Research Article

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MACRO-MICROSCOPIC PROFILING OF AZINJIL, AAVARAM AND MARUKKARAI VER PATTAI (ROOT BARK) OF SIDDHA

Kallingil Gopi Divya ^{1*}, Mattumal Rubeena ¹, Brindha Sundaramoorthy ², Erni Bobbili ², Remya A ², Koppala Narayana Sunil Kumar ³

¹Research Assistant, Department of Pharmacognosy, SCRI, Central Council for Research in Siddha, Ministry of AYUSH, Govt. of India, Arumbakkam, Chennai 600106, India

²Lab Technician, Department of Pharmacognosy, SCRI, Central Council for Research in Siddha, Ministry of AYUSH, Govt. of India, Arumbakkam, Chennai 600106, India

³Research Officer & HOD, Department of Pharmacognosy, SCRI, Central Council for Research in Siddha, Ministry of AYUSH, Govt. of India, Arumbakkam, Chennai 600106, India

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*Corresponding author

E-mail: minnu.kg@gmail.com

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ABSTRACT

Siddha system of therapeutic medicine is largely practiced in the South India and is identified to be very closely related to Tamil civilization. A large number of plants are used for the manufacturing of formulations which are prescribed for various ailments. Root barks possess wide chemical compounds which has immense biological activity and medicinal values. Transformation of the crude drugs into medicine requires quality assurance to the upmost. Aim: Macro-microscopic detailing of three important root bark drugs in Siddha, *Alangium salviifolium* (L.f.) Wangerin (Azinjil verpattai) *Senna auriculata* (L.) Roxb. (Aavaram verpattai) and *Catunaregam spinosa* (Thunb.) Tirveng (Marukkarai ver pattai) has been proposed in the current research. Methodology: The root barks were subjected to morphological, anatomical and powder microscopic studies, with the help of Nikon ECLIPSE E200 trinocular microscope attached with Nikon COOLPIX5400 digital camera, following pharmacopoeial procedures. Results: Macroscopically the barks differed in their organoleptic characters. All the three samples showed distinct cork, cortex and phloem region but differed in the number of layers. Stones cells were present in only *C. auriculata* and *C. spinosa*. *C. auriculata* and *C. spinosa* contained tannin cells. The powder microscopy revealed the presence of different types of crystals in the three species druses in *C. auriculata*, prismatic in *C. spinosa* and *C. catunaregam* is rhomboidal. Discussion: The colour, cracks, fissures and fractures possess affordable useful characters in addition to the macro microscopic character for the identification. The detailed morphology, anatomy and powder microscopic characteristic features of root barks will prove to be an essential database for the accurate identification of these drugs. Conclusion: As these root barks form the major ingredients of a large number of Siddha formulations the study will substantiate to be a great contrivance in their correct identification.

Keywords: *Alangium salviifolium*, authentication, *Catunaregam spinosa*, *Senna auriculata*, standardisation, powder microscopy, quality control.

INTRODUCTION

With passing time there is a considerable amount of interest being generated towards the traditional medicinal systems due to their effective treatment and the minimal side effects. Siddha the most important medicinal system prevalent in Southern India has widened its spectrum to a large number of people. Plants, along with metals and minerals, form the source of raw drug for this system of medicine. The plant parts used include root, stem, leaves, flowers and fruits. Nearly hundred roots along with the root bark are used for the preparation of 293 formulations in Siddha for curing many diseases^{1,2}. The current study has been taken up to evaluate the macro-microscopy of three important root barks namely *Alangium salviifolium*, (Azinjil verpattai), *Cassia auriculata* (Aavaram verpattai) and *Catunaregam spinosa* (Marukkarai ver pattai) used in various Siddha medications.

Alangium salviifolium (Alangiaceae) is a deciduous, rambling shrub/ small tree distributed over the plains and foothills throughout the greater parts of India. It is known for its purgative, anthelmintic, astringent, pungent and emetic properties, and is used in fever, skin diseases, biliousness and colic³. The oil from the root bark proves to be effective in acute rheumatism and has revealed anti tubercular activity against mycobacterial strains

Mycobacterium phlei and M-607 and antifungal activity against *Helmintho sporium sativum*⁴. Root barks of *A. salviifolium* externally used for as an antidote against snake/ scorpion, rabbits, rats, bite and dogs⁵.

