Pharmacognostical studies and antibacterial activity of the leaves of *Murraya koenigii*

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ABSTRACT

The present study deals with the macro and microscopical studies as well as antibacterial studies of *Murraya koenigii* Linn.leaf. Some distinct characters were observed while studying their transverse sections. Macroscopically, the leaf's shape was lanceolate measuring 4.9 cm long and 1.8 cm broad with a reticulate venation
Microscopically, the midrib and laminar region showed a distinct epidermis. The collenchyma was thick walled followed by loosely arranged parenchymatous cells containing oil and starch grains. Physiochemical and preliminary phytochemical studies of the leaf were also carried out. The antibacterial studies confirmed that the methanolic extract was quite effective for *S.typhi* and *E.coli* at 100 µg/ ml and 200 µg/ ml respectively. The present study might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in the present scenario lacking regulatory laws to control quality of herbal drugs and also to find out the antibacterial activity.

Keywords: antibacterial, leaf, macroscopical, methanolic extract, microscopical, *Murraya koenigii*.

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INTRODUCTION

Murraya koenigii (L) is an aromatic, more or less deciduous shrub or a small tree upto 6 mm in height found throughout India up to an altitude of 1,500 m[1]. The plant grows best in tropical and subtropical climates, in sunny to semi shaded locations. They are very frosts sensitive. It is traditionally used as as an antiemetic, antidiarrhoeal, ferifuge and blood purifirer[4,7]. It is used as a flavoring agent in Indian dishes (Curry). *M.koenigii* (Rutaceae) is commonly known as Methi neem.

No report is available on micro-morphological as well as antibacterial activity of this drug, hence the present study was undertaken to explore pharmacognostical investigation of *Murraya koenigii* leaf.

MATERIAL AND METHODS

The plant specimens for the study were collected from the botanical garden of N.I.E.T, Gr.Noida, UP, India. It was identified and authenticated by Dr.Anjula Pande, Taxonomist, Pusa (India). Care was taken to select healthy fully grown plant. The samples collected were dried under the sunlight.

Macroscopy

The leaves were examined for size, shape, color, odour, taste, venation, etc.

Micoscopy

The outer epidermal membrane layer was cleared in chloral hydrate and warmed for half an hour. It was then stained with phloroglucinol and conc.HCl and mounted with glycerine and observed under a compound microscope[6]. The presence/ absence of the following were observed: Epidermal cell, Stomata (Types and distribution) and epidermal hair (types of trichomes and distribution). The transverse sections of the fresh leaves through the lamina and midrib as well as small quantity of powdered leaves were also clearly mounted and observed.

Photomicrograph

Microscopic descriptions of selected tissues were photographed using Radical RXLR-3T 28378

Phytochemical Examination

Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites.[2,5]

Quantitative Investigation

Quantitative leaf microscopy to determine Palisade ratio, Stomata number, Stomata index, Vein islet number and vein termination number were carried out on epidermal strips.

Physical Evaluation

The powdered leaves were determined for Total ash, acid-insoluble, water soluble ash value, sulphated ash value and water soluble and alcohol soluble extractive value.[10]

Fluorescence study

It is an essential parameter for first line standardization of crude drug. The crude drug was subjected to this study and its fluorescence pattern was noted. The powdered material was treated separately with different reagents and exposed to visible, UV light (Short and long) to study their fluorescence behaviour.[9]

Antibacterial Activity[2,8]

Preparation of Methanolic Extract

Hundred grams of the dried leaves of *Murraya koenigii* were boiled in a soxhlet with 300 ml of methanol for 24 h.The entire extract of *Murraya koenigii* leaves was evaporated to dryness in a rotary evaporator.

Microorganisms

Test micro-organisms (*S.aureus, E.coli & S.typhi*) were obtained from the laboratory stock culture. The test micro-organisms were cultured on nutrient agar slants at 37°C for 18 h. The stock cultures were maintained on nutrient agar slants at 4°C.

Antibacterial Activity

The antibacterial activity of the crude extract was determined following the method described in IP, 1996. (Table 1)

RESULTS AND DISCUSSION:

Macroscopy

The following characteristics of fresh leaves were noted:

Colour : Green
Odour : Characteristic
Taste : Characteristic
Shape : Lanceolate
Size : 4.9cm long, 1.8 cm broad
Extra feature : 24 leaflet having reticulate venation

Microscopy

The T.S.of leaf of *M.Koenigii* showed the following structures:

Table 1.Antibacterial Activity

S.No.	Standards (µg/ ml)	Test (µg/ ml)(Methanolic extracts of leaf)
1	10	5
2		10
3	20	50
4		100
5		200

CONTROL: Distilled water with 0.1% Tween 80

MIDRIB PORTION

Epidermis-Single layered, parenchymatous, uniseriate, unicellular covering with trichome

Collenchyma- Below epidermis is a compactly thick walled collenchymatous cells followed by loosely arranged parenchymatous cells containing oils and starch grains.

Vascular Bundle- It consists of a xylem vessels and parenchymatous phloem cells followed by a multilayered thick walled parenchymatous cells containing cellulose.

