Drug Invention Today
ISSN: 0975-7619
Research Article
www.ditonline.info

Phytochemical and Pharmacognostical Studies on *Murraya koenigii* (L)spreng. Roots

Rakesh K Sindhu* and Sandeep Arora
Chitkara College of Pharmacy, Chitkara University, Chandigarh-Patiala NH-64, Rajpura, Patiala-140401, Punjab, India

The *Murraya koenigii* plant is widely used as herb, spice, condiments and also used to treat various types of ailments in Indian traditional system. Worlds about 80% population relies upon herbal products, because they have been considered as safe, effective and economical. The present study was aimed to set standard parameters for standardization of *Murraya koenigii* roots. In the present study microscopy of root and roots powder carried out, ash values, extractive values, fiber content, bitterness value, haemolytic activity, tannin content and roots powder fluorescence nature also observed using different chemical reagents. The alcoholic and aqueous extracts were screened for presence of amino acid and carbohydrates. The preliminary phytochemical screening of petroleum ether extract, ethyl acetate extract, chloroform extract, ethanol extract and aqueous extract was performed. The presence of alkaloids, flavonoids, carbohydrates, and sterol in various extracts were observed. This is first ever pharmacognostical study carried out on *Murraya koenigii* roots.

Keywords: *Murraya koenugii*, Pharmacognostical study, Roots powder, Extracts and Phytochemical Studies

INTRODUCTION

Murraya koenigii (L.) Spreng family Rutaceae, this family represents approximately 150 genera and 1600 species[1,2]. It is commonly known as curry patta in Hindi and is extensively used as a spice and condiment in India and other tropical countries[3].

This plant traditionally used for cooling, anthelmintic, analgesic, piles, allays heat of the body, reduces inflammation and itching. It is also useful in leucoderma and blood disorders. An infusion of the toasted leaves in used to stop vomiting[4]. The plant has been reported to possess. Anti-oxidative Property, Cytotoxic activity[5], Antimicrobial, antibacterial and other microbiological Activity[6,7]. Anti-diabetic and cholesterol reducing property[8,9.10], Antiulcer activity[11] positive inotropic effect[12].

Pharmacognostical evaluation is the initial step in the standardization of crude drugs. The detailed pharmacognostical studies give valuable information regarding the morphology, microscopical and physical characteristics of the crude drugs[13]. There are a number of crude drugs where the plant source has not yet been scientifically identified. Hence pharmacognostic study gives the scientific information regarding the purity and quality of the plant drugs [14]. This is firstever pharmacognostical investigations on *Murraya koenigii* roots as per WHO guideline and other standard parametrs.

MATERIALS AND METHODS

The plant of *Murraya koenigii* roots were collected during the month of the July 2009 from Jhansla, Patiala (Punjab), North India. The plant material was taxonomically identified and authenticated by Dr. H.B. Singh, Head, Raw materials Herbarium and Museum division, with ref. no. NISCAIR/RHMD/Consult/2010-11/1638/236. The voucher specimen has been deposited in the herbarium section of the Phytochemistry and Pharmacognosy Division, Chitkara College of pharmacy, Chitkara university, Panjab for further reference.

The root was dried under shade, sliced into small pieces, pulverised using a mechanical grinder and stored in an air tight container for further use.

Morphology: The crude drug was evaluated for organoleptic properties shape, size. colour, odour, taste, fracture and texture were noted [Plate no.1].

Plate no. 1(Morphology of root)

Microscopy of Root: Microscopy of plant material is performed to distinguish it from the allied drugs and adulterant. The dried root was soaked overnight in water to make it smooth enough for transverse section. Paraffin wax embedded specimens were sectioned using the rotatory microtom (Weswox Optik). The thickness of section was 10-12 µm. The very fine section was selectively subjected to staining reaction with staining reagent safranin (1%) and light green (0.2%). Slides were cleared in xylol and mounted in DPX mountant. Photomicrographs were taken using trinocular microscope (Olympus) [15]. [Plate no. 2]

Corresponding Author:
Rakesh K Sindhu, Chitkara College of Pharmacy, Chitkara University, Chandigarh-Patiala NH-64, Rajpura, Patiala-140401, Punjab, India
Received 30-010-2011; **Revised** 04-11-2011; **Accepted** 23-12-2011

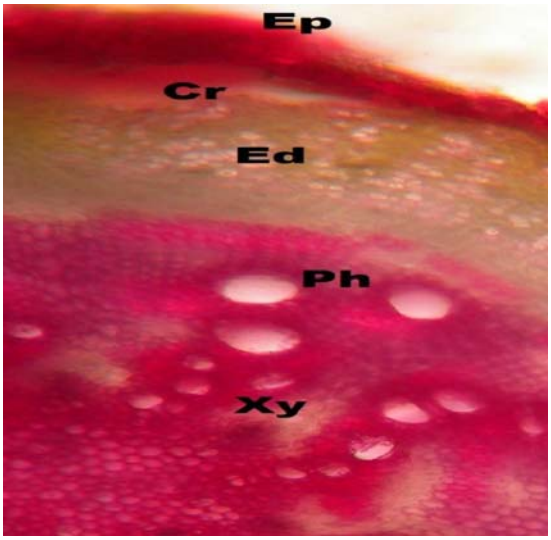


Plate no.2
Ep. = Epidermis, Cr. = Cortex, Ed. = Endodermis,
Ph. = Phloem, Xy. = Xylem

