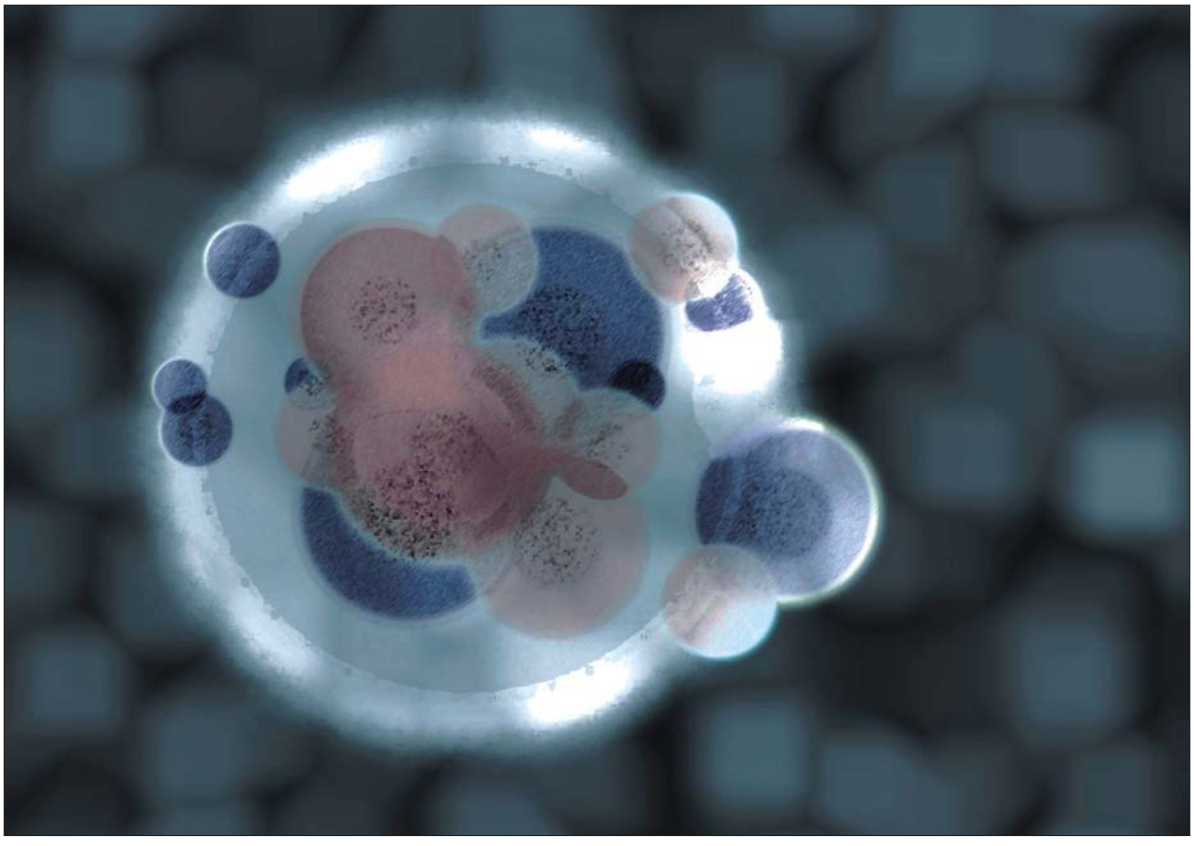

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Morphoanatomy of Stems of *Murraya koenigii* Spreng

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Abstract: The main aim of this study is to develop standard pharmacognostic parameters for stems of *Murraya koenigii* (L.) Spreng (Rutaceae). Pharmacognostic evaluation included examination of morphological and microscopic characters, determination of ash values, extractive values, micrometric measurements, powder characters and fluorescent analysis. The studied morphoanatomical characters of the stem would be an useful tool for the identification this herbal drug.

