Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 1161-1163
Received: 24-07-2020
Accepted: 25-08-2020

Shanthini Nachiar G
Department of Pharmacognosy, C L Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India

Sheela T
Department of Pharmacognosy, C L Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India

Sameema Begum
Department of Pharmacognosy, Nandha College of Pharmacy, Erode, Salem, Tamil Nadu, India

Corresponding Author:
Shanthini Nachiar G
Department of Pharmacognosy, C L Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India

Extraction, fractionation, isolation and characterization of a Carbazole Alkaloid Mukonicine from the leaves of *Murraya koenigii*

Shanthini Nachiar G, Sheela T and Sameema Begum

Abstract
The dried powdered leaves of *M. koenigii* was defatted with petroleum ether by continuous hot percolation using Soxhlet apparatus then extracted with ethanol and concentrated. The concentrated extract was digested with hydrochloric acid and filtered. The ensure was washed with water until acid free and dried. The dark green residue was extracted with benzene and the concentrated. About 6g of the residue was obtained as yield was subjected to column chromatography to isolate the active constituents. Column chromatography was prepared by mixing 150g of alumina in to a slurry with petroleum ether and pouring mixture in to the glass column. After the absorbent has settled, a filter paper disc kept on the top of the column. So that the adsorbent layer is not disturbed during the introduction of the sample or mobie phase. The residue charged on the coumn and eluted successively by gradient elution technique with petroleum ether, benzene and chloroform. About 5ml of eluate collected in a test tube and subjected to TLC analysis in order to identify the presence of compounds. The eluates which gave the same Rf value were pooled together and it was subjected to TLC analysis for the confirmation of the presence of a single compound.

Keywords: Column size 60cm × 4cm, adsorbent-neutral alumina, TLC, silica gel G (Merck) was used. Jasco FT/IR 410 for IR, Bruker 300MHz spectrometer for NMR

Introduction
Murraya koenigii, commonly known as curry leaf or kari patta in Indian dialects, belonging to Family Rutaceae which represent more than 150 genera and 1600 species *Murraya koenigii* is a highly values plant for its characteristic aroma and medicinal value. It is an important export commodity from India as it fetches good foreign revenue [1-4]. A number of chemical constituents from every part of the plant have been extracted. The most important chemical constitutents responsible for its intense characteristic aroma are P-gurjunene, P-caryophyllene, P-elemene and O-phellandrene. The plant is rich source of carbazole alkaloids Bioactive coumarins, acridine alkaloids and carbazole alkaloids from family Rutaceae. *M. koenigii* is widely used in Indian cookery for centuries and have a versatile role to play in traditional medicine. The plant is credited with tonic and stomachic properties. Bark and roots are used as stimulant and externally to cure eruptions and bites of poisonous animals. Green leaves are eaten raw for cure of dysentery, diarrhoea and for checking vomiting. Leaves and roots are also used traditionally as bitter, anthelmintic, analgesic, curing piles, inflammation, itching and are useful in leucoderma and blood disorders Several systematic scientific studies are also being conducted regarding the efficacy of whole plant or its parts in different extract forms for the treatment of different diseases. *M. koenigii* contains a number of chemical constituents that interact in a complex way to elicit their pharmacodynamic response [6-10] A number of active constituents responsible for the medicinal properties have been isolated and characterized. This plant has been reported to have anti-oxidative, cytotoxic, antimicrobial, antibacterial, anti ulcer, positive inotropic and cholesterol reducing activities.

Material and Methods
A.R grade ethanol, benzene, petroleum ether chloroform were used for extraction, column chromatography and TLC. For column chromatography alumina neutral was used, for thin layer chromatography silica gel G was used. Jasco FT/IR 410 for IR spectra and Bruker 300MHz spectrometer for NMR were used. A column size 60cm x 4cm (length and diameter) used for the chromatography. The adsorbent used was neutral alumina for column chromatography.
Column chromatography was prepared by mixing 150gm of alumina in to slurry with petroleum ether and pouring the mixture in to the glass column.

