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Original Research Paper

PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDIES ON THE LEAVES OF *MURRAYA KOENIGII* (L) SPRENG

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ABSTRACT

The leaves of *Murraya koenigii* (L) Spreng (*Rutaceae*) are reported to have great medicinal value such as antibacterial, anti-inflammatory, antifeedant etc. Pharmacognostic evaluation including examinations of morphological and microscopic characters, determination of leaf content, ash value, powder analysis and extractive values were carried out. Phytochemical screenings including qualitative chemical examinations were performed. The leaf had reticulate venation and dentate margin with asymmetrical base. The stomata were distributed on both the sides. Phytochemicals such as carbohydrates, alkaloids, sterols, tannins, volatile oils, saponins, anthroquinone glycosides and flavanoids are reported. Phytoconstituents in various extracts gives us clue for further investigation.

Keywords: *Murraya koenigii*, Pharmacognostic, Physiochemical Studies, Phytoconstituents, Quantitative Leaf Microscopy.

INTRODUCTION

The plants belonging to *Rutaceae* are herbs, shrubs and trees with glandular punctuate, commonly strongly smelling herbage comprising about 150 genera and 1,500 species that are further characterized by the common occurrence of spines and winged petioles. Shrubs or trees are up to 4 m tall and is found in evergreen areas and in moist forest. Leaves of *Murraya koenigii* (L) Spreng (Mitha neem) are commonly used as flavoring agent in Indian curry preparation since ancient times. The Indian variety

Murraya koenigii (L) Spreng and Chinese variety *M. paniculata* are the two species available and both have some common medicinal properties. The leaves are imparipinnate with obliquely ovate or rhomboid shape. It has acuminate apex with irregularly crenate or dentate margin. Phytochemically leaves found to contain alkaloid[1,2] and volatile oil.[3] Leaves are said to possess anti-inflammatory, antifeedant activity.[4] The medicinal value of the leaf has been reported as antibacterial.[5] Phytoconstituents such as Glycozoline[6], Xanthotoxin[7], Sesquiterpine and volatile oils [8] are reported. The leaves are also used as antidysentric, externally cures eruptions, anti vomiting, tonic and stomachic purposes. The effects of leaves on blood glucose and plasma insulin levels in alloxan induced diabetic rats have been detected.[9] The fresh curry leaves have chlorophyll, terpenes and antioxidants which may also contribute in the hypoglycemic effect and increased insulin secretion. The use of fresh curry leaves would have been considered instead of dry powder. Inspite of its various medicinal uses no systematic studies on pharmacognostic activity have been reported.

Fig 1: Morphology of *Murraya koenigii* plant (**A**) along with adaxial and abaxial surfaces of the leaf (**B**).

Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all 'plant parts to be potential sources of medicinal substances.[10] There is a need for documentation of research work carried out on traditional medicines.[11] With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used asmedicine. The process of standardization can be achieved by stepwise pharmacognostic studies.[12] These studies help in identification and authentication of the plant material. Correct

identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics.[13]

However, available literature revealed that no pharmacognostic study has been carried out on the leaves of *Murraya koenigii*; hence the present investigation was undertaken. The objective of the present study is to evaluate various pharmacognostic standards like macroscopy and microscopy of fruit; ash values, extractive values, microscopical characteristics of powdered fruit and preliminary phytochemical analysis of the leaves. Since the plant, *Murraya koenigii*, is useful in traditional medicine for the treatment of several ailments, it is important to standardize it for use as a drug.

