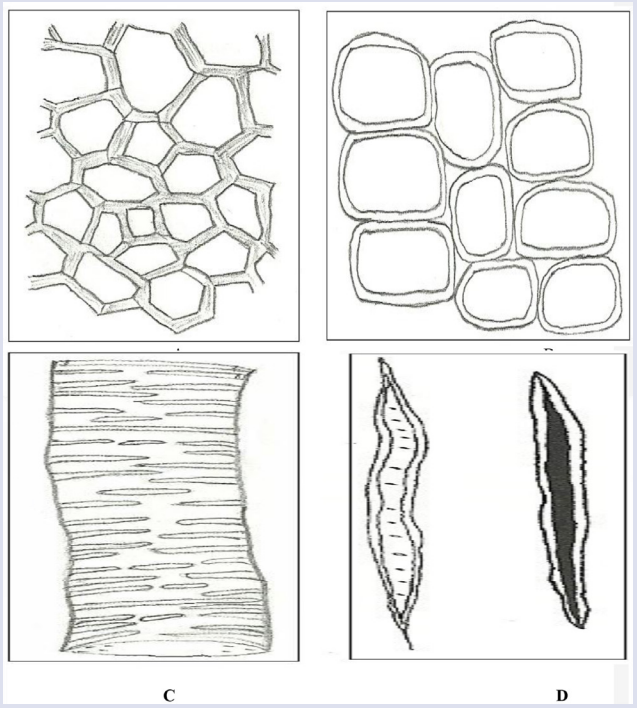

Figure 6: Powder characters of root of *Anthocephalus cadamba* (Line drawing).
(A- Cork cells; B- Parenchyma; C- Vessels; D- Fibers)

Table 1: Preliminary phytochemical profiles of various extracts of *Anthocephalus cadamba*

Test	Pet. Ether	Chloroform	Methanol	Aqueous
Steroids and sterols	+	+	+	-
Triterpenoids	+	+	-	-
Alkaloids	-	+	+	-
Saponins	-	+	+	+
Flavonoids	-	-	+	+
Carbohydrates	-	-	-	+
Gums and mucilages	-	-	-	+
Proteins and amino	-	-	-	+
Tannins and phenolic compounds	-	-	+	+

+ = present; - = absent

Table 2: Physicochemical values for *Anthocephalus cadamba* root powder

Parameter	Value (% w/w)
Total ash	4.26 ± 0.678
Acid insoluble ash	1.1 ± 0.246
Water soluble ash	2.418 ± 0.354
Sulphated ash	5.735 ± 0.745
Ether soluble extractive	2.052 ± 0.264
Ethanol soluble extractive	5.037 ± 0.273
Water soluble extractive	10.0 ± 0.375
Loss on drying	5.576 ± 0.624

Values are expressed as mean ± SD (n = 4)

secondary metabolites. A systematic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism in addition to its macro and microscopic studies.

A. cadamba is often confused with other species due to their relative similarities. The species has been taxonomically described as distinct species by earlier workers.[30] The root finds its application in several other traditional and folklore systems of medicine around the globe that have been previously described and surprisingly no pharmacopoeial standards are available for them in the literature. Owing to its importance in applications, the present study was designed and conducted. From the present study, it can be concluded that the macroscopic and microscopic findings together will help future investigators in proper identification of the plant. Further, the powder microscopy, preliminary phytochemical screening and physicochemical parameters would aid in standardization of the plant material. The wide spectrum of biological activity of this plant is due to presence of several phytoconstituents that needs to be studied further.

CONCLUSION

From the ongoing studies, it can be concluded that the above macroscopic and microscopic studies together may be used as a tool for identi-

fication of *Anthocephalus cadamba* with its pharmacognostic characteristics, discriminating it from its other species diversity.

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ABBREVIATIONS

WHO: World Health Organization: **DPX:** Diphenyle xylene; **Pet. Ether:** Petroleum Ether; **SD:** Standard Deviation: ***A. cadamba****: Anthocephalus cadamba*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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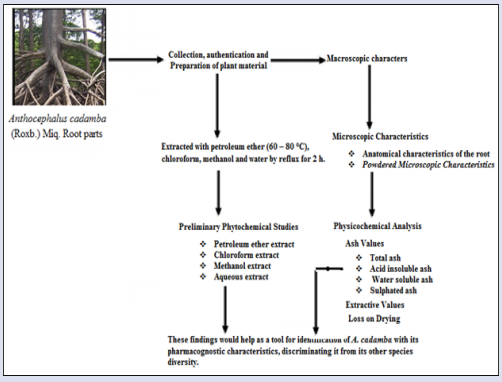
GRAPHICAL ABSTRACT



SUMMARY

- The standardization of a crude drug is an integral part of establishing its correct identity and is of paramount importance in justifying their acceptability in modern systems of medicine. The most established information with regard to the use of herbal preparations currently available in the public domain is in the form of pharmacopoeial monographs. These documents publish standardized parameters for their identification. Published monographs in a pharmacopeia are the most practical approach for quality control of an herbal drug. The preparations made from the root of *Anthocephalus cadamba* are currently being used in several traditional and folklore systems of medicine for the treatment of various diseases without standardization. These findings would help as a tool for identification of *Anthocephalus cadamba* with its pharmacognostic characteristics, discriminating it from its other species diversity and aid in the preparation of an herbal monograph for the species.

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