

RESEARCH ARTICLE

Phytochemical analysis of inevitably important plant *Murraya Koenigii* from upper plateau of Chikhaldara (Melghat) India

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The medicinal plants are almost the exclusive source of drugs for majority of world population today. People want to use herbal drugs because they are considered as safe, inexpensive and have no adverse effects. Plants are also very useful because they can self-generate and can produce a range of beneficial bioactive products. The leaves of *Murraya koenigii* are used as tonic, stomachic, carminative, internally in dysentery, vomiting. Used as anti-helminthic, analgesic, cures piles, allays heat of the body, thirst, inflammation and itching. A scrutiny of literature reveals some notable pharmacological activities of the plant such as activity on heart, anti diabetic and cholesterol reducing property, antimicrobial activity, antiulcer activity, antioxidative property, cytotoxic activity, anti diarrhea activity, phagocytic activity and many more medicinal values. The present study was aimed to isolate phytochemical constituents from *Murraya Koenigii* showing antioxidant properties. Total 12 compounds were isolated which include Cyclopentanol, α -Pinene, cyclohexyl butyl ester, α -Phellandrene, (-)-Spathulenol, Dibutyl phthalate, α -Sitosterol, 13-Docosanamide, Benzenemethanimine, 1-(3-Methoxy-2-nitrobenzyl) isoquinoline, Tetratriacontane, Vitamin E.

Keywords: *Murraya koenigii*, anti-helminthic, antiulcer activity, antioxidative property, cytotoxic activity, anti diarrhea activity, phagocytic activity, Melghat.

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 1500 species [1]. *Murraya Koenigii*, is a native of India, Sri Lanka and other south Asian countries. It is found almost everywhere in the Indian subcontinent, it shares aromatic nature, more or less deciduous shrub or tree up to 6 m in height and 15-40 cm in diameter with short trunk, thin smooth grey or brown bark and dense shady crown [2]. The *M. koenigii* is having grey color bark, longitudinal striations on it and beneath it white bark is present. Leaves are bipinnately compound, 15-30 cm long each bearing 11-25 leaflets alternate on rachis, 2.5-3.5 cm long ovate lanceolate with an oblique base. Margins irregularly crenate, petioles 2-3 mm long, flowers are bisexual, white, funnel shaped sweetly scented, stalked, complete, ebracteate, regular with average diameter of fully opened flower being in average 1.12 cm inflorescence, terminal cymes each bearing 60-90 flowers. Fruits are ovoid to subglobose, wrinkled or rough with glands. It is having the size of 2.5 cm long and 0.3 cm in diameter and gets purplish black when ripen. Fruits are generally biseeded. Seeds generally occur in green color, 11 mm long, 8 mm in diameter.

Murraya Koenigii is a highly valued plant for its characteristic aroma and medicinal value. A number of chemical constituents from every part of the plant have been extracted. The basic medicinal property of these plants lies in some chemical substances. The main nutrients found in curry leaves are carbohydrates, calcium, phosphorus, iron, magnesium, copper and minerals. It also contains various vitamins like nicotinic acid and vitamin C, vitamin A, vitamin B, vitamin E, antioxidants, plant sterols, amino acids, glycosides and flavonoids. It is traditionally used as a whole or in parts as antiemetics, antidiarrheal, febrifuge, blood purifier, antifungal, depressant, anti-inflammatory, body aches, for kidney pain and vomiting [3]. Curry leaf is a good source of vitamin A, calcium and folic acid. Curry leaves offer antioxidant support and help prevent cancer of the skin and stomach. In a study published in Nutrition Research by Jawaharlal Nehru University in 2003, curry leaves significantly reduced the incidence of cancer cells in the stomach and skin tissues [4].

Research studies conducted by Mylarappa B. Ningappa *et al.* at Jawaharlal Nehru Center for Advanced Scientific Research, Molecular Parasitology and Protein Engineering Laboratory in Bengaluru, India have indicated that curry leaves or *Murraya Koenigii* is a good source of antioxidants. The presence of various vitamins like vitamin A, B, C and E help in reducing oxidative stress and free radical scavenging activity. The present study documents phytochemical constituents from *Murraya Koenigii* showing antioxidant properties.

METHODOLOGY

Collection of plant material

The fresh leaves of *Murraya Koenigii* were collected from upper plateau of Chikhaldara District Amravati (Maharashtra) in the month July to September.

Antioxidant activity of *Murraya Koenigii* :-

The radical scavenging activity of plant extracts against 2,2-Diphenyl-1-picryl hydroxyl radical were determined by UV visible spectrophotometer carry 60 (Agilent). Antioxidant present in plant were quantified employing Folin's reagent i.e. DPPH. DPPH assay is often used to evaluate the ability of antioxidant to scavenge free radicals which are known to be a major factor in biological damage caused by oxidative stress.

