



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(6): 779-784
Received: 23-09-2018
Accepted: 24-10-2018

Aju BY
Department of Botany,
University of Kerala,
Thiruvananthapuram, Kerala,
India

Rajalakshmi R
Department of Botany,
University of Kerala,
Thiruvananthapuram, Kerala,
India

Mini S
Department of Biochemistry,
University of Kerala,
Thiruvananthapuram, Kerala,
India

Pharmacognostical evaluation of curry leaf (*Murraya koenigii*)

Aju BY, Rajalakshmi R and Mini S

Abstract

Medicinal plants have been used in the traditional health care system. *Murraya koenigii* is a medicinal plant with great therapeutic potential and is used for the treatment of many diseases. Plants are the richest sources of various drugs. Pharmacognostical studies helps to standardize the plants with therapeutic potential. The present study is to standardize *Murraya koenigii* pharmacognostically which will help for the proper characterization of the drugs derived from this plant. Dried leaf powders as well as fresh leaves were subjected to pharmacognostic analysis. Macroscopic characters (shape, surface and color of the plant samples), Microscopic characters (stomatal index, stomatal number, powder microscopy) organoleptic characters (Color, Texture and odor) were analyzed. Fluorescence analysis of leaf powder and extracts were done. pH, ethanol soluble extractive, water solubility index and water absorption index, loss on drying at 105 °C, total ash value, water soluble ash, acid insoluble ash, sulphated ash, volatile oil, crude fiber, foaming index, swelling index of the plant powder were analyzed using standard procedures. Pharmacognostical standardization was carried out on the basis of macroscopic characters, microscopic characters, physico-chemical constants and fluorescence behavior of the plant powder as well as extracts with various chemical reagents. Pharmacognostical standardization of *Murraya koenigii* leaves can serve as an important source of information to ascertain the identity and to determine the quality and purity of drugs derived from this plant.

Keywords: Pharmacognosy, physico chemical characters, leaf powder, *Murraya koenigii*

1. Introduction

Murraya koenigii belongs to rutaceae, commonly known as “curry leaves” are widely used as a condiment and spice in India and other tropical countries. *Murraya koenigii* is one of the plant species with potential medicinal properties. The whole plant and different parts of the plant are used to cure many human ailments. The ethnobotanical profile of *Murraya koenigii* reveals that the whole plant is used as febrifuge, stomachic^[1], anti-periodic^[2] and to cure diabetes mellitus, leucoderma, kidney pain and vomiting^[3]. Stem is used as datum for cleaning, strengthen gums and teeth^[4]. Bark is used as hair tonic, stomachic and carminative^[5]. Leaves are used as stomachic, purgative, febrifuge anti-anemic, anti-helminthic^[1, 3, 5, 6, 7] memory enhancer, maintaining the natural skin pigmentation and showed skin lightening and rough skin improving effect, losing weight, hypoglycemic activity^[8, 9]. Fruits used as astringent^[10]. Roots reduce inflammation and itching^[11, 12]. Since *Murraya koenigii* is a rich source of various drugs pharmacognostic standardization is essential for the proper identification of the plant. Pharmacognostical studies can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant materials for future studies.

2. Materials and methods

The leaves of *Murraya koenigii* were collected from Trivandrum. Authentication was done by Dr. G.Valsala Devi, Curator, Department of Botany, University of Kerala. A voucher specimen (Voucher No. KUBH 9914) has been deposited in the herbarium, Department of Botany, University of Kerala for further reference. The collected plant materials were shade dried, powdered and used for further studies.

2.1 Macroscopic characters

The fresh leaves of *Murraya koenigii* were used for macroscopic analysis. Color, shape and texture of the leaves were examined.

2.2 Microscopic characters

The leaf peel is taken and is stained with safranin for quantitative microscopic studies.

Correspondence

Aju BY
Department of Botany,
University of Kerala,
Thiruvananthapuram, Kerala,
India

2.2.1 Stomatal Number

It is the average number of stomata per square mm of epidermis of the leaf. A minimum of ten readings were taken from different locations of the leaf and the average value was calculated.