MATERIALS AND METHODS

Plant collection

The leaves of *Murraya koenigii* were collected in summer season locally from Bhadra Wild Life Sanctuary, Karnataka (Southern India) in May 2010. The taxonomic identification of the plant was confirmed by Dr. Prashant Kumar Jha, Department of Botany, ALN Rao Memorial Medical College, Koppa, (Voucher specimen number HPS121). 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. A separate section was prepared and examined for the identification of starch grains by staining with iodine solution. Powder (# 60) of the dried leaf 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[14] as a part of quantitative microscopy. Stomata number, stomatal index, vein islet and veinlet termination number were determined by using fresh leaves of the plant[15] Total ash, water and alcohol soluble ash, water soluble extractive values were determined[16]

Leaf Macroscopy

Morphological characters of plant like shape of leaves, apex, base, margin etc were examined properly. The following macroscopic characters for the fresh leaves were noted: size and shape, color, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odor and taste.[17, 18]

Leaf Microscopy

The outer epidermal membranous layer (in fragments) were cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope. The presence/absence of the following was observed: epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution), epidermis (upper and lower), hypodermis, spongy parenchyma, stomata number, stomatal index, vein islet and veinlet termination number were determined by using fresh leaves of the plant.[15] Xylem elements and ground tissue were also observed under microscope. The transverse sections of the fresh leaves through the lamina and the midrib were also cleared, mounted and observed.[19]

Quantitative leaf microscopy

Quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, vein – islet number and veinlet termination number were carried out on

epidermal strips. Powered leaves were used to determine the rest physicochemical characters like moisture content, total ash, acid – insoluble ash, water – soluble ash, alcohol and water-soluble extractive values.[20]

Phytochemical studies

The leaf powder was subjected to determine volatile oil content. Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as carbohydrates, alkaloids, phytosterols, glycosides, saponins, flavonoids, proteins, tannins and gum.[21,22]

Fluorescence analysis

To check the fluorescent property of *Murraya koenigii* (L.) Spreng, powdered leaf material obtained whish is used for to analysis under ultra violet light. Alcohol, 50% sulphuric acid, 10% sodium hydroxide, 50% Nitric acid are the various chemical and organic reagents used to perform fluorescence analysis.[16]

RESULTS AND DISCUSSION

Macroscopy and Microscopy

The macroscopical studies revealed the shape of leaves of *Murraya koenigii* (L.) Spreng as obliquely ovate or somewhat rhomboid with acuminate obtuse or acute apex, bipinnately compound with exstipulate in alternate arrangement. The petioles were of 20 to 30 cm in length. The leaf had reticulate venation and dentate margin with asymmetrical base (Fig. 1). Under the compound microscope, the stomata were found distributed on abaxial surface while the adaxial surface was without stomata. The type of stomata was noted as anomocytic one. The Uniseriate multicellular trichomes were observed on both surfaces, more frequent on upper surface of midrib portion. The wall of trichome was found ridged. The transverse section of leaf exposed a layer of epidermis composed of rectangular cells as outermost covering on both upper and lower layer. The upper epidermis was enveloped with deposition of cuticle. In midrib portion, epidermis was followed by 1-4 layers of collenchymatous hypodermis in continuation with 2-5 layers of chlorenchyma cells filled with cholorophyll contents. Beneath this, ground tisuue portion lies. This portion is composed of oval to polygonal parenchyma cells and is traversed with vascular bundle. Sandy and prismatic crystals of calcium oxalate were found in this region.

Towards the vascular bundles, bundle of fibres are present on upper side. Xylem and phloem portion of vascular bundle consist of their basic elements (Fig 2). In lamina portion, upper epidermis was followed by 1-2 layers of palisade parenchyma cells continued with spongy parenchyma cells with intercellular spaces (Fig. 3). The whole mesophyll portion is filled with cholorophyll contents. Spiral type of vessels was seen across the mesophyll portion (Fig. 4).

Physico and Phytochemical studies

The quantitative microscopic studies revealed the values like palisade ratio, stomata number, stomata index, vein-islet number and veinlet termination number as displayed in table 1.