LAMINA

Upper Epidermis- Single layered polygonal straight cells covered with cuticle.

Mesophyll-Upper palisade cells were single layered, elongated, compactly arranged followed by spongy

Table 2. Phytochemical Investigations

S.No.	Secondary Metabolites	Results
1	Volatile oils	+
2	Carbohydrates	+
3	Glycoside	+ (Cardiac glycoside, Anthraquinone, coumarin glycoside
4	Proteins	+
5	Vitamins	+ (Vitamin A)

+ = the constituent is present

Table 3. Quantitative Investigation:

Parameters	Result
Stomatal number	Upper Epidermis-3 per sq mm Lower epidermis- 2 per sq mm
Stomatal index	Both surfaces-40-60
Palisade ratio	1:10
Vein islet number	18
Vein Termination Number	8

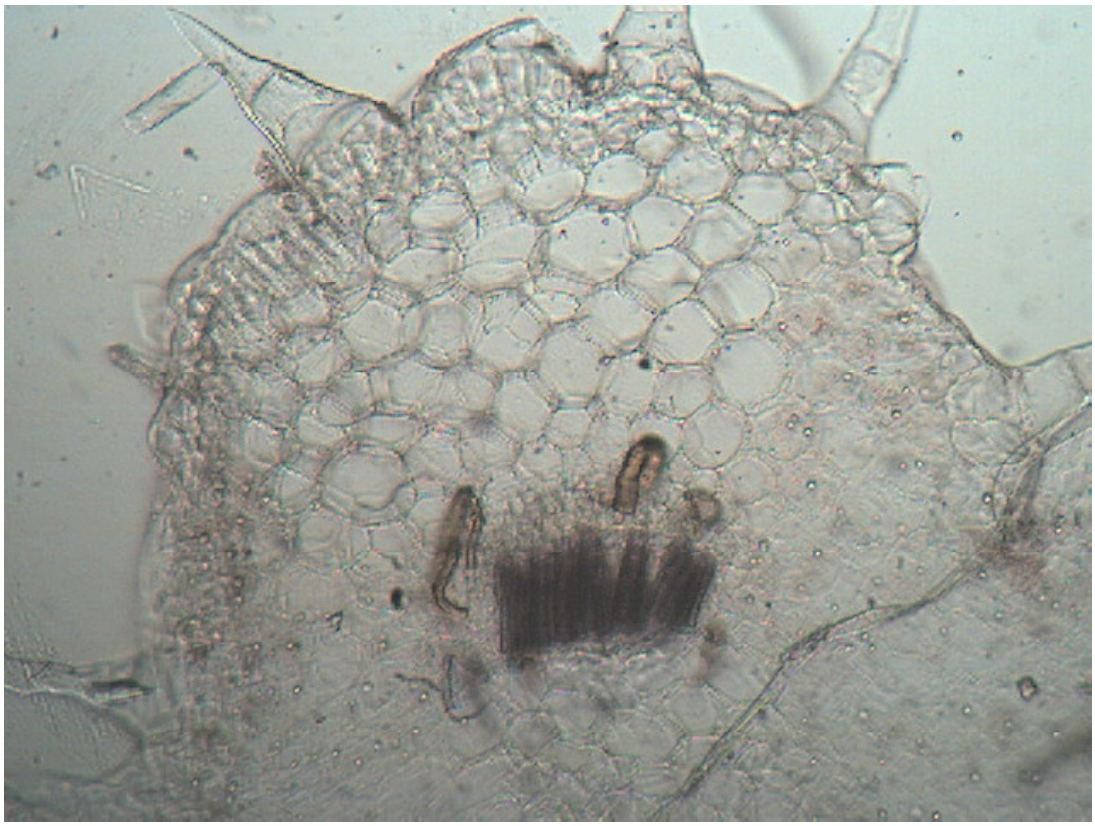

Figure 1. T.S.of leaf (Midrib Portion)

Figure 2. Vascular Bundles (Xylem Vessels)

