Pharmacognostic Evaluation of Leaves and Stem of *Murraya koenigii*

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Abstract

*Murraya koenigii* has been widely used as medicine in indigenous system of medicine. It is used as an analgesic, febrifuge, stomachic, carminative and for the treatment of dysentery skin eruption. The present study was planned for details pharmacognostical studies of *Murraya koenigii* leaves and stem. Macroscopical characters and microscopical characters of leaves and stem were perceived. The Physicochemical properties (total ash, acid insoluble ash, water soluble ash, water soluble extractive, methanol soluble extractive, moisture content and foaming index) of bark powder were studied. The values of physicochemical can also be used for standardization of *Murraya koenigii*. These studies provided referential information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario of lack of regulatory laws to control quality of herbal drugs.

1 Introduction

Vast ethnobotanical knowledge exists in India from ancient time. The dictionary of Indian folk-medicine and ethno botany includes 2532 plants. India has about 45,000 plant species and many of them have been studied for their medicinal properties. About 2000 figures are available in the literature and commonly 500 species are used by indigenous systems. Even though early (4500-1500 BC) origins and a long history of usage in the last two centuries, the Ayurveda had received very little official support and hence less attentions were noticed from good medical practitioners and researchers. A large extent of work is now being done on the Botany, Pharmacognosy, Biotechnology, Chemistry and Pharmacology of herbal medicines. The importance of ethnomedicine has been realized and work is being done on psycho energetic plants, domestic remedies and plants sold by street drug vendors.

According to World Health Organization, medicinal plants are the best source to obtain a variety of newer herbal drugs. The status of herbal medicine has been fast growing all over the world during the last few decades. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. During the twentieth century, when exploring the natural environment, man has made great discoveries that have enabled him to use a considerable number of natural resources.

Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken.

*Murraya koenigii*, belongs to the family Rutaceae, commonly known as curry-leaf tree, is a native of India, Sri Lanka and other south Asian countries. It is found almost everywhere in the Indian subcontinent, it shares aromatic nature, more or less deciduous shrub or tree up to 6 m in height and 15-40 cm in diameter with short trunk, thin smooth grey or brown bark and dense shady crown. A number of chemical constituents from every part of the plant have been extracted. The most important chemical constituents responsible for its intense characteristic aroma are P-gurjunene, P-caryophyllene, P-elemene and O-phellandrene. The plant is rich source of carbazole alkaloids. Bioactive mahanine, koeine, koenigine, koenidine, girinimbol, girinimbine, koinimbine, O-methyl murrayamine A, O-methyl mahanine, isomahanine, bismahamine, bispyrayafoline, coumarins, acridine alkaloids and carbazole alkaloids are reported to be present in *Murraya koenigii*. 
Traditionally, it is used as an analgesic, febrifuge, stomachic, carminative and for the treatment of dysentery skin eruption\(^6\). It is reported to possess antioxidant, antibacterial, antifungal, larvicidal, anticarcinogenic, hypoglycemic, anti-lipid peroxidative, hypolipidemic and antihypertensive activity\(^7\). Hence, we planned to conduct a study on pharmacognostical screening of *Murraya koenigii* leaves and stem to supplement useful data in regard to its correct identity of this plant, as this plant is broadly used in indigenous system of medicine.

2 Material and Methods

2.1 Plant material

The fresh leaves and stem of *M. koenigii* were collected from Rajiv Academy for Pharmacy, Mathura, U P. India in the month of Feb 2014 and were identified by, Mr. Bhuwnandra Singh, Assistant Professor of Rajiv Academy for Pharmacy, Mathura.

2.2 Processing of Plant material

Fresh leaves were collected and dried under shade for 15 days, and were powered using mechanical grinder. This powered material is used for further analysis. The plant was morphologically examined for shape of leaves, apex, base, margin etc. Separate sections of leaves and stem were prepared and examined for the identification of starch grains by staining with iodine solution. Powder of the dried leaves was used for microscopic 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 as a part of quantitative microscopy. The total ash value, water and alcohol soluble ash value and water soluble extractive values were determined.

2.3 Pharmacognostical evaluation

2.3.1 Morphological studies

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

2.3.2 Microscopic studies

Care was taken to select healthy plants and for normal organs. The leaves and stem were cut and removed from the plant and fixed in 5 ml of Formalin, 5 ml of Acetic acid, and 90 ml of 70% v/v Ethyl alcohol (FAA). After 24 hours of fixing the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the method. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks\(^8\)\(^9\)\(^10\).

2.3.3 Sectioning

The paraffin embedded specimens were section with the help of rotary microtome. The thickness of the sections was 10-12 micrometers. Dewaxing of the sections was by customary procedure.\(^6\) The sections were stained with toluidine blue as per the method published. Since toluidine blue is a polychromatic stain, the staining results were remarkably good, and some cytotochemical reactions were also obtained. The rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies. Wherever necessary sections were also stained with safranin and fast green and iodine (for starch)\(^11\).

2.3.4 Photomicrograph

Microscopic description of tissues or supplemented with micrographs were necessary photographs of different magnifications were taken with Nikon Labphot 2 microscopic unit. For normal observations bright field was used for the study of crystals, grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark backgrounds. Magnifications of the figures are indicated by the scale bars\(^12\).

2.4 Physicochemical evaluation

The dried bark of *M. koenigii* was subjected to standard procedures according to WHO Guidelines for the determination of various physicochemical parameters. The following parameters were determined:

2.4.1 Determination of Ash values

Ash values are helpful in determining the quality and purity of a crude drug, especially in the powdered form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination.

2.4.1.1 Total ash value

Accurately about 3 g of air-dried powder of bark of *M. koenigii* was weighed in a tared silica crucible and incinerated at a temperature not exceeding 450 °C until free from carbon, cooled and weighed and then the percentage of total ash with reference to the air-dried powdered drug was calculated.

