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Research Article

Pharmacognostical evaluation of *Murraya* roots

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ABSTRACT

Pharmacognostical evaluation is the first and foremost step to determine identity and assess the quality and purity of the crude drug. *Murraya koenigii* Spreng (Rutaceae), commonly known as Curry leaf plant or Mitha Neem in Hindi is a highly valued plant for its medicinal value and characteristic aroma. Leaves of the plants are extensively used as spice and condiment in India and other tropical countries. Present research work includes study of macroscopic, microscopic study of the root include anatomy of the thin root, anatomy of the thick root and microscopy of the powdered root. Physiochemical studies were done by using WHO recommended parameters. Photographs at different magnifications were taken with Nikon Labphot-2 microscopic unit. These findings will be useful towards establishing pharmacognostical standards and preparation of monograph of the root of *Murraya koenigii*.

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INTRODUCTION

Murraya koenigii is commonly known as Mitha Neem in Hindi and is extensively used as a spice and condiment in India and other tropical countries[1]. Leaves, roots and barks are tonic, stomachic and carminative. Juice of the roots provides relief from the renal pain[2]. An infusion of the toasted leaves is used to stop vomiting[3]. The plant has been reported to possess anti-oxidative property, cytotoxic activity [4], and antimicrobial, antibacterial properties. [5-6], antidiabetic and cholesterol reducing property [7-9]. The plant is rich source of carbazole alkaloids [10].

MATERIALS AND METHODS

Collection & Authentication of the plant materials:

The species for the propose study, that is *Murraya koenigii* (Linn) Spreng roots were collected from the village Mangladevi of Yavatmal District of Maharashtra. Care was taken regarding the age and health of the plant to obtain a best condition root part.

The species for the proposed study was identified as *Murraya koenigii* (Linn) Spreng and authenticated by Dr. P. Jayraman, Botanist Plant Anatomy Research Centre (PARC) Chennai.

The roots were washed properly with water to remove the mud or dust if any; initially roots were dried in sunlight for an hour and shade dried completely. The dried roots were then powdered by means of wood grinder and passed through sieve no-60 to get the coarse powder, which was used for powder microscopy, extraction with different solvents and physic-chemical studies.

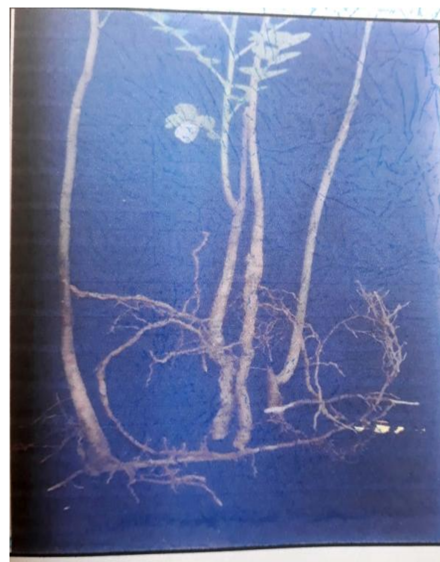

Figure 1: View of Murraya Koenigii Plant (Root)

Macroscopic and Organoleptic studies:

The macroscopic study of a medicinal plant is helpful in rapid identification of the plant material and also plays an important role in the standardization of drug.

The plant (root part) was studied for morphological characters including size, shape, color, odor, taste and extra features.

Microscopic Studies:

The samples were cut and immediately fixed in formalin 5mL + acetic acid 5mL + 90mL of 70% ethyl alcohol. After fixing the samples for 24 hours, they were dehydrated and clarified successively in graded series of tertiary butyl alcohol (TBA) as per the schedule given by Sass[12]. The specimens were infiltrated with paraffin wax (melting point 58-60^{0} C) until TBA solution attained super-saturation and casted into paraffin blocks.