Key words: *Murraya koenigii*, curry leaf, morphoanatomy, Rutaceae, powder microscopy, micrometry

INTRODUCTION

The plant *Murraya koenigii* (L.) Spreng (Rutaceae), commonly known as Curry leaf-tree is used in the treatment of various diseases by traditional medical practitioners. This plant is reported to have stimulant, antidysentery (Adebajo *et al*., 2004), antidiabetic (Grover *et al*., 2003), antioxidant, hypolipidaemic and antiatherosclerotic properties (Vinuthan *et al*., 2007). Traditionally the leaves, bark and roots of *Murraya koenigii* (L.) Spreng. are used as tonic and stomachic. The bark and the roots are used as a stimulant, cure eruptions and the bites of poisonous animals. The stems are very popular for cleaning the teeth and are said to strengthen the gums and the teeth. Also the stems are used as bitter, anthelminitic, febrifuge, anti-inflammatory, foul ulcer, in treatment of vomiting, dysentery and flatulence (Parmar and Kaushal, 1982; Nadkarni, 1995). The constituents identified in the plant are alkaloids (Chakrabarty *et al*., 1997), glycosides, flavonoids (Adebajo and Reisch, 2000), minerals (Narendhirakannan *et al*., 2005) and volatile oils (Parmar and Kaushal, 1982). A complete study on Pharmacognostic aspects has not been reported for the stems of *M. koenigii* till now. Though the plant species could be easily distinguished on the basis of the flowers, it becomes very difficult when the crude drug is in the form of dried and cut pieces. Therefore, present investigation was planned to have a detailed study on its pharmacognostic parameters.

MATERIALS AND METHODS

Plant materials: The plant *M. koenigii* was collected from Mandsaur and positively identified by Dr. H. S. Chattree, Botanist, Govt. Arts and Science College, Mandsaur. Voucher specimen (BRNCP/M/002/2006) was deposited in the herbarium of Department of Pharmacognosy, BRNCP, Mandsaur.

Pharmacognostic studies

Morphological studies: Morphological studies were done using simple microscope. The shape, size, color, taste and odor of stems were determined.

Microscopic studies: Microscopic studies were done by preparing thin hand sections of stem. The sections of stems were cleared with chloral hydrate solution and then stained with phloroglucinol and hydrochloric acid and mounted in glycerin. Separate sections were prepared and stained with iodine solution for the identification of starch grains. The photomicrographs of sections were taken with the help of CXR III camera. Powders (No. 60) of the dried stems were used for the observation of powder microscopical characters. The powdered drug was separately treated with phloroglucinol-HCl solution, glycerin and iodine solution to determine the presence of lignified cells, calcium oxalate crystals and starch grains.

Micrometry: Eyepiece micrometer was calibrated using stage micrometer and the factor was calculated. With the help of eyepiece micrometer measurements were done in

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transverse section of stem and in powder. The powder was previously treated with chloral hydrate solution and stained with phloroglucinol and HCl for phloem fibre, with lactophenol and iodine for starch grains (Shanta *et al*., 2006).

Fluorescence study: Fluorescence study is an essential parameter for first line Standardisation of crude drug. The crude drug was subjected to this studies and its fluorescence pattern were noted. The powder material was treated separately with different reagents and exposed to visible, ultraviolet light (short and long U.V.) to study their fluorescence behavior (Shanta *et al*., 2006; Goel *et al*., 1971).

Physicochemical parameters: Total ash, water-soluble ash, acid insoluble ash and sulphated ash were determined. Petroleum ether (60-80^0), acetone, chloroform, alcohol and water-soluble extractive values were determined to find out the amount of components soluble in various solvents. Moisture content and crude fibre content were also determined (Kokate, 1994; Anonymous, 1985).

RESULTS AND DISCUSSION

Macroscopic examinations: *Murraya koenigii* is an aromatic and small tree up to 6 m in height and 15-40 cm in diameter. The young stems are green in color with sweet aromatic odor and characteristic taste. The outer surface is smooth, soft and glabrous.

The mature stems of *Murraya koenigii* are dark brown (unpeeled) and Cremish brown (peeled) in color with slight aromatic odor and characteristic taste. The outer surface is smooth and hard. The fracture of bark is splintery.

Microscopic examinations: The stem of *Murraya koenigii* has a circular transaction and shows following features (Fig. 1).

Epidermis: It is single layered, parenchymatous, uniseriate, unicellular, tangentially elongated surrounded by thick cuticle (Fig. 2). The diameter of epidermal cells is 7-8-15 µm (Table 1). Epidermis exhibits 5-6 unicellular, uniseriate, covering trichomes (Fig. 3). The length and width of trichome are 115-138-161 and 15-16-17 µm, respectively (Table 1).

Oil gland: Just below the epidermis, there are 6-10 schizolysigenous oil glands (Fig. 4, 5) present, having inner diameter of 46-76-115 µm and outer diameter of 61-94-130 µm (Table 1).