2.4.1.2 Acid insoluble ash

The ash obtained in the above method was boiled with 25 ml of dilute HCl for 5 minutes. The residue was collected on ash less filter paper and washed with hot water, ignited, cooled and weighed. The percentage of acid insoluble ash with reference to the air-dried drug was calculated.

2.4.1.3 Water soluble ash

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The total ash obtained was boiled with 25 ml of distilled water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at allow temperature. 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 with reference to the air-dried drug was calculated.

2.4.2 Moisture content

The powdered drug sample (3 g) was placed on petridish and dried at 105 °C for 4-5 hand weighed. The drying was continued until two successive readings matched each other or the difference between two successive weighing was not more than 0.25% of constant weight.

2.4.3 Foreign matter

A 100 g of the plant material was spread in a thin layer and the foreign matter was sorted into groups by visual inspection and using a hand lens. The remainder of the sample was sifted through a no.250 sieve; dust was regarded as mineral admixture. The sorted foreign matter was weighed. The content of each group was calculated in grams per100 g of air dried sample.

2.4.4 Foaming index

About 1 g of the powdered sample was transferred to a conical flask containing 100 ml of boiling water. Decoction was prepared and filtered. The decoction was poured into 10 stoppered test tubes (height 16 cm, diameter 16 mm) in successive portions of 1 ml, 2 ml, 3 ml up to 10 ml and the volume of liquid in each test tube was adjusted to10 ml with water. The test tubes were stoppered and shaken in a lengthwise motion for 15 seconds. The test tubes were allowed to stand for 15 minutes and the height of the foam was measured.

2.4.5 Determination of extractive values

Determination of extractive values is useful for evaluation of crude drug. It give idea about the nature of the chemical constituents present in a crude drug.

2.4.5.1 Methanol soluble extractive value

Macerated 5 gm accurately weighed coarse powdered drug with 100 ml of methanol in a stoppered flask for 24 h, shaking frequently during first 6 h. Filtered rapidly through filter paper taking precaution against excessive loss of methanol. Evaporated 25 ml of alcoholic extract to dryness in a tared dish and weighed it. Calculated the percentage w/w of methanol soluble extractive with reference to the air-dried drug using following formula.

\[
\% \text{ Methanol soluble extractive value} = 80 \times (\text{Wt. of residue})
\]

2.4.5.2 Water soluble extractive value

The procedure as above was followed using chloroform water I.P. instead of methanol.1314.

3 Results and Discussions

3.1 Macroscopic examinations

3.1.1 Macroscopic examinations of stem

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 aromatic odor and characteristic taste. The outer surface is smooth, soft and glabrous.

The mature stem of Murraya koenigii are dark brown (unpeeled) and Cremish brown (peeled) in color and characteristic taste. The outer surface is smooth and hard. The fracture of bark is splintery.

3.1.2 Macroscopic examinations of leaves

The leaves are imparipinnate with obliquely ovate or rhomboid shape. It has acuminate apex with irregularly crenate or dentate margin. The petioles were of 20 to 30 cm in length.

3.2 Microscopic examinations

3.2.1 Microscopic examinations of stem

The stem of Murraya koenigii has a circular transaction and shows following features (Fig 1)

It is single layered, parenchymatous, uniseriate, unicellular, tangentially elongated surrounded by thick cuticle. While epidermis exhibited 5-6 unicellular, uniseriate, covering trichomes.

Just below the epidermis, there are 6-10 schizolysigenous oil glands were observed (Fig 2)

The unicellular trichomes (Fig 3) and medullary rays (Fig 4) were seen.

Continuous strands of 4-6 layers of compactly arranged parenchymatous, polygonal cells constitute the cortex region (Fig. 1). The cortex region shows the presence of lignified sclerenchymatic cells. The vascular system consist of a cylinder of xylem produced towards the inside and a cylinder of phloem outward along with bi or triseriate medullary rays. Pith consists of walled polygonal, parenchymatous cells bearing starch grains (Fig. 5).

3.2.1 Microscopic examinations of leaves

The unicellular trichomes are present in upper epidermis and various cells are seen in vascular bundle (Fig 6).
3.3 Physicochemical parameters

*Murraya koenigii* barks powder was preliminary evaluated by determining physical constants like total ash, water soluble ash, acid insoluble ash, methanol and water soluble extractive, foreign matter content and foaming index (Table 1). The total ash, acid insoluble ash and water soluble ash of *Murraya koenigii* barks were found to be 11.33% w/w, 5.33% w/w and 1.97% w/w (Table 1). The extractive values were determined to find out the amount of soluble compounds. The methanol and water soluble extractive values of barks of *Murraya koenigii* were found to be 7.75% w/w and 9.56% w/w. The leaves exhibited more amount of water soluble component compared to methanol extracts (Table 1). The foreign matter content of barks was found to be 0.5% w/w (Table 1). The foaming index of barks was found to be 111.1 (Table 1).

<table>
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<th>Parameters</th>
<th>Values</th>
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<td>Loss of drying</td>
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<tr>
<td>Total Ash</td>
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<tr>
<td>Acid insoluble ash</td>
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<td>Foreign matter</td>
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<tr>
<td>Foaming index</td>
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4 Conclusions

The present study on pharmacognostical investigation of *Murraya koenigii* leaves and stem will be providing useful information in regard to its correct identity and help to differentiate from the closely related other species of *Murraya*. 

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5 Competing interests
No conflicts of interest are evident.

6 Author’s contributions
VCP carried out experimental part. BS and AA participated in literature review, draft the manuscript and plant collections. All authors read and approved the final manuscript.

7 References