Antioxidant activity (DPPH free radical scavenging activity) of Methanolic extract

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was determined by the method described by Shen *et al.* The diluted working solutions of the test extract were prepared in methanol. Ascorbic acid was used as standard in 1000-5000 µg/ml solution. 3.94 mg of DPPH was prepared in 100ml methanol and 2.96 ml of this solution was mixed with 40 µl of sample solution and standard solution separately. These solution mixtures were kept in dark for 20 min and optical density was measured at 517nm using UV-Visible Spectrophotometer (carry 60 Agilent). DPPH solution was used as blank. The optical density was recorded and percentage inhibition was calculated using the formula given below

$$\% \text{ of DPPH radical scavenging activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Abs control is the absorbance of DPPH radical and methanol. Abs sample is a absorbance of DPPH radical + sample extract was measure absorbance values were corrected for free radical decay using blank solution.

Abs control is the absorbance of DPPH radical and methanol. Abs sample is the absorbance of DPPH radical + sample extract was the measure. Absorbance values were corrected for free radical decay using blank solution. And IC_{50} value can calculate by using calibration curves verses percentage of inhibitions.

Preparation of plant extract

The plant were dried over ambient temperature and the dried sample were grind properly and dried powder sample was extracted in Soxhlet extractor by using solvent Methanol at 65°C, extracts were concentrated by gradually evaporating the respective solvent on rotary evaporator. The concentrated extract was collected in sterile bottles and kept in a cool and dark place prior to analysis [5].

Isolation of bioactive chemicals by column chromatography

Column chromatography was performed on a classic 20cm long and 2 cm diameter glass column packed with silica gel G Merck, Germany. The concentrated extract of *M. koenigii* (20 mL) was applied to the column by use of a pipette and the column was eluted sequentially with 90% Benzene and 10% Ethanol each fraction collected was tested prior GC-MS study [2].

GC-MS Analysis of *Murraya Koenigii*

Gas Chromatography:-

Gas chromatography of the plant extract was carried out on a 6890 Gas chromatography model 5765 equipped with direct injector and split ratio set to 10:1 (DB-5) (5% phenyl polysioxane, 30m length 250u internal diameter; 0.25um film coating) fused capillary column. Helium was carrier gas at 1.0 ml min. The oven temperature program was to start at 35°C hold for 2 min. then temp. at 20°C per min. to 300°C and hold for 5 min. Injector and detector temperature were 220°C and 230°C respectively. Injection size was 0.02 ul [6].

Gas Chromatography and Mass Spectroscopy

A JEOL GC mate II bench top double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS- 2000 software was used

for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan with a 0.2 seconds inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750 at 1 second per scan.

Identification of chemical constituents

Identification of the chemical constituents was done on the basis of retention index (RI) using a mass spectra library search NIST and by comparing the mass spectral and retention data with literature [7]. The relative amount of individual component were calculated based on the GC peak area (FID response) without using a correction factor.

RESULTS

Simple Reads Report:

Collection Time: 9/9/2016 3:37:26 PM

Method:

Version 5.0.0.999

Instrument: Cary 60

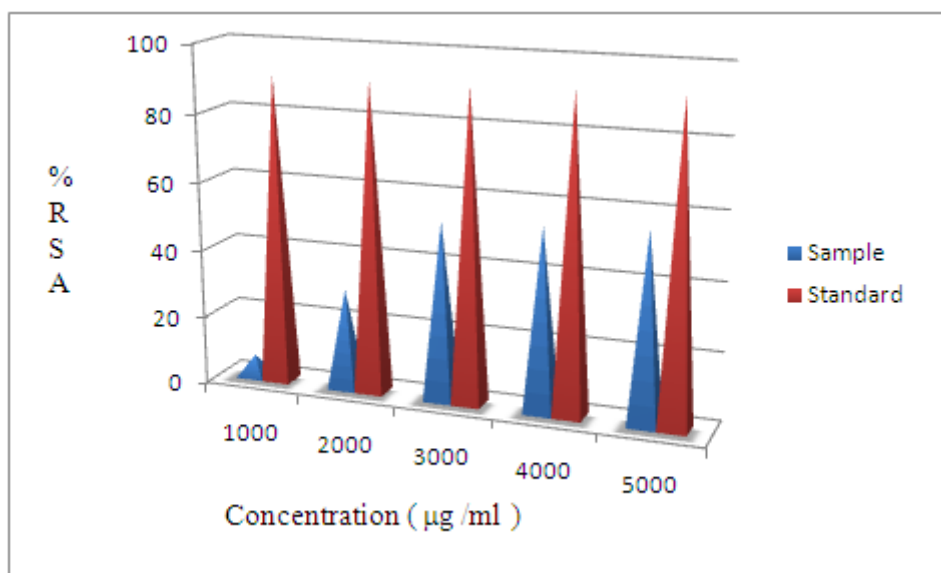
Ave Time (sec) 1.000

Reading	Abs	Nm
DPPH(Control)	0.6963	517.0
M. Koenigii-1	0.6519	517.0
M. Koenigii-2	0.4895	517.0
M. Koenigii-3	0.3304	517.0
M. Koenigii-4	0.3216	517.0
M. Koenigii-5	0.3094	517.0

The radical scavenging activity of the *Murraya Koenigii* leaf extract was tested using stable free radical DPPH (Deep purple colour) as DPPH has the advantage of being unaffected by certain side reaction Graph-1 show the DPPH radical scavenging activity of *Murraya Koenigii* extract with ascorbic acid as reference where the IC_{50} value for the *Murraya Koenigii* extract was calculated by using graph pad prism non linear curve fit Sigmoidal, 4PL, X is log(concentration) and it was found to be IC_{50} = 39.06 lower than 100. The lower the IC_{50} values show the higher antioxidant activities.

Table 1: Simple read report of sample *Murraya Koenigii* and Ascorbic Acid (Standard) at different concentration

Types of Sample	Concentration Ug/ml	% Radical Scavenging activity
<i>Murraya Koenigii</i> Plant Extract (Sample)	1000	6.37656
	2000	29.69984
	3000	52.5491
	4000	53.8130
	5000	55.5651
Ascorbic Acid (Standard)	1000	90.82423
	2000	90.98361
	3000	91.22268
	4000	91.97404
	5000	92.34973

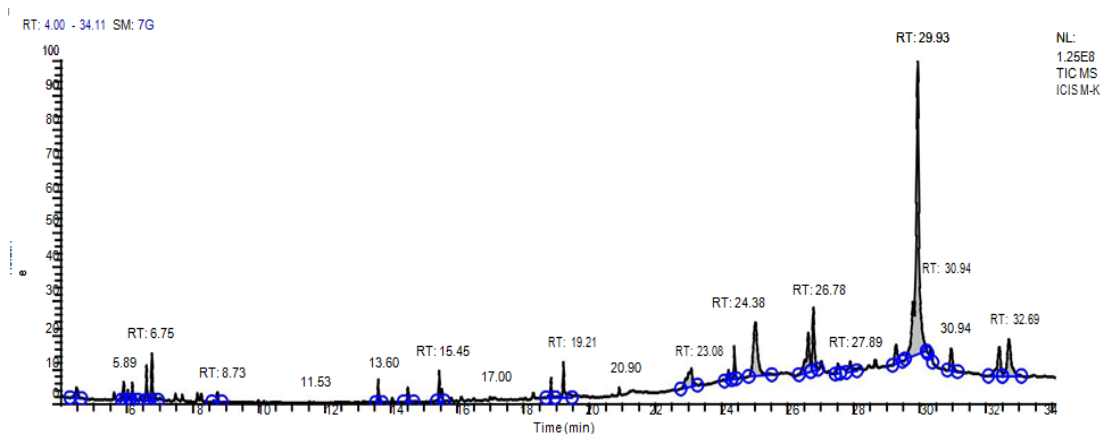
**Graph-** DPPH radical scavenging activity of *Murraya Koenigii***GC-MS of the sample**

GC-MS chromatogram analysis of the Methanolic extract of *Murraya koenigii* Fig. showed major 12 peaks which indicating the presence of various phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library. The various phytochemicals which contribute to the medicinal activities like antimicrobial, antifungal, antiviral and antioxidants. The mass spectra of all the phytochemicals identified in the whole plant the most prevailing compounds were α -Sitosterol (9.37%) is one of