Rotary microtome was used to section the paraffin embedded specimens. Each section thickness was 10-12 um. De-waxing of the sections was the customary procedure[13] (Johansen, 1940). The section was stained with toluidine blue as per the method published by O'Brien et.al. (1964)[14]. Wherever necessary sections were also stained with safrannin, fast green and I-KI for starch.

Photomicrographs of the transverse sections (root) and powder section were taken with the help of Nikon Labphot-2 microscopic unit.

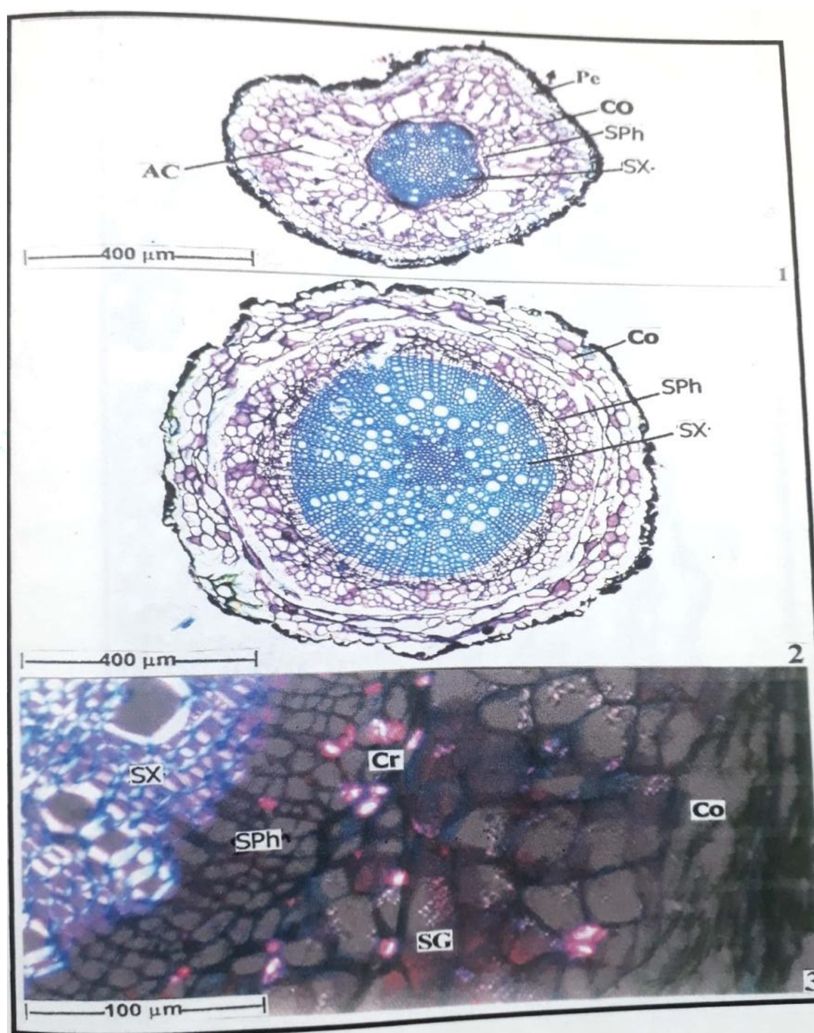

Figure 2: Anatomy of the thin root & crystal distribution

1. T.S. of Thin (Lateral) root view
2. T.S. of Thin Tap root
3. Crystals in the Secondary Phloem, Starch in the cortical cells.

