

GC/MS Determination of Bioactive Components of *Murraya koenigii*¹Hema R., ²S. Kumaravel and ³K. Alagusundaram¹Senior Research Fellow, Department of Food Quality and Testing, IICPT²Scientist, Department of Food Quality and Testing, IICPT³Director, Indian Institute of Crop Processing Technology (IICPT), Thanjavur, TamilNadu, India
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Abstract: In this study, the bioactive components of *Murraya koenigii* leaves have been evaluated using GC/MS. The chemical compositions of the ethanol extract of *Murraya koenigii* were investigated using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of ethanol extract of *Murraya koenigii* revealed the existence of 1-Methyl-pyrrolidine-2-carboxylic acid (69.00%), Ethyl α -D-glucopyranoside (13.36%), Isolongifolone, 4,5-dehydro- (3.68%), ζ -HIMACHALENE (2.88%), 1,2-Ethanediol, monoacetate (2.79%) 1,2-Benzenedicarboxylic acid, diisooctyl ester (2.55%). The results of this study offer a platform of using *Murraya koenigii* as herbal alternative for the current synthetic antimicrobial agents.

[Hema R., S. Kumaravel and K. Alagusundaram. **GC/MS Determination of Bioactive Components of *Murraya koenigii***. Journal of American Science 2011;7(1):80-83]. (ISSN: 1545-1003). <http://www.americanscience.org>.

Key words: *Murraya koenigii*, GC/MS, Bioactive components

Introduction

Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties (de-Fátima *et al.*, 2006). Distinguished examples of these compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides (Shahidi 2000 and Shahidi, *et al.*, 2008). Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant because these may serve as talented sources of new antibiotic prototypes (Meurer-Grimes *et al.*, 1996; Koduru *et al.*, 2006). It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations (Mathekaga and Meyer, 1998).

Murraya koenigii is an aromatic leaf often used in Indian cuisine. It is a tropical to sub-tropical tree in the family Rutaceae, which is native to India. The name itself in Tamil is pronounced as 'kariveppilai' (kari-curry, veppu- neem and ilai-leaf) which is the literal translation of curry leaves. The Tamil name means "leaf that is used to make curry" and present in almost all the dishes of Tamil nadu in addition to coriander leaves, a state of south India. Often used in curries, the leaves generally go by the name "curry leaves", though they are also called "sweet neem leaves." It is an unavoidable content of curries in South

India, where without curry leaves, curry seems to be tasteless. Curry leaves are also entirely unrelated to bay leaves and basil leaves, which are aromatic leaves from the Mediterranean.

It is a small tree, growing 4-6 m tall, with a trunk up to 40cm diameter. The leaves are pinnate, with 11-21 leaflets, each leaflet 2-4 cm long and 1-2 cm broad. They are highly aromatic. The flowers are small, white, and fragrant. The small black shiny berries are edible, but their seeds are poisonous.

The leaves are highly valued as seasoning in which it is usually fried along with the chopped onion in the first stage of the preparation. In their fresh form, they have a short shelf life, and they don't keep well in the refrigerator. They are also available dried, though the aroma is largely inferior. Although most commonly used in curries, leaves from the curry tree can be used in many other dishes to add spice.

The leaves of *Murraya koenigii* are also used as a herb in Ayurvedic medicine. Their properties include much value as an anti-diabetic (Arunselvan *et al.*, 2006; Yadav *et al.*, 2002; Vinuthan *et al.*, 2004; and Achyut *et al.*, 2005), antioxidant (Arunselvan *et al.*, 2007; Vinuthan *et al.*, 2004; Singh *et al.*, 1978; Goutam *et al.*, 1974; Deshmukh *et al.*, 1986; Baliga *et al.*, 2003), antimicrobial (Abhishek Mathur *et al.*, 2010; Vinuthan *et al.*, 2004; Singh *et al.*, 1978; Goutam *et al.*, 1974; Deshmukh *et al.*, 1986; Baliga *et al.*, 2003), anti-inflammatory (Muthumani *et al.*, 2009), hepatoprotective (Pande *et al.*, 2009), anti-hypercholesterolemic (Iyer *et al.*, 1990 and Khan *et al.*, 1996), as well as efficient against colon carcinogenesis (Iyer *et al.*, 1990) etc. Curry leaves are also known to be good for hair, for keeping it healthy and long.

Materials and Methods

Plant material and extraction procedure

Leaves of *Murraya koenigii* were bought fresh from local market, Thanjavur. 10gm powdered plant material was soaked in 20ml of Absolute alcohol overnight and then filtered through a Whatman® No. 41 filter paper (pore size 20 - 25 μ m) along with 2gm Sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and was concentrated to 1ml. The extract contains both polar and non-polar phytochemicals.

Gas Chromatography–Mass Spectrometry (GC/MS) analysis

GC/MS analysis of this extract was performed using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) equipped with a Elite-1 fused silica capillary column (30 m \times 0.25 mm ID. \times 1 μ Mdf, composed of 100% Dimethyl poly siloxane). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the

carrier gas at a constant flow rate of 1 ml/min. and an injection volume of 2 μ l was employed (split ratio of 10:1). Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver5.2.0

Results and Discussion

Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and Structure of the components of the test materials were ascertained.