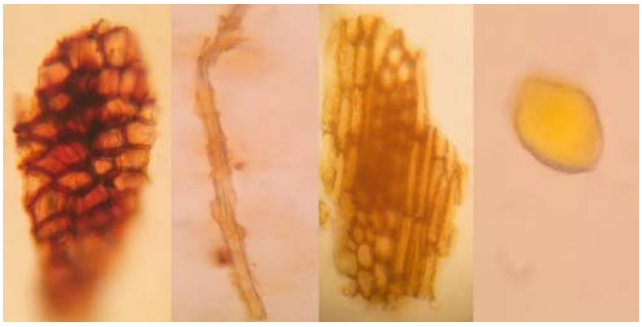
Histochemical Colour Reaction: Presence of different organic compounds in root of the plant is confirmed by using various histochemical tests. Care was taken to ascertain relative concentration of these chemicals by degree of colour produced in different tissues. The transverse section of fresh root was treated with different chemical reagents for colour tests viz. phloroglucinol, millon's reagent, iodine solution followed by sulphuric acid, dragendroff's reagent, wagner's reagent, sulphuric acid solution, libberman-burchard reagent, acetic anhydride and sulphuric acid solution, ferric chloride, iodine solution, caustic alkali, aqueous potassium hydroxide, chloroform with sulphuric acid, aniline sulphate and sulphuric acid[16] [Table no. 1].

Table.1: Behavior of transverse section with different chemical reagents

Sr. No.	Reagents	Test for	Nature of change in histochemical zone	Degree of change
1.	Phloroglucinol+HCl	Lignin	Xylem vessels become pink	++
2.	Millon's reagent	Proteins	Yellow colour	+
3.	Iodine solution followed by H_2SO_4	Cellulose	Cellulose wall become violet	+
4.	Dragendroff's reagent	Alkaloid	Brown colour	++
5.	Wagner's reagent	Alkaloid	Dark yellow colour	++
6.	H_2SO_4 solution	Sterol	Red colour	++
7.	Libberman – Burchard reagent	Terpenes	Pink colour	+
8.	Acetic anhydride and H_2SO_4 solution	Strerol	Black colour	-
9.	$FeCl_3$ solution	Tannins	Dark green to black colour	-
10.	Iodine solution	Starch	Light bluish	+
11.	Caustic alkali+HCl	Calcium oxalate	No change	-
12.	Aqueous. KOH solution 10%+ H_2SO_4	Suberin	Light brown	-
13.	Chloroform+ H_2SO_4	Sterol	Red colour	+

++ High; + Moderate; - Absent

Powder studies:
Microscopic study: The shade dried root was mechanically pulverized to coarse powder and sifted through 40 mesh sieve. To study the ingredients of powder, a pinch of powder was taken on slide and mounted with phloroglucinol, hydrochloric acid and glycerin. The slide was observed under microscope[17] [Plate no. 3].



A B C D

Plate no. 3
A= Parenchymatous cells, B= Fibre, C= Medullary ray, D= Oil vacule

Colour reactions: To study the behavior of root powder with different chemical reagents, a pinch of powder was treated with different chemical reagents as 1N hydrochloric acid, sodium hydroxide, acetic acid, 5% ferric chloride, picric acid, nitric acid with ammonia solution, 5% iodine, 1N nitric acid and powder as such were performed, change in colour was observed[18] [Table no. 2].

Table.2: Behavior of root powders with different chemical reagents

Sr. No	Treatment	Color of powder
1	Powder as such	Light Yellow
2	Powder + 1 N HCl	Dark brown
3	Powder + 1N NaOH	Light brown
4	Powder + Acetic Acid	Reddish brown
5	Powder + 5% Ferric chloride	Light Cream
6	Powder + Picric acid	Fluorescent light yellow
7	Powder + HNO_3 + Ammonia solution	Light Brown
8	Powder + 5% Iodine	Dark brown
9	Powder + 1N HNO_3	Brown

Fluorescence behavior of powder: Many herbs show fluorescence behavior when cut surface or powder is exposed to UV light and this can help in their identification. To study the fluorescence nature of root powder, a pinch of powder was treated with different chemical reagents viz.1N hydrochloric acid, 1N sodium hydroxide, 1N sodium hydroxide in methanol, picric acid, 1N nitric acid, acetic acid, acetone, 50% sulphuric acid, nitric acid in ammonia solution and observed under day light, long UV (365 nm) and short UV light (254 nm) [19] [Table no. 3]

Table.3: Fluorescence nature of root powder under UV and visible radiations

Sr. no.	Treatment	Long UV (365 nm)	Short UV (254 nm)	Visible
1	Powder as such	Fluorescent yellow	Light brown	Light Yellow
2	Powder + 1N HCl	Dark green	Light brown	Light brown
3	Powder + 1N NaOH	Dark brown	Light brown	Reddish brown
4	Powder + 50% HNO3	Dark brown	Light brown	Brown
5	Powder + Acetic acid	Yellowish	Light yellow	Light brown
6	Powder + Picric acid	Fluorescent yellow	Dark brown	Light yellow
7	Powder + 1N NaOH in methanol	Light greenish	Dark brown	Light brown
8	Powder + $FeCl_3$	Cream browm	Light brown	Light brown
9	Powder + 1N NaOH in	Light	Light	Light

	methanol + Nitrocellulose in amyl acetate	yellowish	greenish	brown
10	Powder + 1N HCl + Nitrocellulose in amyl acetate	Light brown	Brown	Brown
11	Powder + 1N NaOH + Nitrocellulose in amyl acetate	Light greenish	Light brown	Light brown

Ash Values[20]

Total ash: Total ash is produced by incinerating the drug at the temperature possible to remove all of the carbon. A higher temperature may result in the conversion of carbonates to oxides. The total ash usually consists of carbonates, phosphates, silicates and silica which includes both physiological ash, which is derived from the plant tissue itself and non-physiological ash which is the residue of the adhering material to the plant, e.g., sand and soil. About 2 g of air-dried powdered drug was accurately weighed and taken in a silica crucible and incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was cooled and weighed. The percentage of total ash was calculated with reference to the air dried drug. [Table no. 4]