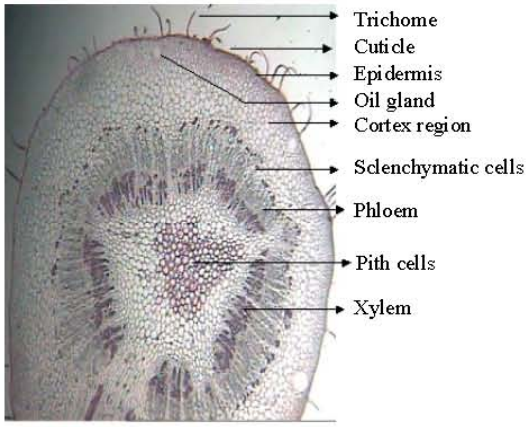

Fig. 1: Transverse section of the young stem of *Murraya koenigii* Spreng showing primary growth at x 100

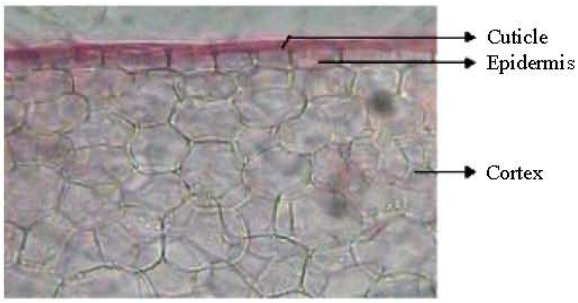

Fig. 2: Transverse section showing cuticle, layer of epidermis and cortex region in young stem at x 450

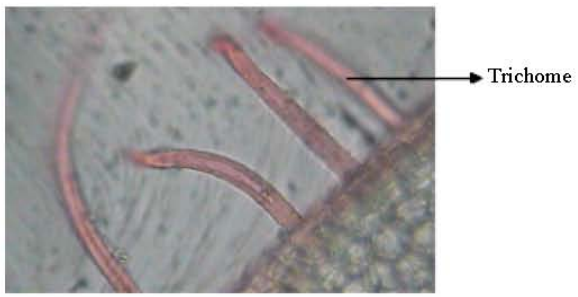

Fig. 3: Transverse section showing trichomes in young stem at x 450

Cortex: Continuous strands of 4-6 layers of compactly arranged parenchymatous, polygonal cells constitute the cortex region of 292-298-303 µm (Fig. 2). The diameter of individual cell is 19-35-49 µm (Table 1). The cortex region shows the presence of lignified sclerenchymatic cells of 30-40-50 µm (Fig. 6, Table 1).

Vascular bundle: The vascular system consists of a cylinder of xylem produced towards the inside and a

Table 1: Microscopical measurements (Micrometry)

Plant parts	Measurements (µm)
Oil gland (inner diameter)	46-76-115
Oil gland (outer diameter)	61-85-130
Trichome length	115-138-161
Trichome (width)	15-16-17
Epidermis layer (young) diameter	7-8-15
Cork region	64-75-98
Cortex region	292-298-303
Individual cell of cortex diameter	19-35-49
Sclerenchymatous cells	30-40-50
Distance b/w two medullary rays	61-59-92
Phloem region	65-64-84
Xylem region	73-97-119
Pith region	38-64-95

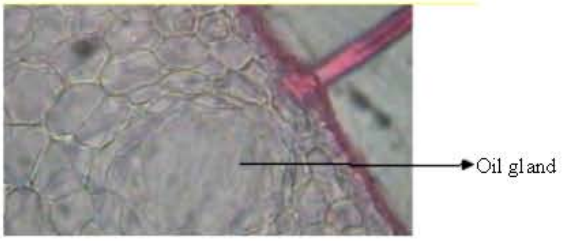

Fig. 4: Transverse section showing oil gland in cortex region in young stem at x 450

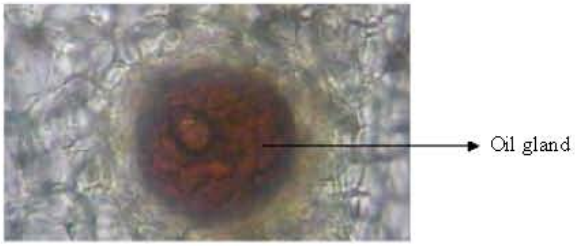

Fig. 5: Transverse section showing oil filled gland in cortex region in young stem at x 450

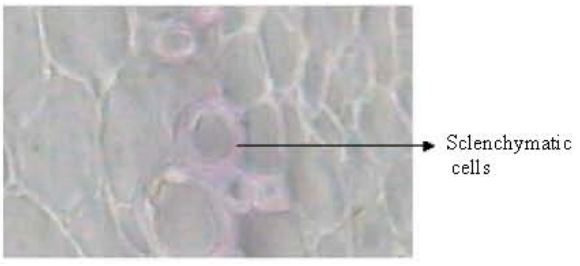

Fig. 6: Transverse section showing sclerenchymatous cells in young stem at x 450

cylinder of phloem outward along with bi or triseriate medullary rays. Vascular bundles are of collateral, conjoint and open type (Fig. 7, 8). The total region of xylem and phloem is of 73-97-119 and 38-64-95 µm, respectively. The distance between two medullary rays is 61-59-92 µm (Table 1).