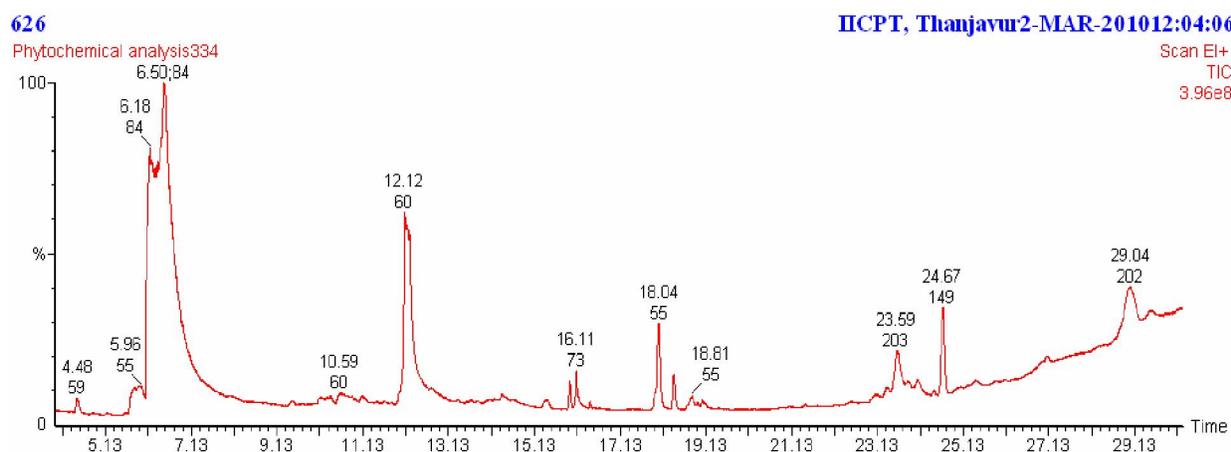


Figure 1: Chromatogram obtained from the GC/MS with the extract of *Murraya koenigii*

Table 1. Total ionic chromatogram (GC–MS) of ethanol extract of *Murraya koenigii* obtained with 70 eV using a Elite-1 fused silica capillary column with He gas as the carrier.

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	4.48	Propane, 1,1,3-triethoxy-	C ₉ H ₂₀ O ₃	176	0.56
2	5.96	1,2-Ethanediol, monoacetate	C ₄ H ₈ O ₃	104	2.79
3	6.50	1-Methyl-pyrrolidine-2-carboxylic acid	C ₆ H ₁₁ NO ₂	129	69.00
4	12.12	Ethyl α -D-glucopyranoside	C ₈ H ₁₆ O ₆	208	13.36

5	15.41	Pentadecanoic acid, 14-methyl-, methyl ester	C ₁₇ H ₃₄ O ₂	270	0.39
6	16.11	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	0.81
7	16.43	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	0.11
8	18.04	Oleic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296	2.54
9	18.39	Phytol	C ₂₀ H ₄₀ O	296	0.72
10	18.81	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	0.60
11	23.59	ζ-HIMACHALENE	C ₁₅ H ₂₄	204	2.88
12	24.67	1,2-Benzenedicarboxylic acid, diisooctyl ester	C ₂₄ H ₃₈ O ₄	390	2.55
13	29.04	Isolongifolene, 4,5-dehydro-	C ₁₅ H ₂₂	202	3.68

Thirteen compounds were identified in *Murraya koenigii* leaf extract by GC-MS analysis. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and Concentration (%) are presented in (Table 1 and Fig 1). The prevailing compounds were 1-Methyl-pyrrolidine-2-carboxylic acid (69.00%), Ethyl α-d-glucopyranoside (13.36%), Isolongifolene, 4,5-dehydro- (3.68%), ζ-HIMACHALENE (2.88%), 1,2-Ethanediol, monoacetate (2.79%) 1,2-Benzenedicarboxylic acid, diisooctyl ester (2.55%).

Table 2: Major Phyto-components and its biological activities obtained through the GC/MS Study of *Murraya koenigii* have been listed

Sl. No.	Retention Time	Peak Area %	Name of the Compound	Active biological activity
1.	6.50	69.00	1-Methyl-pyrrolidine-2-carboxylic acid	Used in the formulation of drugs by both oral and transdermal delivery routes
2.	12.12	13.36	Ethyl α-d-glucopyranoside	Preservative
3.	16.11	0.81	n-Hexadecanoic acid	Antioxidant, hypocholesterolemic nematocide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
4.	16.43	0.11	Hexadecanoic acid, ethyl ester	Antioxidant, hypocholesterolemic nematocide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
5.	18.04	2.54	Oleic acid, methyl ester	5-Alpha-Reductase-Inhibitor, Allergenic, Alpha-Reductase-Inhibitor, Anemiagenic, Antiallopecic, Antiandrogenic, Antiinflammatory, Antileukotriene-D4 (Anti-platelet activating factor), Cancer-Preventive, Choloretic, Dermatitigenic Flavor, Hypocholesterolemic, Insectifuge Irritant, Percutaneostimulant, Perfumery, Propecic
6.	18.39	0.72	Phytol	Cancer-Preventive
7.	18.81	0.60	9,12-Octadecadienoic acid (Z,Z)-	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematocide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge
8.	23.59	2.88	ζ-HIMACHALENE	Used in flavouring of spirit drinks
9.	24.67	2.55	1,2-Benzenedicarboxylic acid, diisooctyl ester	Used as Softeners, Used in preparation of perfumes and cosmetics, Used as plasticized vinyl seats on furniture and in cars, and clothing including jackets, raincoats and boots. Used in textiles, as dyestuffs, cosmetics and glass making.
10.	29.04	3.68	Isolongifolene, 4,5-dehydro-	Anti-proliferative

The biological activities listed are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

Acknowledgement

I would like to thank wholeheartedly Sh. S. Kumaravel, Scientist, Department of Food Quality and Testing, Indian Institute of Crop Processing Technology and Dr. K. Alagusundaram, Director, Indian Institute of Crop Processing Technology for guiding and supporting me, throughout.

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11/22/2010