Water soluble ash: Water-soluble ash is that part of the total ash content which is soluble in water. The total ash obtained was boiled for 5 min with 25 ml of water, the insoluble matter was collected in an ash less filter paper, incinerated at a temperature not exceeding 450°C, subtracted the weight of the insoluble matter from the weight of the ash and calculated the percentage of water soluble ash with reference to the air dried drug. [Table no. 4]

Acid-insoluble ash: Acid insoluble ash is determined by treating the total ash with dilute hydrochloric acid and weighing the residue. This limit particularly indicated contamination with siliceous materials such as earth and sand by comparison with the total ash value for the same sample differentiation can be made between contaminating material and in the natural ash of the drug. The total ash obtained was boiled with 25 ml of 2 N hydrochloric acid for 5 min, the insoluble matter was collected in an ash less filter paper, washed with hot water, ignited, cooled in dessicator and weighed. The percentage of acid-insoluble ash with reference to the air dried drug was calculated. [Table no. 4]

Table 4: Ash values

sample	11.25%
Water soluble ash	10.57%
Acid insoluble ash	8.75%
Sulphated ash	7.025%

Suphated ash: About 1 g of air dried powder drug was treated with dilute sulphuric acid before ignition in a tared silica crucible to a constant weight. The ash obtained was weighed. Percentage of sulphated ash was calculated with reference to the air-dried drug [Table no. 4].

Extractive Value[20]: Extractive value is used as a means of evaluating crude drug which are not readily estimated by other means. It is employed for that material for which no suitable chemical or biological assay method exist.

Petroleum ether extractive: Accurately weighed 5 g of the air dried powdered drug was macerated with 100 ml of petroleum ether (60-80°) in a stoppered flask for 24 h. The mixture was vigorously shaken at regular intervals. After 24 h the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100°C till it was completely dried and weighed. The percentage of petroleum ether soluble extractive was calculated with reference to air dried material [Table no. 5].

Table 5: Extractive values

Sr. No.	Solvent	Extraction period (h)	Color of extract	Extractive value (%) w/w
1.	Petroleum ether (60-80°C)	24	Florescent Yellow	3.5
2.	Chloroform	24	Light Yellowish	5.3
3.	Ethyl acetate	24	Light brown	3.2
4.	Ethanol	24	Brown	8.5
5.	Aqueous	24	Dark brown	4.5

Chloroform extractive: Accurately weighed 5g of the air dried powdered drug was macerated with 100 ml of chloroform in a stoppered flask for 24 h. The mixture was vigorously shaken at regular intervals. After 24 h the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100°C till it was completely dried and weighed. The percentage of chloroform soluble extractive was calculated with reference to air dried material [Table no. 5].

Ethyl acetate extractive: Accurately weighed 5 g of the air dried powdered drug was macerated with 100 ml of ethyl acetate in a stoppered flask for 24 h. The mixture was vigorously shaken at regular intervals. After 24 hthe solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100°C till it was completely dried and weighed. The percentage of benzene soluble extractive was calculated with reference to air dried material [Table no. 5].

Ethanol extractive: Accurately weighed 5 g of the air dried powdered drug was macerated with 100 ml of ethanol in a stoppered flask for 24 h. The mixture was vigorously shaken at regular intervals. After 24 h the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100°C till it was completely dried and weighed. The percentage of ethanol soluble extractive was calculated with reference to air dried material [Table no. 5].

Water extractive: Accurately weighed 5 g of the air dried powdered drug was macerated with 100 ml of water in a stoppered flask for 24 h. The mixture was vigorously shaken at regular intervals. After 24 h the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100°C till it was completely dried and weighed. The percentage of water soluble extractive was calculated with reference to air dried material [Table no. 5].

Determination of Crude Fibre Content: 2 g of powdered drug was extracted with diethyl ether and added 200 ml of boiling dilute sulphuric acid (1.25%) to the ether exhausted marc in a 500 ml flask. The mixture was refluxed for 30 min, filtered through filter paper and the residue was washed with boiling water until the effluent washing was acid free. Rinsed the residue and placed back into the flask with 200 ml of boiling sodium hydroxide solution (1.25%) and refluxed the mixture again for 30 min., filtered through ashless filter paper and washed the residue with boiling water until the last

washing was neutral. It was then dried at 110°C to constant weight and then ignited to constant weight. The ash was cooled in dessicator, weighed[21].

Calculations as follows

$$\% \text{ Crude Fibre} = \frac{\text{Weight of the ash obtained}}{\text{Weight of the drug sample}} \times 100$$

The results are represented in Table no. 6.

Loss on Drying: This parameter is used to determine the amount of moisture present in a particular sample. The powder drug (2 g) sample was placed on a tared evaporating dish. The tared evaporating dish was dried at 105 ± 1°C until constant weight and weighed. The drying was continued until two successive readings match each other[20] [Table no. 6]

Determination of Swelling Index[22]: Swelling properties of many medicinal plants shows specific therapeutic or pharmaceutical utility e.g. gums, pectin, or hemicellulose. One g of plant material was accurately weighed, placed into 25 ml glass stoppered measuring cylinder. 25 ml water was added and shaken the mixture thoroughly in every 10 min for one h, allow standing for 3 h at room temperature. Measured the volume in ml occupied by plant material calculated the mean value of individual determination, related to one gm of plant material [Table no. 6].

Determination of Foaming Index[22]: The medicinal plant materials contain saponins that cause the persistent foam formation when an aqueous decoction is shaken. The foaming ability of plant material and their extract is measured in term of foaming index. 1 g of powdered root was accurately weighed and transferred in to a 500 ml conical flasks containing 100 ml water and boiled for 30 min, cooled and filtered into 100 ml volumetric flask and made the volume with water. The decoction was poured into 10 stoppered test tubes in successive portion of 1 ml, 2 ml, 3 ml, etc up to 10 ml and adjusted the volume of each test tube with water to 10 ml and shaken them in lengthwise motion for 15 sec. Allowed to stand for 15 min and measured the height of the foam. The results were assessed as follows:

If height of foam in every tube was less than 1 cm the foaming index was considered less than 100. If height of the foam was more than 1cm in every tube the foaming index was over than 1000. In such case repetitions was done by using a new series of dilutions of decoction in order to obtain the result. If height of foam in any test tube was 1 cm, the volume of the plant material decoction in that tube (a) was used to determination of index.

$$\text{Formula used for calculation of foaming index} = \frac{1000}{a}$$

a = Volume of decoction was used for preparing the dilution in tube where foaming height was 1cm measured. The results are represented in Table 6.