The investigation of the chemical constituents on root bark showed presence of alangine⁶, alangine A, B, alanginine⁷, akharkantine, ankoline, lamarkine and five alkaloidal bases^{8,9}. In Siddha system Naalpattapunnukupugai (smoke) from a powder prepared using *A. salviifolium* and several other drugs is used to heal chronic ulcers, Puraityodum virana thailam (a medicated oil) is used in the treatment of ulcer with sinuses, carcinoma, scrofula/tubercular glands in the neck, cancer of the cheek, deep abscess of the thigh and carbuncle².

Cassia auriculata (Caesalpinaceae) is a fast-growing, profusely branched, tall, evergreen shrub of 1.2 – 3.0 m in height reaching a height of even 6 m is found in the dry zones of southern, western and central India extending up to Rajasthan in the north³. The root is used in skin diseases¹⁰, as astringent¹¹, jaundice¹², to control urination¹³, skin diseases^{14,15}, joint pain¹⁶ and sores of the lips and mouth¹⁷. The ethanolic extract of the root yielded a flavone glycoside identified as 7,4'-dihydroxyflavone-5-O- β -D-galactopyranoside¹⁸. Trace element like iron, copper and zinc has

been reported in leaves¹⁹. The root shows interferon like activity against Ranikhet disease virus²⁰.

Cassia auriculata is used in the preparation of Karungali thailam (medicated oil for topical application) which proves its use in the treatment of tuberculosis². Aavarai kudineer (water based oral decoction) and 'SUGNIL' a product for Diabetes Mellitus²¹. Marunthugalin vegathinalundana kolaarukaluku kudineer with *C. auriculata* as one of its ingredients is being in use to cure stomach pain due to side effects of certain drugs, amoebic dysentery and bloody stools².

Catunaregam spinosa (Rubiaceae) is a thorny, rigid shrub or tree, up to 9 m in height and 1.2 m in girth with a bole of 2-3 m found throughout India up to an altitude of 1900 m. The species which occurs in India is common as an under growth in the Sal forests of the sub-Himalayan tract and elsewhere. Root is found to possess insecticidal and insect repellent properties³. Root is used in snake bite²², boils²³, post-delivery pains²⁴, anti-fertility agent²⁵, insecticidal²⁶, in skin diseases, herpes²⁷, gonorrhea and diseases of the urinary tract²⁸. Scopoletin was isolated from the root and root bark²⁹ and D-Mannitol from the root³⁰. The root bark yielded three long-chain fatty esters of triacontanoic acid²⁹.

In Siddha system of medicine, a formulation named Patai Cankaran which is prepared using root bark of *Catunaregam spinosa* is used to cure Ringworm¹.

MATERIALS AND METHODS

Botanically authenticated root barks of *Alangium salviifolium*, *Cassia auriculata* and *Catunaregam spinosa* were procured from Mettur. The macroscopy of the samples was documented by Nikon COOLPIX5400 digital camera. Sectioning of samples preserved in FAA (Formalin-5 ml, Acetic acid-5 ml, 70% Ethyl alcohol-90 ml) were done to reveal the anatomy of the barks and the rest were dried, powdered, passed through sieve 60, followed by preservation in an air-tight covers for powder microscopy. Transverse sections of the root barks were hand cut using a 7° clock platinum blade stained with safranin and photographed using Nikon ECLIPSE E200 trinocular microscope attached with Nikon COOLPIX5400 digital camera under bright field light. Magnifications were indicated by the scale-bars. A pinch of the powdered root barks was mounted in glycerine on a clean microscopic slide. Slides were observed under Nikon ECLIPSE E200 trinocular microscope and diagnostic characters were identified, photographed and documented³¹. The powder was

tested for the presence of starch by treating with iodine solution and for tannins by treating them with ferric chloride solution¹ and the results documented.