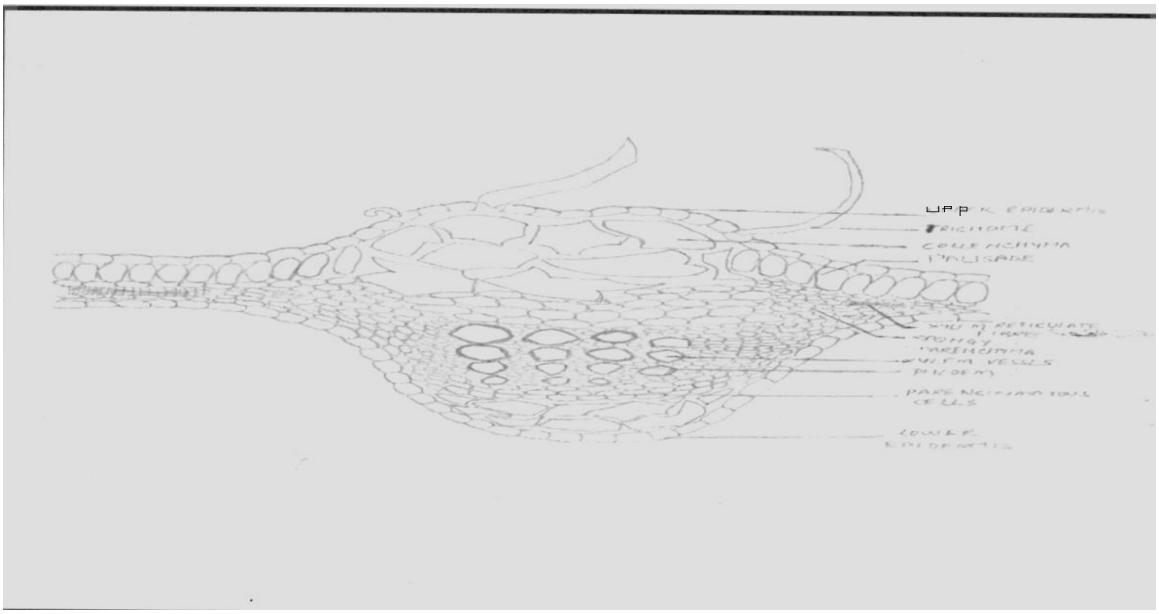

Figure 3. Complete T.S of Leaf (Both lamina and midrib portion)

parenchyma, thin walled, closely arranged intracellular spaces.In between these cells xylem reticulate fibres were seen.

Lower Epidermis- Single layered, polygonal, straight cells were found.

Phytochemical Investigations:

The following phytochemical constituents were seen as shown in Table 2

Quantitative investigation:

The results for Palisade ratio, Stomatal number, Stomatal index, Vein islet number and vein termination number

Table 4. Physical Evaluation:Ash Value

Parameters	Result
Total ash value	4.86% w/w
Acid insoluble ash value	0.37% w/w
Water soluble ash value	1.47% w/w
Sulphated ash value	9.5% w/w

Extractive Value

Parameters	Result
Alcohol soluble extractive value	3.04 w/w
Water soluble extractive value	0.26 w/w

Table 5. Fluorescence Analysis of Powder

Treatment	Visible (400–800 nm)	U.V.short (254 nm)	U.V.long (365 nm)
As such	Yellow	Yellow	Brownish yellow
Methanol	Yellow	Yellow	Brownish yellow
1N NaOH	Greenish yellow	Yellow	Dark yellow
Methanol + NaOH (1:1)	Yellow	Light Yellow	Dark yellow
Ethanol	Yellow	Black	Dark yellow
Conc.HCl	Brownish yellow	Light yellow	Brownish yellow
H_2SO_4 (66 %)	Yellow	Black	Black
Conc H_2SO_4	Black	Black	Black
Nitric acid	Dark brown	Yellow	Brown

Table 6. Results of Antibacterial Activity

Sample	Conc	*S.typhi*	*S.aureus*	*E.coli*
Standard	10 µg/ ml	3 mm	4 mm	6 mm
(Amoxicillin)	20 µg/ ml	4 mm	4 mm	8 mm
Test	5 µg/ ml	16 mm	20 mm	17 mm
(Methanolic	10 µg/ ml	19 mm		19 mm
Extract)	50 µg/ ml	21 mm		17 mm
	100 µg/ ml	17 mm		18 mm
	200 µg/ ml			

etc carried out on epidermal strips has been shown in Table 3

Physical Evaluation:

The following were the results as shown in Table 4

Fluorescence:

The powder showed the following results when exposed to different reagents and to visible and UV light.

Antibacterial Activity:

In the antibacterial activity screening the extract inhibited the growth of *S.typhi* and *E.coli* with a moderate zone of inhibition. (Table 6).The zone of inhibition of the methanolic extracts were the same (100 µg/ ml) against *S.typhi* and *E.coli*.

DISCUSSION:

Standardization of herbal drugs means a systemic approach to quality control. Establishment of the pharmacognostic profile of the leaves of *M.Koenigii* will assist in standardization which can guarantee quality, purity and identification of sample Different parameters like macroscopy, Microscopy, Phytochemical screening, microbial assay etc were done for standardization.

Significance of different parameters:

Stomatal no. is significant in the evaluation of a leaf drug and affected by factors like age of the plant and size of the leaf. Stomatal index is relatively constant and therefore, of diagnostic significance for a given species and also used for the differentiation of allied or closely related species of the same genus in air dried,as well as fresh conditions. Vein islet and vein termination number is also used as a characteristic for the identification of allied species. Palisade ratio furnishes an important data for leaf drug evaluation and can be successfully applied for the studies of several dicot leaves of medicinal property.

Ash value is helpful in determining the quantity and purity of a crude drug. Loss on drying determines the moisture content and fluorescence study is an essential parameter for first line standardization of crude drug.

Finally, antimicrobial activity can be determined by comparing zone of inhibition of standard solution and test solution (here, methanolic leaf extract).

CONCLUSION:

The pharmacognostic studies of the leaves of *Murraya koenigii* were studied successfully.

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