Determination of Tannins[22]: Tannins are complex substances. They occur as mixture of polyphenols that are difficult to separate and crystallize. They are capable of turning animal hide into leather by binding proteins to form water insoluble substances that are resistant to proteolytic enzyme. Powdered root 2 g of each root was accurately weighed and placed into conical flask. Added 150 ml of distilled water and heated over boiling water for 30 min, cooled, transferred the mixture to 250 ml volumetric flask and diluted to volume with water. Allowed the solid material to settle down and filtered the liquid through filter paper, discarded the first 50 ml of filtrate. Evaporated 50 ml of extracts of root, to dryness, dried the residue in an oven at 105 °C for 4 h and weighed (T1). Took 80 ml of root extract, added 2 g of hide powder and shaken for 1 h. Filtered and evaporated 50 ml of clear filtrate to dryness. Dried the residues in an oven at 105 °C and weighed (T2). This is the amount of plant material that does not bind to hide powder. 2 g of hide powder was dispersed in 80 ml of water and shaken well for 1 h. Filtered and evaporated 50 ml of clear filtrate to dryness. Dried the residues in an oven at 105 °C and weighed (T0).

Formula used for calculation

$$\text{Tannins percentage: } = \frac{[T1-(T2-T0)]\times 500}{w}$$

Where w = the weight of the plant material 2 g. The results are given in Table no. 6.

Determination of Bitterness Value[22]: Medicinal plant materials have a strong bitter taste act as appetizing agents. The bitter properties of plant materials are determined by comparing the threshold bitter concentration of an extract of the materials with that of a dilute solution of quinine hydrochloride.

Stock and diluted quinine sulphate solutions: Accurately weighed 0.1 g quinine hydrochloride (R) was dissolved in safe drinking water to produce 100 ml. 5 ml of this solution was further diluted to 500 ml with safe drinking water. This stock solution of quinine hydrochloride (S_q) contained 0.01 mg/ml. Nine serial dilutions were made each containing 0.042, 0.044, 0.046, 0.048, 0.050, 0.052 0.054, 0.0.54 and 0.058 ml solution of S_q and volume made up to 10 ml with safe drinking water and obtaining a concentration of 0.1, 0.20, 0.3 upto 1 mg/ml.

Stock and diluted solutions of the plant material: The stock solution was prepared of the concentration of 10 mg/ml in distilled water (S_T). 10 test tubes were used for serial dilution with 1 ml, 2 ml, 3 ml to 10 ml of (S_T) and final volume made up with safe drinking water to 10 ml.

Method: After rinsing the mouth with safe drinking water, 10 ml of the most dilute solution was tasted while swirling it in the mouth mainly near the base of tongue for 30 sec. After 30 sec the solution was spit out and it was ascertained for I min whether a delayed sensation for bitterness existed. Then mouth was rinsed with safe drinking water. The next highest concentration was not tasted until at least 10 min. The lowest concentration at which material continues to provoke a bitter sensation after 30 sec was referred the threshold bitter concentration. After a first series of test, rinsed the mouth thoroughly with safe drinking water until no bitter sensation remains, wait at least 10 min before carrying out second test.

$$\text{Formula used for bitterness calculation} = \frac{2000\times C}{A\times B}$$

Where

A = Concentration of stock solution (S_q) mg/ml

B = Volume of (S_T) ml tube with threshold bitter concentration

C = Quantity of quinine hydrochloride (in mg) tube with threshold bitter concentration. The results are given in Table no. 6.

Determination of Haemolytic Activity[22]: Haemolytic activity of plant material is carried out for detection of saponins. It is determined by comparison of plant material extract and reference material saponin which has activity of 1000 unit per g. The erythrocyte suspension was prepared by filling a glass stoppered flask to one tenth of its volume with sodium citrate (36.5 g/L). Sufficient volume of blood freshly collected from healthy rat was introduced to it and shaken immediately. 1 ml of citrated blood was further diluted with 50 ml phosphate buffer of pH 7.4. The reference solution was freshly prepared by dissolving 10 mg glycyrrhizinic acid, (Himedia) in phosphate buffer pH 7.4 to make 100 ml.

Preliminary test: The alcoholic and aqueous extract (1g) of root 0.1 ml, 0.2 ml, 0.5 ml and 1ml were taken and adjusted the volume in each tube with phosphate buffer to 1 ml. In each tube 1 ml of 2% blood suspension was added. Gently inverted to mix the tubes, to avoid the formation of foam. Tubes were shaken after 30 min interval. Then allowed to stand for 6 h at room temperature. Examined the tubes and recorded the dilution at which total haemolysis had occurred, as indicated by clear, red solution. The alcoholic extract of root has shown haemolytic activity in highest concentration i.e. 1 ml. Therefore further dilutions were done as follows.

A serial dilution of alcoholic extract of root was prepared by using 13 test tubes in a concentration of 0.40, 0.45, and 0.50 up to 1 mg/ml and adjusted the volume in each tube with phosphate buffer to 1 ml. I ml of 2% blood suspension was added in each tube. Tubes were observed for haemolysis after 24 h. A serial dilution of glcyrrhizinic acid was prepared in the same manner. Calculated the quantity of glcyrrhizinic acid (g) that produces total haemolysis.