2.2.2 Stomatal Index

It is the percentage in which the number of stomata to the total number of epidermal cells, each stoma being counted as one cell. Stomatal index was calculated using the following equation,

$$S.I = (S/E+S) \times 100$$

Where,

S.I = Stomatal Index

S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area.

3. Powder microscopic observations

The powdered leaves were sieved and used for powder organoleptic and microscopic analysis. The powder was treated with phloroglucinol and observed under microscope.

4. Physico-chemical Constants

The standard procedures^[13, 14, 15, 16] were followed to calculate the physico-chemical constants.

4.1 Loss on drying at 105 °C

Test for loss on drying determines the moisture content and volatile oil present in the drug. i.e., volatile oil and water drying off from the drug.

A 100 ml beaker was accurately weighed. 4 g of leaf powder was taken in a beaker and weighed. The beaker was placed in a hot-air oven and dried at 105 °C for 5 hours. It was cooled in a desiccator and weighed. The process was repeated until constant weight is obtained. The percentage of loss in weight of the sample was calculated.

Calculation

$$\text{Percentage of loss on drying at } 105\text{ }^{\circ}\text{C} = \frac{\text{Loss in weight of the sample} \times 100}{\text{Weight of the sample taken}}$$

4.2 Total ash

The total ash method was designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

A silica crucible was ignited, cooled and weighed. 2 g of powdered leaf was taken in the crucible and accurately weighed. The powder was incinerated until free from carbon in a muffle furnace, cooled and weighed. The percentage of total ash was calculated.

Calculation

$$\text{Percentage of total ash} = \frac{\text{Weight of ash}}{\text{Weight of sample taken}} \times 100$$

4.3 Acid-insoluble ash

Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the

remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth.

Total ash was taken in the crucible. 25 ml 6N hydrochloric acid was added and boiled for five minutes. Filtered with an ash less filter paper. Washed with hot water until the filtrate was free from acid. After filtration, transferred the filter paper containing the insoluble matter into the same crucible and ignited in a muffle furnace to constant weight. The % of the acid-insoluble ash was calculated.

Calculation

$$\text{Percentage of acid insoluble ash} = \frac{\text{Weight of acid insoluble residue} \times 100}{\text{Weight of sample taken}}$$

4.4 Water-soluble ash

Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

The total ash was taken in the crucible. Added 25 ml water and boiled for five minutes. Filtered with an ash less filter paper. Washed with hot water. Transferred the filter paper containing the insoluble matter into the same crucible and ignited to constant weight. Weight of water soluble ash was obtained by subtracting the water insoluble residue from the total ash content. Calculated the % of the water soluble ash with reference to the air dried drug.

Calculation

$$\text{Percentage of water soluble ash} = \frac{\text{Weight of water soluble ash} \times 100}{\text{Weight of sample taken}}$$

4.5 Sulphated Ash

Silica was heated to redness for 10 minutes, allowed to cool in a desiccator and weighed. Put 1 to 2 g of the substance, accurately weighed, into the crucible, ignited gently at first, until the substance was thoroughly charred. Cooled, moistened the residue with 1 ml of *sulphuric acid*, heated gently until white fumes were no longer evolved and ignited at 800° ± 25° until all black particles were disappeared. Conduct the ignition in a place protected from air currents. Allowed the crucible to cool, a few drops of sulphuric acid were added and heated. Ignited as before, allowed to cool and weighed.

4.6 Alcohol-soluble extractives

This method determines the amount of chemical constituents extracted with ethyl alcohol from a given amount of medicinal plant material.

4 g of coarsely powdered leaf was accurately weighed. Transferred it in a glass stoppered conical flask and added 100 ml alcohol (approximately 95%). Shaked for 6 hours. Allowed to stand for 18 hrs. Filtered rapidly. A 50 ml beaker was taken and weighed. Pipetted out 25 ml of the filtrate to the beaker. Evaporated to dryness on water bath and kept it in air oven at 105 °C for 6 hours. Cooled in desiccator for 30 minutes and weighed. The percentage of alcohol soluble extractives was calculated.