After the adsorbent has settled, a filter paper disc kept on the top of the column. So that, the adsorbent layer is not disturbed during he introduction of the sample or mobile phase. The residue (5g) charged on the column and eluted successively by gradient elution technique with petroleum ether, benzene and chloroform. About 5ml of eluates collected in the test tube and subjected to TLC analysis in order to identify the presence of compounds. The eluates which gave the same Rf value were pooled together and it was subjected to TLC analysis for the confirmation of the presence of a single compound. The results were tabulated in the table 1

Table 1: TLC analysis for the confirmation of the presence of a single compound

Fraction number	Eluent	Nature of the residue	Examination on TLC
1-20	Pet.Ether	No residue	No spot
21-40	Pet-ether-benzene (50:50)	No residue	No spot
41-47		No residue	No spot
48-62	Benzene	A pale yellow residue	Single spot
63-70		No residue	No spot
71-90	Benzene-chloroform (50:50)	No residue	No spot
91-110	chloroform	No residue	No spot

Identification of the Isolated Compound
Chemical test: Conc.H2so4-violet color
Solubility: Soluble in ethanol, Benzene and insoluble in water
Melting point: 230 ^{0}C

TLC OF The Isolated Compound

Fig 1: Represents a brown spot of a isolated compound

TLC
Adsorbent: Silica gel G
Detecting Reagent: Conc.H2s04
Solvent system: Benzene: Chloroform (1:1)
Rf value: 0.4
The residue was subjected to IR and NMR spectral studies to identify the chemical nature of the compound.

Result and Discussion
Carbazole alkaloid isolated from the ethanolic extract of the leaves of *M. koenigii* by column chromatography [13]. The isolated compound was subjected to TLC (Rf-0.4) and melting point (230◦) determination and the compound characterized by IR and H NMR spectral studies. The results for the interpretation of the IR and NMR spectra are as follows.
Mukonicine isolated from the ethanolic extract of the leaves by column chromatography. The isolated compound was identified by TLC and melting point determination and characterized by IR and NMR spectral studies.

Spectral Studies: [14-20]
Infra red spectra
OH and NH stretching vibration at 3427.27cm^{-1}
C-H stretching at 2850.59
C-H bending at 1376.12
C-O stretching at 1111.89
C=C stretching in aromatic nuclei at 1633.59 and 1462.91

Proton NMR spectra
Aromatic proton at 7.263ppm
Olefinic protons (HC=H) group at 5.345ppm
Six protons of two aromatic –OMe group at 3.5ppm
CH3-C group at 1.55ppm
Three protons of an aromatic C-Me group at 2.3ppm.