Calculated the haemolytic activity of the medicinal plant material using the following formula:

$$\text{Haemolytic activity} = \frac{1000 \times a}{b}$$

Where 1000 = the defined haemolytic activity of saponin (R).
a = quantity of saponin (R) that produces total haemolysis.
b = quantity of plant material that produces total haemolysis (g).
The results are represented in Table no. 6.

Table 6: Crude fiber content, Loss on drying, swelling index, Foaming index, Tanins contents, Bitterness value, Heamolytic value.

Parameter	Observation
Crude fiber content	7.53 %
Loss on drying	10 %
Swelling index	No significant result
Foaming index	No significant result
Tannins	19
Bitterness value	2.5 unit / g
Haemolytic activity	30.34 %

Determination of Microbial Count[22]**:**
Total viable aerobic bacterial count Culture media
Soybean casein digest agar medium

Ingredients	Quantity (g)
Pancreatic digest of casein	15
Papaic digest of soybean meal	05
Sodium chloride	05
Agar	15
Water	q.s. 1000 ml

Mixed all the contents and sterilized it by autoclaving at 121°C. Adjusted the pH to 7.3 ± 0.2.

Soybean casein digest medium

Ingredients	Quantity (g)
Pancreatic digest of casein	17
Papaic digest of soybean meal	03
Sodium chloride	05
Dibasic potassium phosphate	2.5
Dextrose	2.5
Water	q.s. 1000 ml

All The ingredients were dissolved in distilled water and warmed slightly. After cooling it to room temperature, the pH was adjusted to 7.1 ± 0.2. It was sterilized by autoclaving at 121°C for 30 minutes.

Method: Each Sample (10ml) in separate was transferred to 100 ml of SCDM and mixed well in an incubator shaker at 125 rpm for 1-4 hours, for revivification of microorganisms. 1 ml of sample was pipetted out from SCDM broth medium into pre-sterilized petri-plates (180°C for 2 hours) and 15-20 ml of soybean casein SCDAM was added. The contents were mixed properly for uniform distribution and the SCDAM plates were incubated in a bacteriological incubator at 35°C for 48-96 h. After incubation total number of bacterial colonies was counted using colony counter and CFU / ml was calculated using the following formula:

$$\text{CFU / ml} = \frac{\text{Total counted colony on agar plates} \times \text{dilution}}{\text{Initial sample weight taken}}$$

Determination for E. coli Culture media:
Macconkey agar medium

Ingredients	Quantity (g)
Pancreatic digest of gelatin	17
Peptone	03
Lactose	10
Sodium chloride	05
Bile salts	1.5
Agar	13.5
Neutral red	30
Crystal violet	01
Water to	q.s. 1000ml

The ingredients were boiled in water for 1 minute to affect solution and after adjustment of pH to 7.1 ± 0.2, sterilization was done.

Macconkey broth medium

Ingredients	Quantity (g)
Pancreatic digest of gelatin	20
Lactose	10
Dehydrated ox bile	05
Bromocresol purple	10
Water	q.s 1000 ml

The adjustment of pH to 7.3 ± 0.2 was done and it was sterilized.

Method: Aseptically 10ml of sample was transferred to 100ml lactose broth/soyabean casein digest broth medium and the media was incubated at 37ºC for 24 h. The flask was examined for growth and the contents were mixed by gentle shaking. 1 ml of the enriched culture was pipetted into the tubes containing 10ml MacConkey,s broth and incubated at 35ºC for 26 h. Concomitantly, streaking on the surface Mac Conkey,s agar medium was done using a loopful of enriched culture and the plates were incubated at 37ºC for 24 h [Table no. 7].

Total fungal count Culture media:

(i) Soyabean casein digest agar medium
(ii) Soyabean casein digest medium

Method: The culture media were prepared as above and 10ml of each sample was transferred to 100 ml of SCDM and mixed well for 1-4 in incubator shaker at 125 rpm for revivification of microorganism. 1 ml of sample was pipetted out from SCDM broth medium into pre-sterilized petri plates (180ºC for 2 h) and 15-20 ml of SCDAM was added. The contents were mixed properly for uniform distribution and the SCDA plates were incubated in BOD incubator at 25ºC for 5-7 days. After incubation total number of fungal colonies was counted with the help of colony counter and CFU per ml was calculated using formula:

$$\text{CFU / ml} = \frac{\text{Total counted colony on agar plates} \times \text{dilution}}{\text{Initial sample weight taken}}$$

Determination for *Pseudomonas aerginosa* Culture media: Cetrimide agar medium

Ingredients	Quantity (g)
Pancreatic digest of gelatin	20
Magnesium chloride	1.4
Potassium sulphate	10
Cetrimide	0.3
Agar	13.6
Glycerin	10
Water	q.s. 1000 ml

The ingredients were boiled in water for 1 minute to affect solution and after adjustment of pH to 7.1 ± 0.2, sterilization was done.

Method: 1ml of the enriched culture was added to the plates containing cetrimide agar media, mixed and incubated at 35ºC to 37ºC for 24 to 48 hours and observations for microbial growth were made [Table no. 7].

Table 7. Microbial determination in various extracts

Extract	Total bacterial count	Total fungal count	*E. coli*	*P.aerginosa*	*S. aureus*
Pet. Ether extract	-	-	-	-	-
Ethyl acetate Extract	-	-	-	-	-
Chloroform extract	-	-	-	-	-
Ethanol extract	-	-	-	-	-
Aqueous. Extract	-	-	-	-	-

+ + High; **+** Moderate; **–** Absent.

Determination for *Staphylococcus aureus* Culture media: Vogel Johnson agar medium (VJA)

Ingredient	Quantity (g)
Pancreatic digest of casein	10
Yeast extract	05
Mannitol	10
Dibasic potassium phosphate	05
Lithium chloride	05
Glycine	10
Agar	16
Phenol red	25
Water	q.s. 1000 ml

All ingredients were made in to solution by heating and cooled to ~45ºC and 20 ml of a 1% w/v solution of potassium tellurite was added to it. The pH was adjusted to 7.4 ± 0.2 and the contents were sterilized.