Calculation

$$\text{Percentage of solubility in alcohol} = \frac{\text{Weight of extract} \times \text{volume of solvent} (100) \times 100}{25 \times \text{Weight of sample taken}}$$

4.7 Water-soluble extractives

This method determines the amount of chemical constituents extracted with water from a given amount of medicinal plant material.

4 g of coarsely powdered leaf was taken and accurately weighed. Transferred it in a glass stoppered conical flask and added 100 ml distilled water. Shake occasionally for 6 hours. Allowed to stand for 18 hrs. Filtered rapidly. A 50 ml beaker was weighed. Pipetted out 25 ml of the filtrate to the beaker. Evaporated to dryness on water bath and kept it in air oven at 105 °C for 6 hours. Cooled in desiccator for 30 minutes and weighed. The percentage of water-soluble extractives was calculated.

Calculation

$$\text{Percentage of solubility in water} = \frac{\text{Weight of extract} \times \text{volume of solvent}(100) \times 100}{25 \times \text{Weight of sample taken}}$$

4.8 Water absorption index

2.5 g of leaf powder was taken in a centrifuge tube and 30 ml of distilled water was added to it at 30 °C and stirred intermittently for 30 minutes. Centrifuged for 10 minutes at 5100 rpm. The supernatant was carefully poured into a petridish and allowed both supernatant and pellet to dry overnight. The water absorption index was calculated.

$$\text{Water absorption index (WAI)} = \frac{\text{Weight of dry solid}}{\text{Weight of plant powder}}$$

4.9 Volatile oil

In order to determine the volume of oil, the plant material was distilled with water and the distillate was collected in a graduated tube. The aqueous portion separates automatically and was returned to the distillation flask. If the volatile oils possess a mass density higher than or near to that of water, or are difficult to separate from the aqueous phase owing to the formation of emulsions, a solvent with a low mass density and a suitable boiling point may be added to the measuring tube. The dissolved volatile oils will then float on top of the aqueous phase.

20 g of coarsely powdered leaf was taken in a 1 litre round bottom flask. 300 ml of water and a few porous pieces were added. The flask was connected to a volatile oil apparatus (Clevenger apparatus). The contents of the flask were heated and boiled for 2 hours. The flask was rotated occasionally to wash down any material that adheres to its sides. The apparatus was allowed to cool for 10 minutes and the volume was read. The % volatile oil was calculated.

Calculation

$$\text{Percentage of volatile oil} = \frac{\text{Volume of volatile oil}}{\text{Weight of drug taken}} \times 100$$

4.10 Determination of crude fiber

5 g of the material was dried to constant weight in an air oven at 105 ± 2°C. Weighed accurately about 2.5 g of the dried material into a thimble and extracted with petroleum ether for 1 hour using a soxhlet flask. Transferred the fat free material to a 1 litre flask. 200 ml of diluted sulphuric acid is taken in a beaker and boiled, transferred to the flask containing the fat free material and immediately connected the flask with a reflux condenser and heated so that the contents of the flask begin to boil within 1 minute. Rotated the flask frequently. Continued boiling for 30 minutes. Removed the flask and

filtered through fine linen held in a funnel and washed with boiling water until the washings are no longer acid to litmus. 200 ml of Sodium hydroxide solution was boiled under reflux condenser. Washed the residue on the linen into the flask with the boiling sodium hydroxide solution. Immediately connected the flask with the reflux condenser and boiled for 30 minutes. Removed the flask and immediately filtered through the filtering cloth. Thoroughly washed the residue with boiling water and transferred to a gooch crucible prepared with a thin compact layer of ignited asbestos. Washed the residue thoroughly first with hot water and then with 15 ml of ethyl alcohol. Dry the gooch crucible with the contents in an air oven maintained at 105 ± 2°C till constant weight. Incinerated in a muffle furnace until all the carbonaceous matter was burnt. Cooled in a desiccator and weighed.