(Ac- Air chambers, Co- cortex, Cr- crystals, Pe- periderm, SG- starch grains, Sph- Secondary phloem, Sx- secondary xylem)

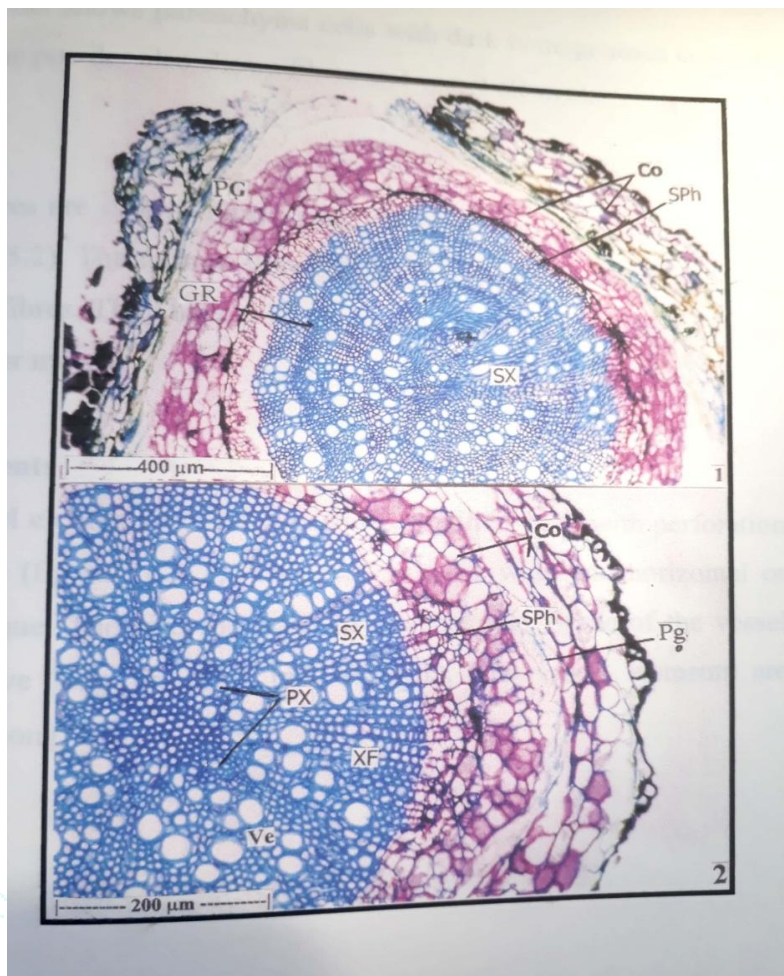

Figure 3: Anatomy of Thick Root

1. T.S. of Thick Root (Half portion) 2. T.S. of Thick Root-an enlarged section.
Co-cortex, Gr-growth ring, Pg-phellogen, Px-protoxylem, SPh-secondary phloem, Sx-secondary xylem, Ve-vessel, XF-xylem fibers

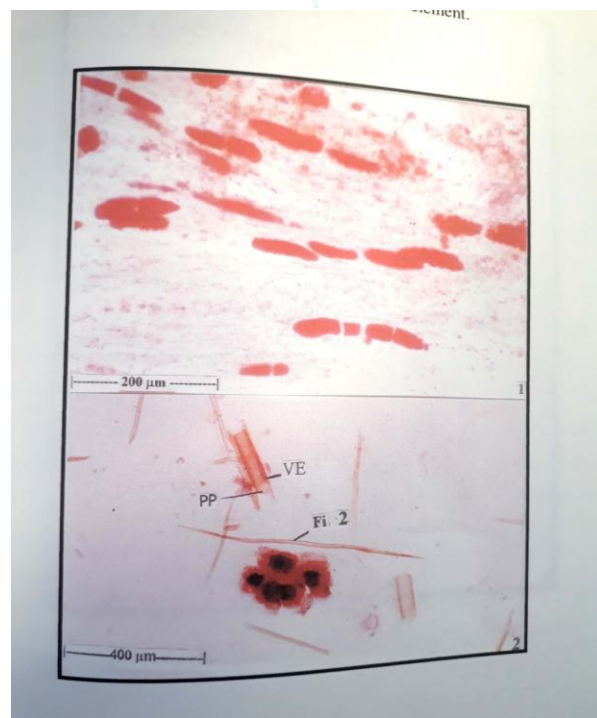

Figure 4: Powder microscopy of the root

1. Parenchyma cells with dark homogeneous content. 2. Vessel element and fibers.
(Fi- fibers, PP- perforation plate, VE- vessel elements)