Method: Enriched culture was streaked on the surface of VJA media and incubated at 35ºC for 24 hours and observed for the presence of growth [Table no. 7].

Paper Partition Chromatography of Amino Acids: Amino acids are the basic units of proteins. The proteins are found in every living cell. The amino acids which can be synthesized by the living cells are called non-essential amino acids, while those which cannot be synthesized are called essential amino acids and must be supplied by diet. The essential amino acids are Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine and Valine. The Non-essential amino acids are Alanine, Aspartic acid, Cysteine, Glutamic acid, Glycine, Proline, Tryptophan, Glutamine, Asparagine, Serine and Tyrosine etc[23].

Carbohydrates are the most abundant organic molecule in nature. These are the carbon compounds that contain large quantities of hydroxyl groups. The simplest carbohydrates also contain either an aldehyde moiety or a ketone moiety. All carbohydrates can be classified as either monosaccharides, oligosaccharides or polysaccharides e.g. Ribose, Ribulose, Xylulose Glucose, Galactose, Mannose, Fructose, Erythrose.

Preparation of extract: Powdered (10 g) roots of Murraya koenigii was weighed and macerated with 100 ml of water and left overnight. The supernatant clear liquid was filtered. The extraction was repeated for three consecutive days so as to exhaust the root of all water soluble extractives. The combined filtrates were concentrated on a water bath and the proteins precipitated by addition of alcohol (95%) were washed with ethanol to remove unbound amino acids. The mother liquor obtained after removing the proteins was concentrated for detection of amino acids in Free State and carbohydrates[24,25].

Paper chromatography: Chromatographic Whatman paper No.1 sheets (Qalligens) were used for paper chromatography. The starting line was marked two centimeter above from the base. To obtain the desired concentration of the extract on the paper, the spots were applied repeatedly at the same point. The spots were kept at a distance of two centimeter apart for the amino acid identification**.** The solvent systems used were n-butanol: glacial acetic acid: water (4:1:5) for amino acids and n-butanol: glacial acetic acid: water (4:2:1) for carbohydrates.

The chamber was saturated in 16 h prior the experiment with respective solvent systems. Care was taken so as not to touch the paper with fingers. The papers were developed in

descending manner. Air dried chromatograms were sprayed with 0.2% w/v solution of Ninhydrin in acetone for amino acids and aniline hydrogen pthalate for carbohydrates, heated at 110^{0}C in oven. Pink to violet colour were visualized for amino acids and yellow to dark brown for carbohydrate[26] [Table no. 8,9].

Table 8: Amino acids analyzed by paper chromatography

Sr. No.	Amino Acids	R_f Value	Amino acids detected
1.	Alanine	0.45	–
2.	2-Aminobutyric acid	0.67	–
3.	Arginine	0.20	–
4.	Aspartic acid	0.19	-
5.	Glutamic Acid	0.22	–
6.	Glycine	0.28	+
7.	Histidine	0.26	–
8.	Lysine	0.19	-
9.	Methionine	0.37	–
10.	Norleucine	0.15	
11.	Ornithine	0.18	-
12.	Phenylalanine	0.29	+
13.	Tyrosine	0.50	+

+ve : Detected; –ve : Not detected

Table 9: Carbohydrates analyzed by paper chromatography

Sr. No.	Carbohydrates	R_f Value	Carbohydrate Detected
1.	Galctose	0.55	+
2.	Maltose	0.45	-
3.	Lactose	0.32	-
4.	D-Ribose	0.42	+
5.	Sucrose	0.54	-
6.	Fructose	0.78	+
7.	D-Xylose	0.65	-
8.	L-Arabinose	0.82	-

+ve : Detected; –ve : absent

R_f values for each spot was calculated

$$R_f = \frac{\text{Distance travelled by the solute from the start}}{\text{Distance travelled by the solvent from the start}}$$

Preliminary Phytochemical Examination:

Preparation of the extract: About 20 g of air dried powdered root was extracted with ethanol in a soxhlet extractor for 72 h. the aqueous extract was prepared by maceration with distilled water for 24 h to obtain the aqueous extract. Concentrated ethanol and aqueous extract in rotary vaccum evaporator. The extracts were screened for the presence of various phytoconstituents [Table no. 3.11].

Test for alkaloids: Stirr a small portion of the solvent free petroleum ether, chloroform, ethyl acetate, alcohol and water extracts separately with a few drops of dilute hydrochloric acid and filter. The filtrates were tested with various alkaloidal reagents such as mayer's reagent (cream precipitate), dragendorff's reagent (orange brown precipitate), and wagner reagent (reddish brown precipitate). Mayer's reagent: Few drops of mayer's reagent were added in each extract and observed formation of the white or cream colored precipitates.

Dragendorff's reagent: Few drops of dragendorff's reagent were added in each extract and observed formation of the orange yellow or brown colored precipitates.

Wagner reagent: Few drops of wagner reagent were added in each extract and observed formation of the reddish brown precipitates.

Test for carbohydrates: Dissolve small quantities of alcoholic and aqueous extracts, separately in 4 ml of distilled water and filter. The filtrate may be subjected to varios tests to detect the presence of carbohydrates.

Molisch's Test: To about 2 ml of extract few drops of α-naphthol (20% in ethyl alcohol) were added. Then about 1 ml of concentrated sulphuric acid was added along the side of the tube. Reddish violet ring appeared at the junction of two layers. Indicates the presence of carbohydrates.

Fehlings Test: 1ml of fehling's reagent (copper sulphate in alkaline conditions) was added to the filtrate of the root extract in distilled water and heated in a steam bath. Brick red precipitates appeared which confirm the presence of carbohydrates.