The physico-chemical analysis of powder exposed the moisture content (loss on drying), total ash, acid insoluble ash, water soluble, alcohol soluble extractives and water soluble extractives are as shown in table 2. The higher percentage of total ash and acid insoluble ash may be due to presence of calcium oxalate crystals or other inorganic salts present in various metabolites. The preliminary screening of presence or absent of primary and secondary metabolites was revealed by qualitative tests (Table 3) as done according to Brain and Turner (1975). The fluorescence analysis of powdered leaf material was subjected to analyse under Long Ultra Violet light after treatment with various chemical

and organic reagents like alcohol, 50% sulphuric acid, 10% sodium hydroxide, 50% nitric acid and water.[16] The florescence behavior was noted as in table 4.

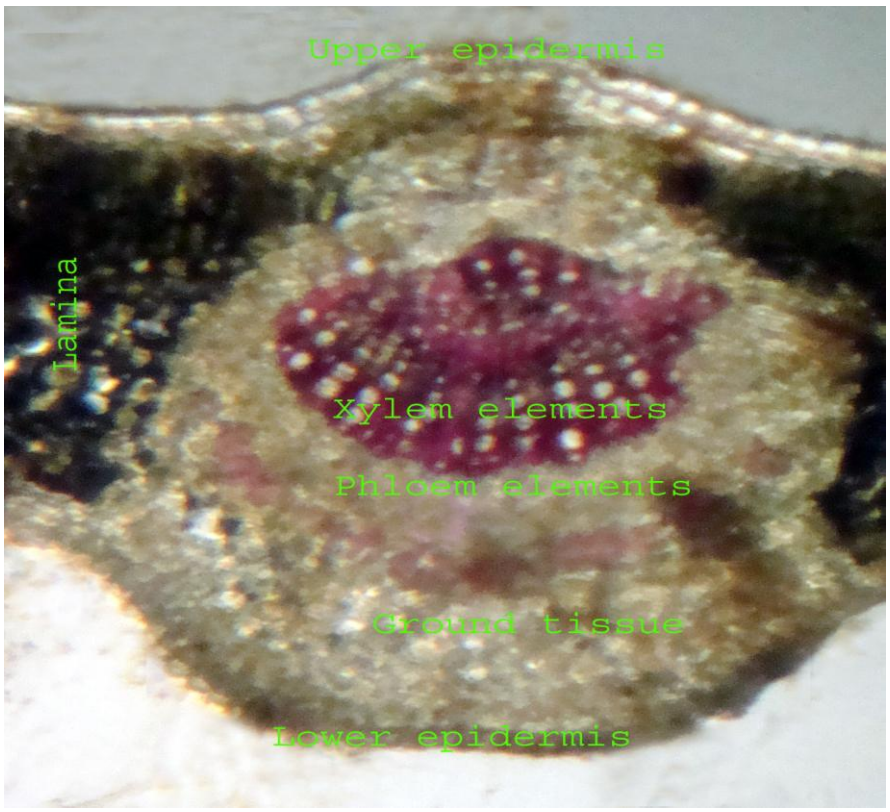

Fig 2: Microscopy of *Murraya koenigii* leaf showing outline of TS across midrib stained with phloroglucinol and HCl.