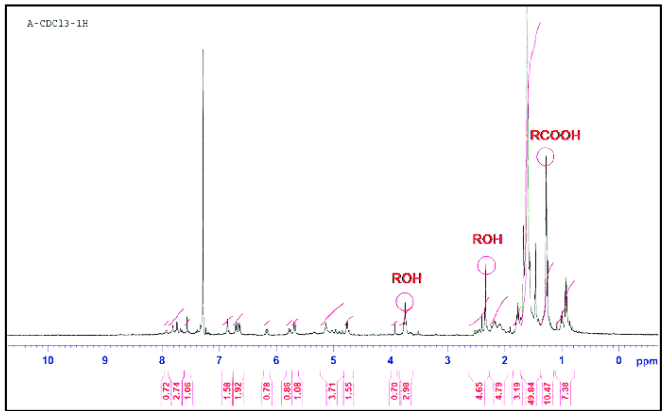


Fig 2: represents the NMR spectra of *M. koenigii*

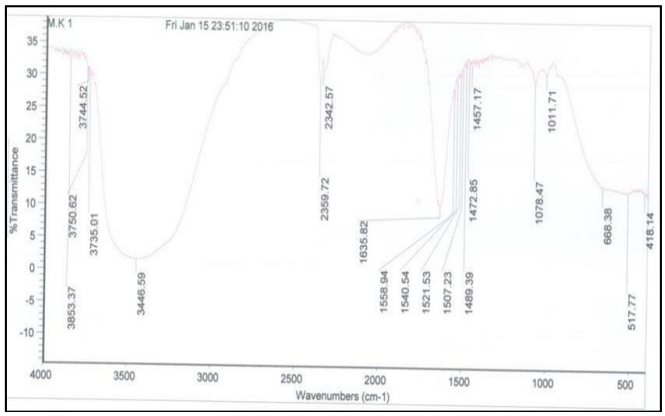
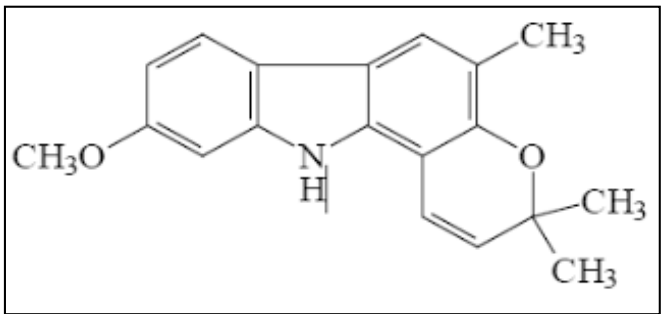


Fig 3: represents the IR spectral studies of *M. koenigii*

Thus, the result obtained from the Melting point, TLC and IR, and [1] H-NMR spectra revealed that the compound isolated may be Mukonicine



References

1. Satyavati GV, Gupta AK, Tendon N. Medicinal Plants of India, Indian council of medical research, New Delhi India. 1987; 2:289-299
2. Kumar VS, Sharma A, Tiwari R, Sushil K. *Murraya koenigii*- a review, JMAPS C.Studies On Medicinal Resources of Rutaceous Plants And Development To Pharmaceutical Chemistry, Natural Med. 2000; 54:117-122. 1999; 21(4):
3. Nadkarni KM, Indian Materia Medica, Edition, Popular Prakashan, Mumbai. 1976; 3(I):196.
4. Kirtikar KR, Basu BD. Indian Medicinal Plants, Edition Oriental Enterprises, Uttaranchal Pradesh. 1981; 2(I):473.
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. 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, Aryavaidyan J. 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, *et al*. *Murraya koenigii* reduces blood cholesterol and glucose level in ob/ob mice. American Journal of Chinese 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. Mukherjee M, Mukherjee S, Shaw AK, Ganguly SN. "Mukonicine-A carbazole alkaloid from Leaves of *Murraya koenigii*" Phytochemistry. 1983; 22(10):2328-2329.
14. Douglas A, Skoog, Donald M, West F, James Haller, Stanley R *et al*. Fundamentals of Analytical Chemistry-An Introduction to analytical separation", 8th edition, Eastern Press (Pvt) Ltd, 2004, 920-921.
15. Beckett AH, Stenkale JB. Practical Pharmaceutical Chemistry 4th edition, CBS publishers and distributers, Delhi, 1977, 2.
16. Robert M. Silverstein and Clayton Bassler G., Spectro metric identification of organic compounds, S. Chand & Com. Ltd, New Delhi, 2005; 103:180.
17. Sharma YR. Elementary organic spectroscopy-Principles and chemical applications S. Chand & Com. Ltd, New Delhi, 2005, 103:108.
18. John R. dyer Application of absorption of aromatic compound 8th ed., PHI publishers, 1991.
19. William Kemp. Organic spectroscopy 3rd ed., MacMillan press Ltd London, 1994.
20. Frank Settle. Hand book of Instrumental techniques for analytical chemistry, Ronald A. Hites Gas chromatography-Mass spectroscopy person education (Singapore) Pvt. Ltd. Delhi. India, 2004, 615.