Test for glycosides: Hydrolysed another small portion of the extract with dilute hydrochloric acid for few hours in water bath and subjected the hydrolysate with liebermann-burchard's, keller-killani, and borntrager's tests to detect the presence of different glycosides.

Keller-Killani Test: 1ml of glacial acetic acid containing traces of $FeCl_3$ and 1 ml of concentrated H_2SO_4 was added to the extract carefully. Colour appeared which confirm the presence of glycosides in the root extracts.

Borntrager's test: 1ml of benzene and 0.5 ml of dilute ammonia solution were added to the extract. A black brown colour was obtained which show the presence of glycosides in the root extracts.

Test for phenolic compound and tannins: Take small quantities of alcohol and aqueous extracts separately in water and test for the presence of phenolic compounds and tannins with dilute ferric chloride solution (5%) and lead acetate test.

Ferric chloride test: On addition of ferric chloride solution (5%), colour was observed in all the three portions due to the presence of phenolic compounds. colour appeared which show the presence of phenolic compound.

Lead acetate test: Few drops of lead acetate solution (5%) were added to the alcoholic extract of the root. White precipitate was appeared which confirm the presence of phenolic compounds.

Test for flavonoids: Ammonia test: Filter paper strips were dipped in the alcoholic and aqueous solutions of the extract and ammoniated. The filter paper changed its colour to yellow which indicates the presence of flavonoids.

Pew test for flavonoids: To 1ml of the each extracts, a piece of metallic magnesium/zinc was added followed by addition of 2 drops of concentrated hydrochloric acid. A brownish colour confirmed the presence of flavonoids in all the extract.

Test for proteins and free amino acids: Added a few ml of alcoholic and aqueous extracts in a few ml of distilled water and subjected to million's, biuret and ninhydrin tests.

Millon's test: To 2 ml of filtrate, 5-6 drops of million's reagent (solution of mercury nitrate and nitrous acid) was added. A red colour precipitate appeared which confirms the presence of proteins and free amino acids.

Biuret test: To the ammoniated alkaline filtrate 2-3 drops of 0.02% copper sulphate solution was added. A red colour was

obtained which confirms the presence of proteins and free amino acids.

Ninhydrin test: To each of the filtrate, lead acetate solution was added to precipitate tannins and filtered. The Filtrate was spotted on a paper chromatogram, sprayed with ninhydrin reagent and dried at 110° C for 5 minutes. Violet spots were seen which confirm the presence of proteins and free amino acids.

Test for saponin: Foam test: Dilute 1 ml of alcoholic and aqueous extracts separately with distilled water to 20 ml and shake in a graduated cylinder for 15 minutes. A one centimeter layer of foam indicates the presence of saponin.

Sodium bicarbonate test: To the few milligrams of extract few drops of sodium bicarbonate were added and shaken well. Formation of honey comb like frothing indicates positive test for saponins.

Test for phytosterol and triterpenes: Liebermann-Burchard's test: The hydro-alcohlic extract was shaken with chloroform and few drops of acetic anhydride were added chloroform extract along with a few drops of concentrated sulphuric acid from the side of the tube. The appearance of blue to brick red colour indicates the presence of sterol and triterpenes.

Hesse's reaction: The residue was dissolved in chloroform (4 ml) and an equal quantity of concentrated sulphuric acid was then along the side of the tube. The formation of the pink colored ring, which is on shaking diffused in both the layers, indicating the presence of sterols in the extract[27,28] [Table no. 10].

Table 10: Prelimenry phytochemical screening

Sr. no.	Plant Constituents Test / Reagent	Pet. Ether extract	Ethyl acetate extract	Chloroform extract	Ethanol extract	Aqueous extract
1.	**ALKALOIDS**	+	-	+	+	-
	Mayer's reagent	+	-	+	+	-
	Dragendroff's reagent	+	+	+	+	-
	Wagner's reagent					
2.	**GLYCOSIDES**	-	-	-	-	-
	Killer-Killani test	-	-	-	-	-
	Sodium nitropruside test	-	+	-	+	-
	Borntrager test					
3.	**CARBOHYDRATES**	-	-	-	+	+
	Molisch's reagent	-	-	-	+	+
	Fehling solution					
4.	**STEROLS**	-	-	-	+	-
	Liebermann-	-	-	-	-	-
	Burchard's test	+	-	-	+	-
	Salkowski test	-	-	-	-	-
	Hesses reaction					
	Hersch reaction					
5.	**SAPONINS**	-	-	-	+	-
	Foam test	-	-	-	+	-
	Sodium bicarbonate test					
6.	**PHENOLIC**	-	-	-	-	-
	COMPOUNDS & TANNINS	-	-	-	+	-
	Ferric chloride solution					
	Lead acetate solution					
7.	**PROTEINS &**	-	-	-	+	-
	AMINO ACIDS	-	-	+	+	-
	Biuret test	+	-	+	+	+
	Millon's reagent					
	Ninhydrin reagent					
8.	**FLAVANOIDS**	-	-	-	+	-
	Shinoda/Pew test	-	-	-	+	-
	Ammonia test					