Calculation

$$\text{Crude fibre (dry basis)} = \frac{M1 - M2}{M} \times 100$$

Where, M1 = mass in g of gooch crucible and contents before ashing.

M2 = mass of gooch crucible containing asbestos and ash

M = mass in gm of dried material taken for test.

4.11 Determination of foaming index

1 g of the plant powder, weighed accurately and transferred to a 500 ml conical flask containing 100 ml of boiling water. Maintained at moderate boiling for 30 minutes. Cooled and filtered into a 100 ml volumetric flask and added sufficient water through the filter to dilute to volume. Poured the decoction into 10 stoppered test-tubes (height 16cm, diameter 16mm) in successive portions of 1 ml, 2 ml, 3 ml, up to 10 ml, and adjusted the volume of the liquid in each tube with water to 10 ml. Stoppered the tubes and shaken them in a lengthwise motion for 15 seconds, two shakes per second. Allowed to stand for 15 minutes and measured the height of the foam. The results were assessed as follows.

- If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100.
- If a height of foam of 1 cm is measured in any tube, the volume of the plant material decoction in this tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.
- If the height of the foam is more than 1 cm in every tube, the foaming index is over 1000. In this case repeat the determination using a new series of dilutions of the decoction in order to obtain a result.

$$\text{Foaming index} = \frac{1000}{a}$$

Where *a* = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

4.12 Determination of swelling index

The swelling index is the volume in ml taken up by the swelling of 1 g of plant material under specified conditions. Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual plant material. Using a glass-stoppered measuring cylinder, the material is shaken repeatedly for 1 hour and then

allowed to stand for a required period of time. The volume of the mixture (in ml) is then read.

Introduce the specified quantity of the plant material into a 25-ml glass-stoppered measuring cylinder. 25 ml of water was added and shaken the mixture thoroughly every 10 minutes for 1 hour. Allowed to stand for 3 hours at room temperature, or as specified. Measured the volume in ml occupied by the plant material, including any sticky mucilage. Calculated the mean value of the individual determinations, related to 1 g of plant material.

4.13 Determination of pH of the plant powder

1 g of plant powder was taken in a conical flask. 10 ml of distilled water was added to the conical flask and mixed well. Allowed it to stand for 5 minutes at room temperature. Measured the pH of the sample using pH meter.

5. Fluorescence analysis

The fluorescence analysis of the powder as well as various extracts of *Murraya koenigii* leaves was carried out by using the standard method [17]. The behavior of the powder with different chemical reagents was also carried out.

6. Results and Discussion

The major problem faced in herbal formulation industry is the identification of an authenticated raw material and in absence of data; one can use any adulterant in the process of formulating the drug. [18] From the present study, important diagnostic characters that might be useful in determining the authenticity and identifying adulteration of the crude drug were assessed. Organoleptic and microscopic analysis along with physicochemical parameters were determined on plant samples in order to establish appropriate data that can be

useful in identifying crude drugs, particularly those supplied in the powder form^[19]. *Murraya koenigii* has many medicinal and therapeutic actions that have been scientifically validated and documented.

Pharmacognostical standardization was carried out on the basis of macroscopic characters, microscopic characters, physico chemical constants and fluorescence behavior of the plant powder as well as extracts with various chemical reagents. Macroscopic characters revealed that leaves are exstipulate and bipinnately compound. Leaflets are lanceolate having reticulate venation. Leaves are smooth with green color. Microscopic study shows that stomata are anomocytic. In this type the stoma remains surrounded by a limited number of subsidiary cells which are quite alike the remaining epidermal cells. The results of microscopic data is shown in Table 1

Table 1: Quantitative microscopic data of *Murraya koenigii* leaves

SI No	Parameters	Value in 1 sq.mm (Average of 10 fields)
1	Stomatal number	
	Abaxial	35.87
	Adaxial	20.46
2	Stomatal index	
	Abaxial	10.23
	Adaxial	5.08

Powder microscopy gives idea about the powder character of the drug and is helped to identify the drug in powder form. The powder is moss green in colour with strong characteristic smell. On microscopical examination the powder (Fig 1-3) showed xylem tracheids, xylem vessels and phloem elements.