Fig. 7: Transverse section showing medullary rays, xylem vessels and xylem parenchyma in young stem at x 450

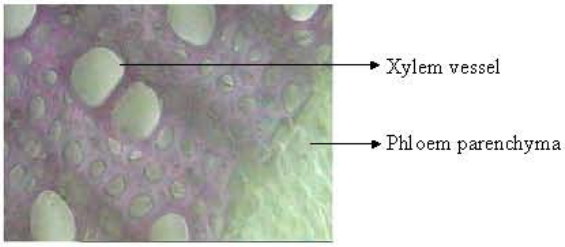

Fig. 8: Transverse section showing xylem and phloem parenchyma cells in young stem at x 450

Pith: Pith consists of thin walled polygonal, parenchymatous cells bearing starch grains (Fig. 9, 10) of 6.89-13.69 µm (Table 1). The total region of pith is of 38-64-95 µm (Table 1).

At the secondary stage the primary cambium (in between the xylem and phloem) produces secondary vascular tissues and the xylem parenchyma soon becomes sclerenchymatous (Fig. 11). For the better protection of the stem at the secondary stage, the cork-cambium originates in the outer side i.e., in the epidermis itself and produces cork cells on the outer side (total cork region is of 64-75-98 µm (Table 1) and a layer of phelloderm (secondary cortex) (Fig. 11) on the inner side. At secondary stage medullary rays become fully developed i.e., penetrates inside the xylem region (Fig. 12). Oil glands remain persistent during secondary growth (Fig. 13). A region of unmodified cells is also observed just below the xylem (Fig. 14).

Powder microscopy: The analysis of powder showed the presence of covering trichome, lignified fibres, starch grains, cork cells, sclerenchymatic cells, biseriate medullary rays, lignified xylem vessles (pitted, reticulate). When treated with iodine, starch grains were also observed in the powder.

The results of micrometry and fluorescence analysis are presented in Table 1 and 2, respectively. The total ash, water soluble, acid insoluble and sulphated ash values of the plant are shown in Table 3. Crude fibre content and

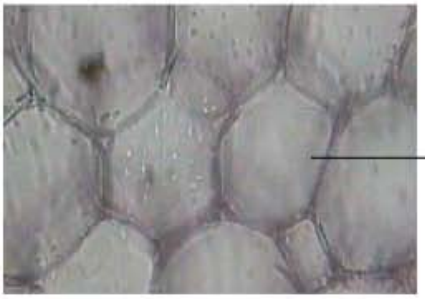

Fig. 9: Transverse section showing pith region in young stem at x 450

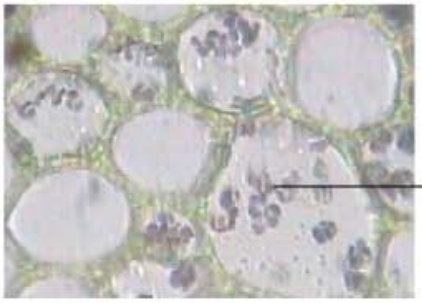

Fig. 10: Transverse section showing starch grains in pith region in young stem at x 450

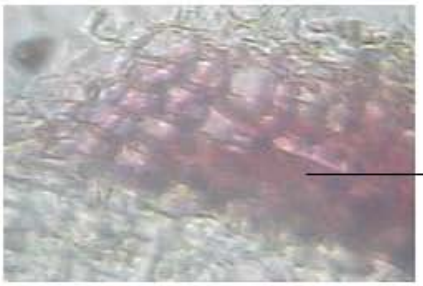

Fig. 11: Transverse section showing bark region in mature stem at x 450

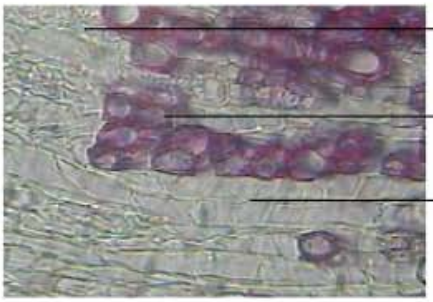

Fig. 12: Transverse section showing developed Medullary rays in xylem region in mature stem at x 450