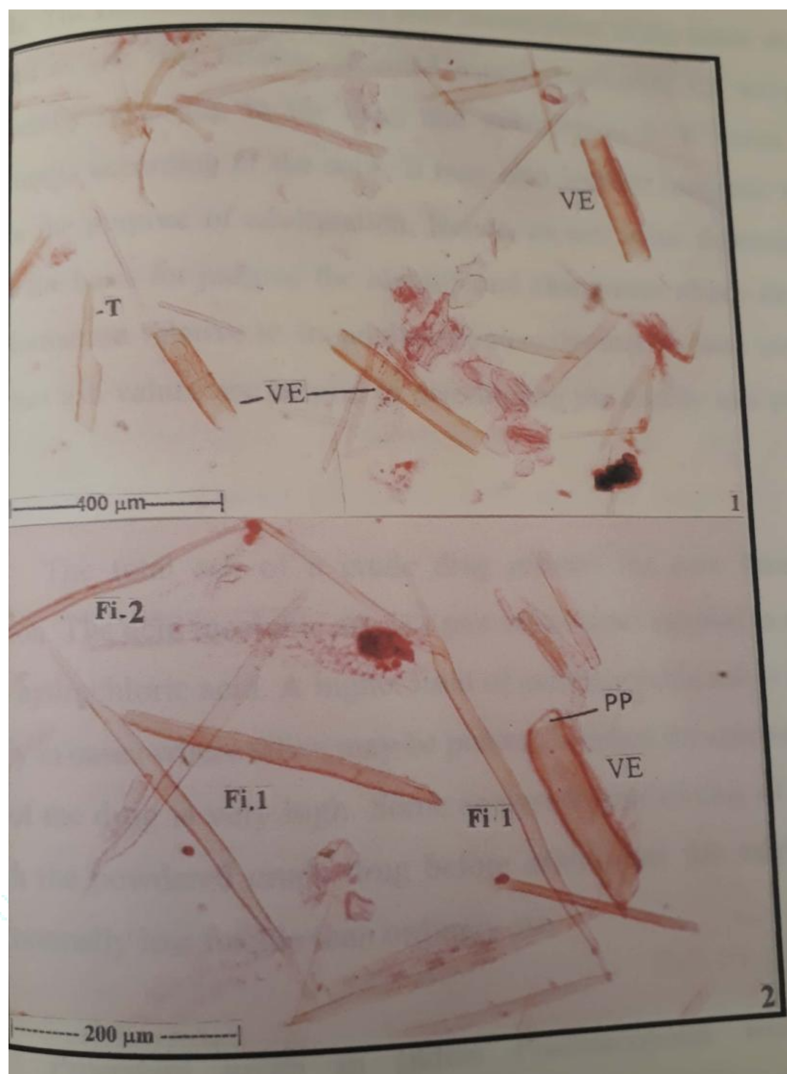

Figure 5: Powder Microscopy of the Root Vessel element and fibers

(Fi- fibers, PP- perforation plate, T-tail, VE- vessel elements.

Physico-Chemical Evaluation

The Physico-chemical parameters such as total ash value, acid insoluble ash value, water soluble ash value, sulphated ash value, extractive values and moisture content were determined as per standard protocol described in WHO guidelines[15, 16]. Procedure given in Indian Pharmacopoeia was used to determine the different ash values.

Total Ash Value:

Accurately weighed about 3 g of air dried powdered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air-dried drug.

Acid Insoluble Ash Value:

The ash obtained as directed under total ash was boiled with 25 ml of 2N hydrochloric acid for 5 min. The insoluble matter was collected on an ash less filter paper, washed with hot water, dried the filter paper, ignited and weighed. Then calculate the percentage of acid insoluble ash with reference to the air-dried drug.