+ve : Detected; –ve : absent

RESULTS AND DISCUSSION

The phytochemical and pharmacognostical evaluation of *Murraya koenigii* roots were performed. The root is a typical root and in transverse section it shows the features of a dicot root i.e. Epidermis, Cortex, Endodermis, Phloem and Xylem. The microscopy of the powder revealed the presence of xylem vessel, fiber, parenchymatous cell and cork cells. Total ash, acid insoluble ash, water insoluble ash and sulphated ash were 11.25%, 10.57%, 8.75%, 7.025% respectively. The extractive values i.e. petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract were3.5 %, 5.3 %, 3.2%, 8.5%, 4.5%. The fiber content was 7.53%. The plant can be used as bitter as its bitterness was found to be 2.5 unit/g. The plant possesses haemolytic activity. The plant extracts were good to be free of microbial contamination. The tannin content was 19. The alcoholic and aqueous extracts were screened for presence of amino acid and carbohydrates. The extracts showed the presence of two amino acids viz. Phenylalanine and glycine and two carbohydrates i.e. galactose, ribose and fructose. The preliminary phytochemical screening of Pet. Ether extract, Ethyl acetate extract, Chloroform extract, Ethanol extract, and aqueous extract was performed. The presence of alkaloids, flavonoids, carbohydrates, and sterol in various extracts were observed. This is first ever pharmacognostical study carried out on the roots of *Murraya koenigii*.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Madhu Chitkara, Vice-Chencellor, Chitkara University, Rajpura, Patiala, Punjab, India and Dr. Ashok Chitkara, Chairman, Chitkara Educational Trust, Chandigarh, India, for support and institutional facilities.

REFERENCES

1. Pillai KG, Gopala. Chikilsamanmjari. Part I. Srichitra Ayurveda Series no. 1, Trivandrum, 1958; 100.
2. Bose MBK, Chandra. Pharmacopoeia Indica. Bishan Singh Mahendra Pal Singh, 23-A Connaught Place, Dehradun, India, 1985; 20.
3. Satyavati GV, Gupta AK, Tandon N. Medicinal Plants of India, vol. 2. Indian Council of Medical Research, New Delhi, India, 1987; 289–299.
4. Mhaskar KS, Blatter E, Caius JF. In; Kirtikar and Basu's Illustrated Indian Medicinal Plants Their Usage in Ayurveda and Unani Medicine, Vol.- 3, Shri Satguru Publication, Delhi, 2000; 656-659.
5. Shah KJ, Juvekar AR. Positive inotropic effect of *Murraya koenigii (Linn.) Spreng* extract on an isolated perfused frog heart Indian Journal of Experimental Biology, 2006; 44:481- 484.
6. Shrinivasan K. Plant foods in the management of diabetes mellitus: spices as beneficial antidiabetic food adjuncts. Int. J. Food Sci. Nutr. Sept 2005, 56(6), 399-414.
7. Manfred F. John MP, Dajaja DS, Douglas AK. Koenoline, a further cytotoxic carbazole alkaloid from *Murraya koenigii*. Phytochemistry, 1985; 24:3041-3043.
8. The Wealth of India, Council of Scientific and Industrial Research, New Delhi, 2003, 317.
9. Ram HNA, Hatapakki BC, Hukkeri IV, J., Aryavaidyan, 2002, 16 (1), 40-44.
10. Kesari AN, Gupta RK, Watal G. Hypoglycemic Effects of Murraya koenigii on Normal and Alloxan- Diabetic Rabbits. Journal of Ethanopharmacolgy, 2005; 97: 247-251.
11. Xie JT, Chang WT, Wang CZ, Mehendale SR, Li, J. Ambihaipahar, R., Ambihaipahar, U., Fong HH, Yuan CS, mrraya koenigii reduces blood cholesterol and glucose level in ob/ob mice. American Journal of Chinise Medicine, 2006, 34(22), 279-284.

12. Rahman MM, Gray AI. A benzoisofuranone derivative and carbazole alkaloids from Murraya koenigii and their antimicrobial activity. Phytochemistry, 2005; 66:1601-1606.

13. Sharma SK. Recent approach to herbal formulation development and standardization. 2004. http//pharmainfo.net.

14. Sindhu RK, Vasudeva N and Sharma SK. Pharmacognostical and preliminary phytochemical investigations on *Verbesina encelioides* Benth. Roots. Journal of Herbal Medicine and Toxicology. 2010; 4, 113-118.

15. Johnsen DA. Plant microtechnique. Mcgraw hills Book Company. New York. 1940; 523.

16. Govil JN, Singh VK, Hashmi S. Medicinal Plants: New Vistas of Research (Part I), Vol. X, 1993; 181-187.

17. Evans WC. Trease and Evans. Pharmacognosy, WB Sacenders Company Ltd. London. 14th Edn.1996; 194.

18. Hashmi S, Ahmad F, Husain W. Botanical and Physico-chemical Standardization of Isqueal (Urginea indica Kunth.), A Multi-Action Drug of Unani System. Hamdard Medicus. 2003; 43:102-106.

19. Kokoski CJ, Kokoski RJ and Salma FJ. Fluorescence of powdered vegetable drugs under UV radiation. Scientific edn. Journal of American Pharmaceutical Association. 1958; 47: 715-717.

20. Anonymous. The Pharmacopoeia of India, Controller of Publication, New Delhi. 1996; A-74.

21. Knevel AL, Digangi FF. Jenkin's Quantitative Pharmaceutical Chem., Seventh Edn, Blakistan Publication, New Delhi. 1977, 223-236.

22. Anonymous. Quality Control methods for medicinal plants material. World Health Organization, Geneva, AIT US publisher and distributor Delhi.1998.

23. Das D. Biochemistry. Academic Publishers. 2005;187-188.

24. Evan WC. Trease and Evans Pharmacognosy. Elsevier Publication, A Division of Reed Elsevier India Private Limited, New Delhi. 2006, 3-4.

25. Sharma SK and Ali M. Amino acid and carbohydrate composition of stem bark of some cultivars of Mangifera indica (Mango). Journal of Indian Chemical Society. 1992; 69:891-892.

26. Stryer L. Biochemistry, forth edetion, Freeman and company, New York. 1997; 463-470.

27. Rangari VD. Pharmacognosy and phytochemistry, carrier publication, first edn, Nashik, India. 2000; 100-101.

28. Kokate CK. Pharmacohnosy. 16th Edn., Nirali Publication, Delhi. 1998; 108-109.

Source of support: Nil, Conflict of interest: None Declared