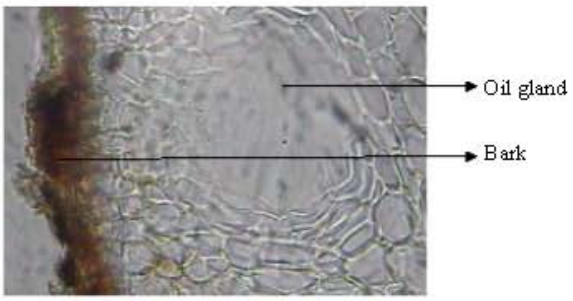

Fig. 13: Transverse section showing bark along with oil gland in mature stem at x 450

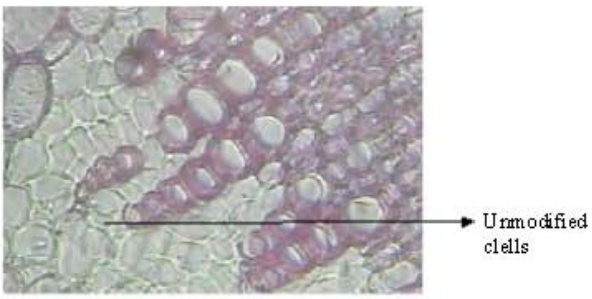

Fig. 14: Transverse section showing unmodified cells below xylem region in mature stem at x 450

Table 2: Results of Fluorescence analysis of powder

Treatments	Visible (400 to 800 nm)	U.V. Short (254 nm)	U.V. Long (365 nm)
As such	Yellow	Yellow	Brownish yellow
Methanol	Yellow	Yellow	Brownish yellow
1 N NaOH	Greenish yellow	Yellow	Dark yellow
Methanol + NaOH (1:1)	Yellow	Light yellow	Dark yellow
Ethanol	Yellow	Brownish yellow	Brownish yellow
Conc. HCl	Brownish yellow	Light yellow	Brown > yellow
H_2SO_4(66%)	Yellow	Cremish yellow	Mud color
Conc. H_2SO_4	Black	Black	Black
Nitric acid	Dark brown	Yellow	Brown

Table 3: Determination of ash values (% w/w)

Particulars	Ash values (% w/w)
Total ash	4.86
Water soluble ash	0.24
Acid insoluble ash	0.37
Sulphated ash	3.83

moisture content of dried powdered stems were determined and found to be, 54.25 and 0.36 (% w/w), respectively. Extractive values (% w/w) of dried powdered stems were determined and comparatively water soluble extractives were found to be more in this species (Table 4).

As there is no pharmacognostic anatomical study on record of this drug which is of great value, present study was taken up with a view to lay down the microscopic standards, which could be used in deciding the

Table 4: Determination of extractive values (% w/w)

Solvents	Extractive values (% w/w)
Pet. Ether	0.26
Chloroform	0.45
Acetone	0.27
Alcohol	3.04
Aqueous	3.87

genuineness of the drug source. The drug was found to contain trichomes and trichomes are epidermal outgrowths of considerable value for taxonomic purposes. These outgrowths play a role in plant defense especially with regard to phytophagous insects, avoiding insect feeding and oviposition responses and the nutrition of larvae. They may be involved in the regulation of temperature and water repellency as well (Duartea and Deburb, 2005). Schizolysigenous oil glands were found below the epidermis and the volatile oils are stored in these glands. Oils filled glands were also found in this study.

The sclerenchymatic ring in the cortex has been described in this study. The sclerenchymatic cell concentration is effective in withstanding environmental pressures, such as damage by wind and to fend off herbivores (Metcalfe and Chalk, 1988). Pith usually consists of parenchyma and serve to store starch or secrete crystals and other ergastic substances (Dutta, 2001). The transverse section of pith region shows the presence of starch grains in *M. koenigii*. These above mentioned arrangements along with bi or triseriate medullary rays would be crucial in the identification of this plant. Ash values, extractive values and fluorescence analysis are few parameters, which normally are adopted to get the qualitative information about the purity and standard of the crude drug. The macro and the micro morphological standards discussed can be considered as a distinguishing parameter to identify and decide the authenticity of this drug and thus can be included as microscopic standards in Pharmacopoeias.

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