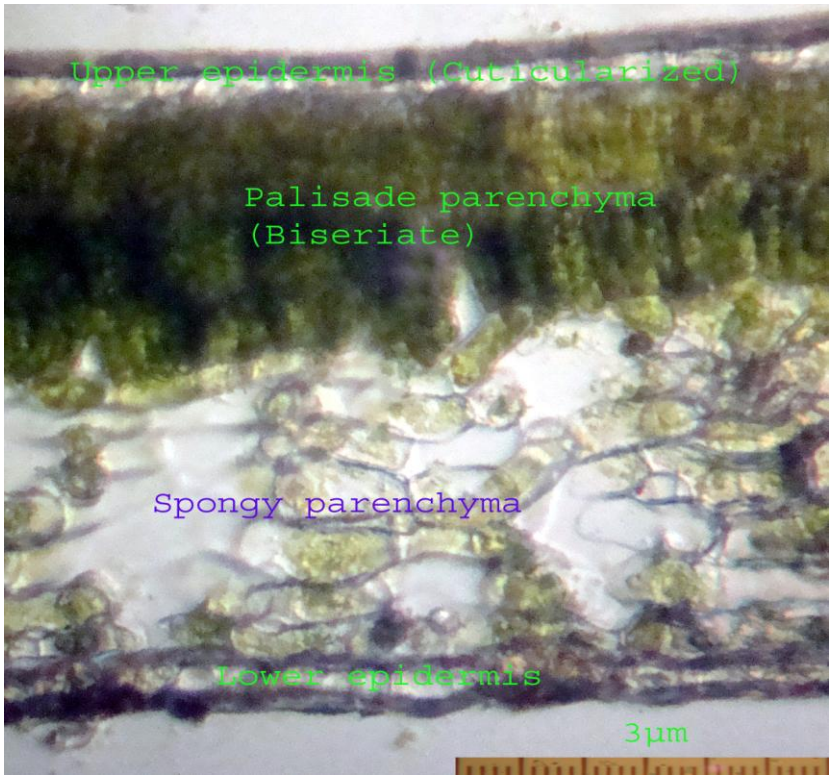

Fig 3: Microscopy of *Murraya koenigii* leaf showing outline of TS across lamina.