Water Soluble Ash Value:

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450 ºC. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

Sulphated Ash Value:

The sulphated ash was determined by incinerating 3 gm of accurately weighed air-dried coarsely powdered drug in a tarred silica crucible that was previously ignited and cooled before weighing at a temperature not exceeding 450 ºC. The residue was moistened with 1 ml of concentrated sulphuric acid, ignited at 800±25 ºC until all black particles have disappeared.

Extractive Value:

Alcohol Soluble Extractive Value:

Alcohol soluble extractive value was performed by the method described by Khandelwal (2007). 5 g of the air-dried drug macerated with 100 ml of alcohol in a closed flask for 24 hours, frequently shaken during first 6 hours and allowed

to stand for 18 hours. Rapidly filtered taking precautions against loss of solvents. Evaporated 25 ml of filtrate to dryness in a tarred flat-bottomed china dish and dried at 105 ºC until constant weight is obtained. The percentage of alcohol soluble extractive with reference to the air-dried drug is calculated. It was then cooled; again sulphuric acid was added and ignited. It was cooled and the percentage of sulphated ash was calculated with reference to air-dried drug.

Alcohol Soluble Extractive Value:

Water soluble extractive value was performed by the method described by Khandelwal (2007). 5g of the air-dried drug macerated with 100 ml of water in a closed flask for 24 h frequently shaken during first 6hrs and allowed to stand for 18 h. Rapidly filtered and evaporated 25 ml of filtrate to dryness in tarred flat-bottomed china dish and dried at 105 ºC until constant weight is obtained. Calculate the percentage of water soluble extractive with reference to the air-dried drug. (Khandelwal, 2007).

Loss on Drying:

The moisture content of a crude drug will be responsible for decomposition of crude drugs by either producing a chemical change or microbial growth. Hence, the moisture content of the drug should be determined and controlled. The moisture content is determined by heating a drug at 105°C in an oven to a constant weight. (WHO- Quality Control Methods for Medicinal Plant Material. Geneva, England: World Health Organization; 2010)

RESULT AND DISCUSSION

Macroscopic/ Organoleptic study of Root:

In Organoleptic evaluation, appropriate parameters like taste, odour, colour, size shape of the roots were studied. Roots observed dark brown to grayish white in colour, characteristic odour with bitter and slightly pungent taste.

Tap root has many secondary roots of different thickness arise from the primary root. Thin, wirg tertiary roots arise in clusters at the tip often primary and secondary roots.

Taste is bitter, slightly pungent, characteristic odour.

Microscopy

a. Thin lateral root is uneven in cross-sectioned outline, measuring 550 um in widest plane and 350 um in narrow plane.

b. Root has dark thick crushed epidermal layer on the surface.

c. The cortex is wide and vascular cylinder has five lobbed xylem and thin sheath of phloem.

d. Thin tap root is circular and 750 um in diameter.

e. Inner cortex has small, compact, polygonal parenchyma cells.

f. Secondary xylem cylinders are has five or six primary xylem strands and sclerotic pith.

g. Xylem fibers are thick walled and occurred in radial lines.

h. Calcium oxalate prismatic crystal seen in the inner cortical cells.

i. The old root has deeply fissured surface. The cortex consists of four or five layers of tangentially elongated thin walled layer of phellogen are seen between the outer and inner cortex.

j. Powder microscopy showed parenchyma cells and vessel elements. Vessel elements are long, narrow cylindrical cells with perforation at both ends. The vessel elements are 180-280 um long.

Physico-chemical Studies:

Physico-chemical characteristics	Values % (w/w)
Total Ash Value	7.85
Acid insoluble ash value	2.26
Water soluble ash value	1.74
Sulphated ash value	3.82
Water soluble extractive value	4.2
Alcohol soluble extractive value	5.8
Loss on Drying	8.6

CONCLUSION

The observed parameters like morphology, microscopy, quantitative and qualitative studies may be useful to establish certain botanical standards for identification and standardization of *Murraya koenigii* (L) Spreng Root for the further studies.

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