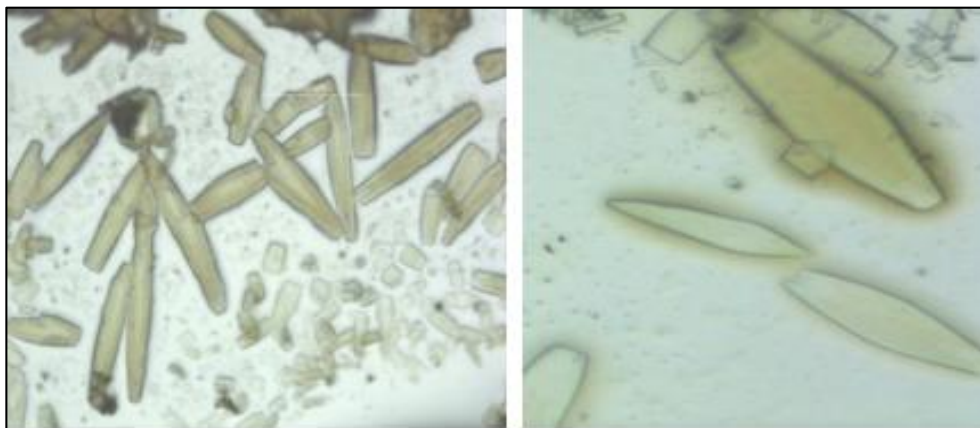


Fig 1: (a & b) showing Xylem tracheids.



Fig 2: Xylem vessel

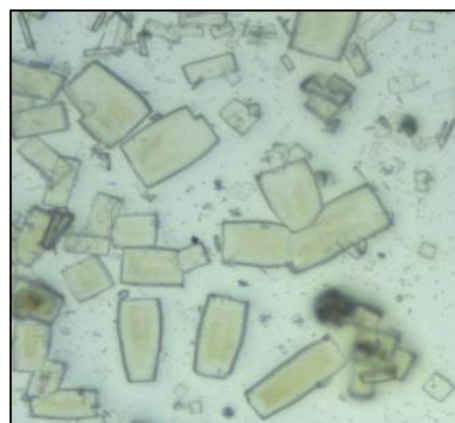


Fig 3: Phloem

The physico-chemical parameters (Table 2) are mainly used in judging the purity and quality of the drug. Ash value of a drug gives an idea of the earthy matter or inorganic composition or other impurities present along with the drug, the ash values are important since ash can be derived from the plant itself (physiological or natural ash) as well as from the extraneous matter, especially sand and soil adhering to the surface of the drug (non physiological ash). Physiological and non-physiological ash together is called as total ash. The total ash may vary within wide limits for specimen of genuine drug due to variable natural or physiological ash, in such cases the ash obtained is treated with acid in which most of the natural ash is soluble leaving the silica as acid-insoluble ash which represents most of the ash from the contaminating soil. The total ash value is 9.67 %, water soluble ash is 5.85 % and acid soluble ash is 20 %. The ash values of the powdered leaves revealed a high percentage of sulphated ash (16.70 %).