RESULTS

Macroscopy

Alangium salviifolium: Slightly glabrous, papery, scaly due to the presence of rhytidoma, irregularly peeling, fibrous, slightly concave inside; width 0.5 to 2 cm, thickness: 0.1 cm (Fig 1.1).

Cassia auriculata: Thin bark gives away to the reddish cortical region, surface irregular, double quill, width 1 to 3 cm, thickness: 0.1 to 0.3 cm (Fig 1.2).

Catunaregam spinosa: rough bark with inner surface showing longitudinal striations, width 1 to 3 cm, thickness 0.1 to 0.2 cm (Fig 1.3, Table 1).

Microscopy

Alangium salviifolium: TS of root bark showed 3 distinct layers of cork followed by broad cortex and phloem. The cork is well developed composed of 3 to 5 successive layers of thick walled cells followed by a broad cortex containing thin walled parenchyma cells with druse crystals of calcium oxalate present randomly; the phloem zone is seen delimiting below made up of phloem elements arranged in narrow radial strip alternating with phloem rays (Fig 2).

Cassia auriculata: The transverse section of the root bark shows the typical anatomical characteristics as layer of symmetrically arranged tabular cork cells arranged in 15 to 20 layers containing reddish brown matter, followed by phellogen/cork cambium which is compactly arranged radially in 10 to 15 layers. The phellogen layer is followed by isodiametric thin walled parenchymatous cells defined as the cortex with scattered reddish-brown oleoresin cells followed by a band of lignified, pitted rectangular and oval sclerenchyma/stone cells. The stone cells originate into secondary phloem which consists of is a wide zone of tissue composed of xylem vessels, sclerenchymatous pericyclic fibres which are present between medullary rays are multiseriate and consists of parenchymatous cells containing which contains starch grains, prisms of calcium oxalate crystals (Fig 3).

Table 1: Comparative morphological characters of root barks

Species	Colour		Odour	Taste	Fracture
	Outer	Inner			
<i>A. salviifolium</i>	Brownish grey	Yellowish brown	Characteristic	Bitter	Irregular
<i>C. auriculata</i>	Greyish brown	Reddish brown	Nil	Acrid	Irregular
<i>C. spinosa</i>	Light brown	Blakish brown	Characteristic	Mild astringent	Incurved, short

Table 2: Comparative microscopic characters of root bark

Species	Cork Layers	Stone cells	Tannins	Crystals	Starch grains
<i>A. salviifolium</i>	3-5	Absent	Not phenolic	Druses	Present
<i>C. auriculata</i>	15-20	Present	Phenolic	Prismatic	Present
<i>C. spinosa</i>	< 20	Present	Slightly phenolic	Rhomboidal	Present

1.1 *Alangium salviifolium*

1.2 *Cassia auriculata*

1.3 *Catunaregam spinosa*

Figure 1. MACROSCOPY OF THE THREE ROOT BARKS

2.1. T.S of *A. salviifolium* root bark - entire

2.2. Cork-enlarged

2.3. Cortex-enlarged

2.3. Medullary rays

Figure 2. TS OF *ALANGIUM SALVIIFOLIUM* ROOT BARK

Ck-Cork; Ct-Cortex; MR-Medullary rays; Pa-Parenchyma with starch; RCr-Rosette Crystal

3.1 T.S of *C. auriculata* root bark – entire

3.2. Cork enlarged

3.3. Phellogen enlarged

3.4. Cells with tannin and starch

3.5. Pericyclic fibres

3.6. Phloem fibres

3.7. Medullary rays

Figure 3. TS OF *CASSIA AURICULATA* ROOT BARK

Ck-Cork; Ct-Cortex; MR-Medullary ray; Per- Pericycle; Pg-Phellogen; PhF-Phloem fibre; TC-Tannin cell;

4.1. T.S of *Catunaregam spinosa* root bark - entire

4.2. A portion of cork-enlarged

4.3. Enlarged portion of cortex showing sclereids and prismatic crystals

Figure 4. TS OF *CATUNAREGAM SPINOSA* ROOT BARK

Ck-Cork; Ct-Cortex; Pa-Parenchyma; PCr-Prismatic crystals; Scl- Sclereids; Sg-Starch grains