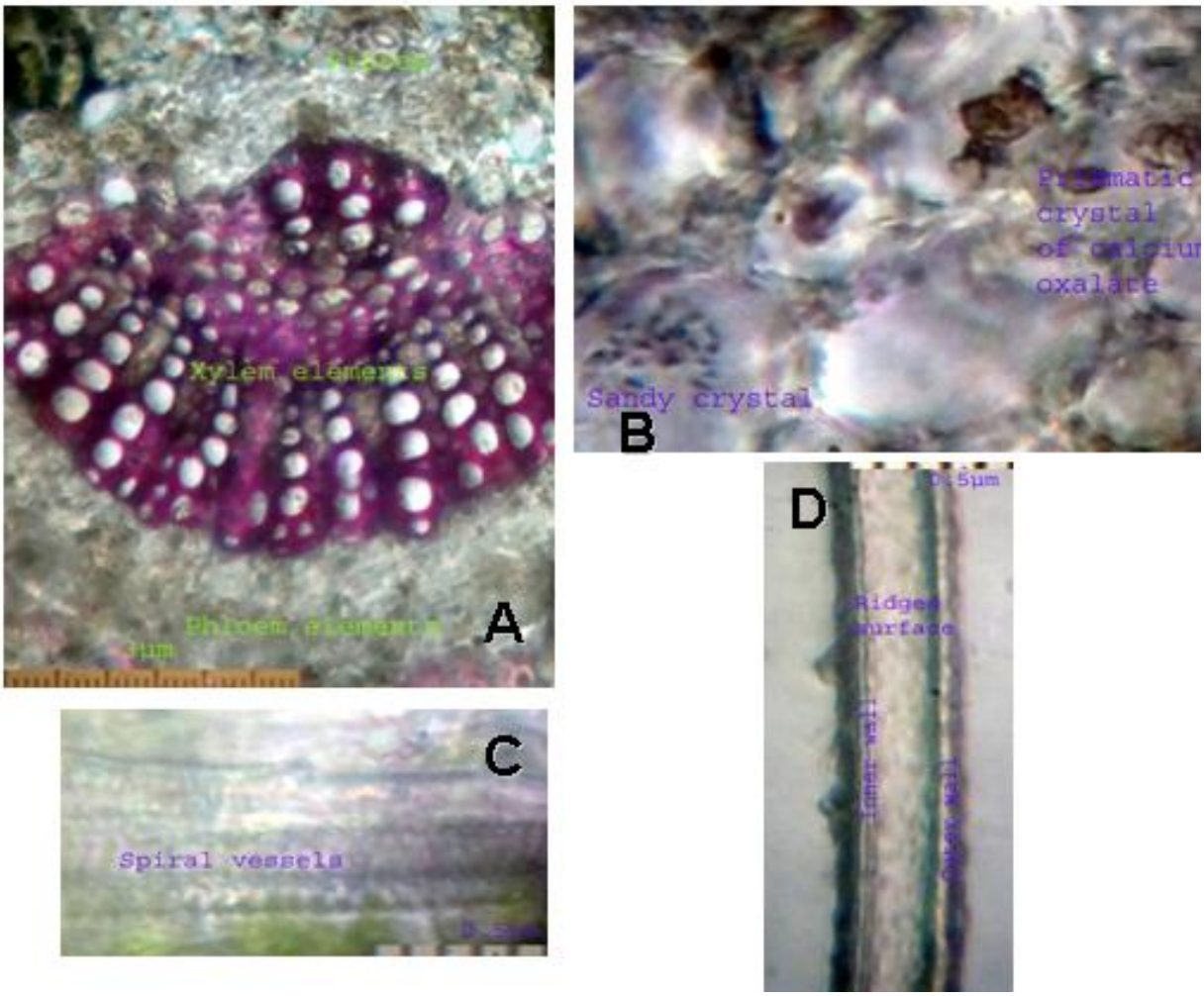

Fig 4: Microscopy of leaf showing - Vascular bundle and fibres (A); Sandy and prismatic crystal of calcium oxalate (B); Spiral vessel of lamina portion (**C**) and Trichome showing ridged wall (D).

Table 1: Quantative leaf microscopy of *Murraya koenigii.*

Parameter	**Range**	**Mean***
Palisade Ratio	11-14	12.85 ± 0.35
Stomatal Number Upper surface	0	0
Stomatal Number Lower surface	67-82	66.31 ± 6.81
Stomatal Index Upper surface	0	0
Stomatal Index Lower surface	13.47-15.42	14.68 ± 0.22
Vein islet number	12-15	13.64 ± 0.42
Veinlet Termination Number	9-12	13.62 ± 0.29

* Mean value of 10 counts

Table 2: Physio-chemical parameters of *Murraya koenigii*.

Parameters	**Values obtained on dry weight basis(w/w)**
Loss of drying	10.19%
Total Ash	11.33%
Acid insoluble ash	5.33%
Water soluble ash	1.97%
Methanol soluble extractives	7.75%
Water soluble extractives	9.56%

Table3: Phytochemical examination of leaves of *Murraya koenigii.*

Qualitative Tests	**Results**
Carbohydrates	++++
Protein	+
Phytosterols	+
Tannin	+
Gum/mucilage	-
Flavonoids	++
Volatile oil	+
Anthroquinone Glycoside	++
Alkaloid	++++
Saponins	++

Table 4: Fluorescence behavior of leaves of *Murraya koenigii*.

Treatment	**Day light**	**UV light**
Powder as such	Pale Green	Fluorescent pale green
Powder in distilled water	Bluish-Green	Fluorescent Bluish-Green
Powder in absolute alcohol	Olive-green	Fluorescent Orange
Powder in 10% NaoH	Light brown	Fluorescent Dark brown
Powder in 50% HNO3	Yellow	Fluorescent Black
Powder in 50% H2So4	Dark green	Fluorescent Yellowish-green

CONCLUSION

The presence of these phytochemicals make the plant useful for treating different ailments and have a potential of providing useful drugs of human use. The quantitative determination of pharmacognostic parameters will help for setting standards for crude drugs. The total ash is particularly important in evaluating the purity of drugs. The pharmacognostic constants for the leaves of this plant, the diagnostic microscopic features and the numerical standards reported in this work could be useful for the compilation of a suitable monograph for it proper identification. And further work aiming towards tracing out of phytochemicals present in it and pharmacological activities are in progress.

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