Table 2: Physico chemical constants of leaf powder of *Murraya koenigii*

Sl No	Parameters	Percentage (%w/w)
1	Loss on drying at 105 °C	19.26
2	Total ash value	9.67
3	Water soluble ash	5.85
4	Acid insoluble ash	0.20
5	Sulphated ash	16.70
6	Ethanol soluble extractive	7.15
7	Water soluble extractive	2.29
8	Water absorption index	1.03
9	Determination of volatile oil	1.5
10	Crude fibre	19.69
11	Foaming index-	Nil
12	Swelling index	11.5

Extractive values give an idea about the chemical constituents present in the drug as well as useful in the determination of exhausted or adulterated drugs. It determines the amount of chemical constituents extracted with ethyl alcohol or with water from a given amount of medicinal plant material. The

results suggest that the powdered leaves have high ethanol soluble extractive value. It revealed that most of the chemical compounds present in *Murraya koenigii* can be extracted with ethanol. *Murraya koenigii* leaves have high ethanol soluble extractive value. So the chemical compounds present in *Murraya koenigii* leaves may be more soluble in ethanol.

The loss on drying reveals the percentage of moisture present in the drug, since moisture facilitates the enzyme hydrolysis or growth of microbes which leads to deterioration. Excess moisture in the crude drug may lead to the breakdown of important constituents and growth of microorganisms during storage of drug. [20] The plant has low moisture content (19.26%). The crude fibre content (19.96%) which was studied can be implied to determine the nutritive value of the leaves. The leaves contain good amount of fibers.

The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index. The foaming index of *Murraya koenigii* leaves revealed that saponin is absent in the leaves of *Murraya koenigii*. Many medicinal plants contain saponins that can cause persistent foam when an aqueous decoction is shaken.

Fluorescence behavior of powder as well as extracts (Table 3.1 & 3.2) in both UV and day light were analyzed. Leaf powder as well as extract showed various colors under UV and day light. Volatile oils are characterized by their odour, oil-like appearance and ability to volatilize at room temperature and are specific for a particular drug. Chemically, they are usually composed of mixtures of organic compounds such as monoterpenes, sesquiterpenes, their oxygenated derivatives and some other aromatic compounds which predominate in volatile oils. They are considered to be the "essence" of the plant material, and are often biologically active, they are also known as "essential oils". The term "volatile oil" is preferred because it is more specific and describes the physical properties. The plant contains very low amount of oils (1.5%). The leaves possess strong characteristic smell even though it contains very low amount of oil. The pH of the plant powder is 5.7. It revealed that the leaves were slightly acidic in nature.

Table 3.1: Fluorescence analysis of leaf powder of *Murraya koenigii*

Sl. No	Reagents	Day light	Short UV (254nm)	Long UV (365 nm)
1.	Powder	Moss green	Cowdung green	Black
2.	Powder + Conc.HCL	Dark green	Black	Black
3.	Powder + Conc.HNO ₃	Orange	Dark green	Black
4.	Powder + Conc.H ₂ SO ₄	Cowdung green	Dark green	Black
5.	Powder + Acetic acid	Brown	Black	Black
6.	Powder + Ethanol	Cowdung green	Dark green	Black
7.	Powder + Methanol	Cowdung green	Dark green	Black
8.	Powder + Acetone	Olive green	Dark green	Dark brown
9.	Powder + 1N NaOH	Dark green	Brown	Black

Table 3.2: Fluorescence analysis of various extracts of *Murraya koenigii* leaves

Sl No	Extracts	Day light	Short UV (254nm)	Long UV (365 nm)
1	Petroleum ether	Olive green	Black	Violet
2	Chloroform	Dark green	Black	Violet
3	Methanol	Yellowish green	Dark green	Violet
4	Hydro alcohol	Brown	Dark green	Violet
5	Water	Brown	Moss green	Violet

Many medicinal plant materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums and those containing an appreciable amount

of mucilage, pectin or hemicelluloses. The swelling index of *Murraya koenigii* leaves is 11.5 %.

7. Conclusion

The present study pharmacognostically standardize *Murraya koenigii*, which will help for the proper identification of the drug. In recent years there has been an emphasis in pharmacognostical standardization of medicinal plants of therapeutic potential. *Murraya koenigii* is a medicinal plant with various therapeutic potential. So the present study can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant materials for future studies.