5.1. Cork cells

5.2. Cortical parenchyma

5.3. Cells with starch

5.4. Medullary ray cells

5.5. Fibres

5.6. Rosette crystals

FIGURE 5. POWDER MICROSCOPY OF *A. SALVIIFOLIUM* ROOT BARK

6.1. Cork cells

6.2. Cells with tannin

6.3. Medullary ray cells

6.4. Fibre

6.5. Crystal fibers

6.6. Parenchyma with starch

FIGURE 6. POWDER MICROSCOPY OF *C. AURICULATA* ROOT BARK

FIGURE 7. POWDER MICROSCOPY OF *C. SPINOSA* ROOT BARK

Catunaregam spinosa: TS of the bark show a zone of cork, followed by a broad secondary cortex and secondary phloem regions. Cork is multilayered, made up of compactly arranged rectangular cells; some cells contain tannin; next to the cork is a multilayered cork cambium, made up of thin walled rectangular cells. Cork cambium is followed by a broad zone of secondary cortex, made up of thin walled, compactly arranged rectangular parenchymatous cells; several concentric rows of stone cells are found traversing the secondary cortex; rhomboidal type of calcium oxalate crystals is found deposited in the lumen of some stone cells; some cells contain tannin; groups of starch grains are found abundantly in the cortical cells. Secondary phloem region is broad, found next to secondary cortex and is made up of thin walled parenchymatous cells; several layers of concentrically arranged stone cells are found in the secondary phloem region also. Medullary rays are uni to biseriate, found extending into the secondary phloem region; the ray cells contain starch grains (Fig 4, Table 2).

Powder microscopy

Alangium salviifolium: Brownish in colour and tastes bitter, showed cork cells in surface view, parenchyma cells from the cortex, starch grains, fibres and rosette crystals (Fig 5).

Cassia auriculata: Powder was reddish brown coloured with acrid taste, showed cork cells, tannin cells, medullary ray cells, crystal fibres and parenchyma with starch grains (Fig 6).

Catunaregam spinosa: Powder dark brown coloured with mild astringent taste, showed cork cells both in sectional and surface view, sclereids in groups and some with prismatic crystals and parenchyma with starch (Fig 7).

DISCUSSION

Bark constitutes the external tissue of stem or roots which are mainly composed of secondary phloem which in turn contains elements like sieve tubes, companion cells, phloem parenchyma, phloem fibers and occasionally stone cells and radially traversing

medullary rays. The external tissue has a rugged and scaly appearance due to the exfoliation of tissue brought about by the formation of phellogen³². The colour, cracks, fissures and fractures possess affordable useful characters for the identification. In the present study also, it is clearly evident that the organoleptic characters of the bark differs with species being brownish gray in *A. salviifolium* light brown in *C. spinosa* and greyish brown in *C. auriculata*. Microscopic study revealed the presence of outer multilayered cork varying from 8 to 15 layers in *C. spinosa* and nearly 12 to 20 layers in *C. auriculata* and 3 to 5 in *A. salviifolium*. The presence of different cell contents can also aid in the delimiting the species as seen in the current study tannin cells were present only in *C. auriculata*; stone cells were seen in *C. spinosa* only; rosette crystals were documented only in *A. salviifolium* whereas prismatic crystals were seen only in *C. auriculata* and *C. spinosa*. The presence of stratified phellem, rhytidome, calcium oxalate crystals and uni to triseriate medullary rays in the root bark of *A. salviifolium* is in accordance to the previous study³³. The detailed macro-microscopic examinations of the three important root bark drugs were carried out, of which *C. auriculata* and *C. spinosa* is the first-hand report.

CONCLUSION

Root barks possess wide chemical compounds which has immense biological activity and medicinal values. Transformation of the crude drugs into commercialized and generalized system requires quality assurance to the upmost. Standardization of crude drugs has become very important for identification and authentication of a drug of natural origin and this detailed macroscopic anatomical and powder microscopic studies will be helpful to verify and identify these three important root barks used in medicine.

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