8. Acknowledgement

The financial assistance from DST/ INSPIRE, New Delhi, India and the facilities provided by University of Kerala are greatly acknowledged.

9. References

1. Xie JT, Chang WT, Wang CZ, Mehendale SR, Li J, Ambihapahar R, *et al.* Curry leaf *Murraya koenigii* Spreng. reduces blood cholesterol and glucose levels in ob/ob mice. *The American Journal of Chinese Medicine* 2006; 34:279-284.
2. Purthi JS. *Spices and Condiments*. New Delhi, India: National Book Trust, 1976.
3. Mhaskar KS, Blatter E, Caius JF. Kirtikar and Basu's *Illustrated Indian Medicinal Plants*, Edn 3, Indian Medical Science Delhi, India. 2000; 1(11):86-96.
4. *The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products*, New Delhi, 2003, 4.
5. Parrota JA. *Healing Plants of Peninsular India*. USA: CASI. Publication, 2001.
6. Kumar VS, Sharma A, Tiwari R, Kumar S. *Murraya koenigii*: A review. *Journal of Medicinal and Aromatic Plant Science*. 1999; 2(1):1139-1144.
7. Gupta S, George M, Singhal M, Sharma GN, Garg V. Leaf extract of *Murraya koenigii* Linn. for anti-inflammatory and analgesic activity in animal models. *Journal of Advanced Pharmaceutical Technology and Research*. 2010; 1:68-77.
8. Joshi BS, Kamat VN, Gawd DH. Structures of Girinimbine, Mahanimbine, Isomahanimbine, Koenimbidine and Murrayacine. *Tetrahedron*. 1970; 26(6):1475-1482.
9. Mandal NA, Kar M, Banerjee SK, Das A, Upadhyay SN *et al.* Antidiarrhoeal activity of carbazole alkaloids from *Murraya koenigii* Spreng Rutaceae seeds. *Fitoterapia*. 2010; 81:72-74.
10. Nayak S. Influence of Ethanol Extract of *Vinca rosea* on Wound Healing in Diabetic Rats. *Online Journal of Biological Sciences*. 2006; 6(2):51-55.
11. Samsam SH, Moatar F. *Natural medicines and plants*. Mashal Publications Tehran, 1991, 123-130.
12. Gahlawat DK, Jakhar S, Dahiya P, nd *Murraya koenigii* (L.) Spreng: an ethnobotanical, phytochemical and pharmacological review.
13. *Indian Pharmacopoeia*. Govt. of India, Ministry of Health and Family Welfare. Delhi: Indian Pharmacopoeial commission, Ghaziabad, 2010.
14. *Quality control methods for medicinal plant materials world health organization* Geneva. 1998; 28-34:45-46.
15. Gomez MH, Aguilera JM. A physico-chemical model for extrusion of corn starch. *J Food Sci*. 1984; 49:40-43:63.
16. *Manual of methods of analysis of foods, Food safety and standards authority of India, Ministry of Health and Family welfare, Govt of India, New Delhi*. 2015, 20-21.
17. Kokashi CJ, Kokashi RJ, Sharma M. Fluorescence of powdered vegetable drugs in ultra- violet radiation. *J Am Pharm Assoc*. 1958; 47:715-717.
18. Soni S, Kondalkar A, Tailang M, Pathak AK. Pharmacognostic and Phytochemical Investigation of *Stevia rebaudiana*, *Pharmacognosy Mag*. 2008; 4(13,1):89.
19. Pandey CN, Raval BR, Mali S, Harshal S. *Medicinal Plants of Gujarat (Gujarat Ecological Education and Research (GEER) foundation, Gandhinagar)*, 2005, 211.
20. Adeshina GO, Onaolapo JA, Ehinmidu JO, Odama LE. Phytochemical and Toxicologic Activity of Leaf Extracts of *Alchornea cordifolia* (Schum and Thonn) Muell. Arg. *Nig. J Pharm. Res*. 2008; 6(1):19.