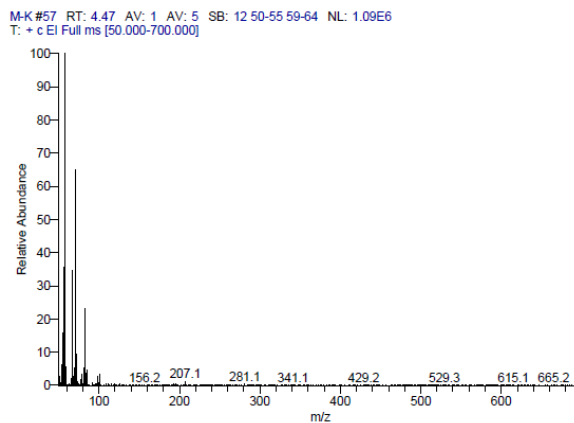
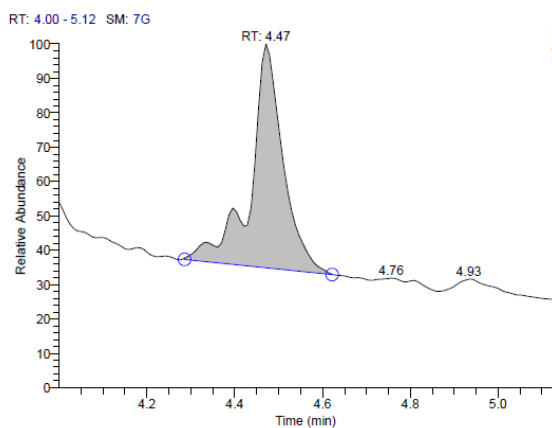
several phytosterols (plant sterols) with chemical structures similar to that of cholesterol. α -sitosterol is being studied for its potential to reduce benign prostatic hyperplasia (BPH) and blood cholesterol levels. 1,3 Docosenamide has an amide compound (4.82%), Benzenemethanimine, 1-(3-Methoxy-2-nitrobenzyl) isoquinoline is a (40.27%). Isoquinoline derivatives as endogenous neurotoxins in the Parkinson's disease. Vitamin E (5.94%) refers to a group of compounds that include both tocopherols and tocotrienols.

Sample Header

Data File: M-K
 Original Data Path: C:\GCMS-data\YEAR 2016\SEP\22
 Sample Type: Unknown
 Sample ID: 1
 Sample Name:
 Acquisition Date: 09/23/16 03:22:58 PM
 Run Time(min): 30.11
 Injection Volume(μl): 1.00
 Scans: 3585
 Low Mass(m/z): 50
 High Mass(m/z): 700
 Instrument Method: C:\GCMS-data\instrument method\GERNAL-gcms-METHOD.meth

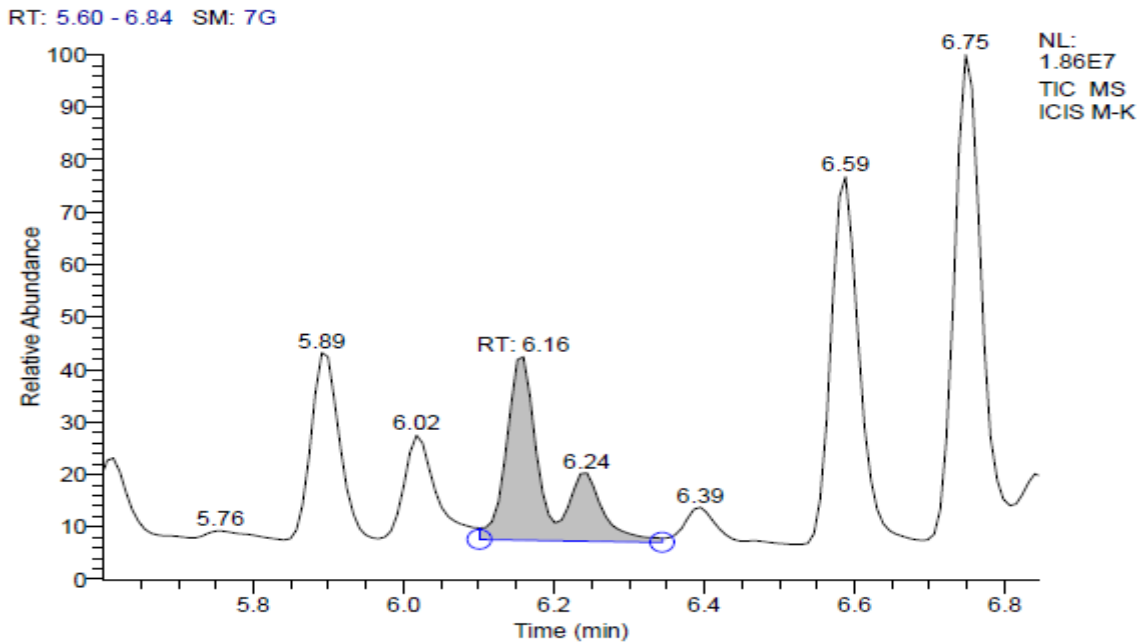


CIL/ SAIF Panjab University Chandigarh



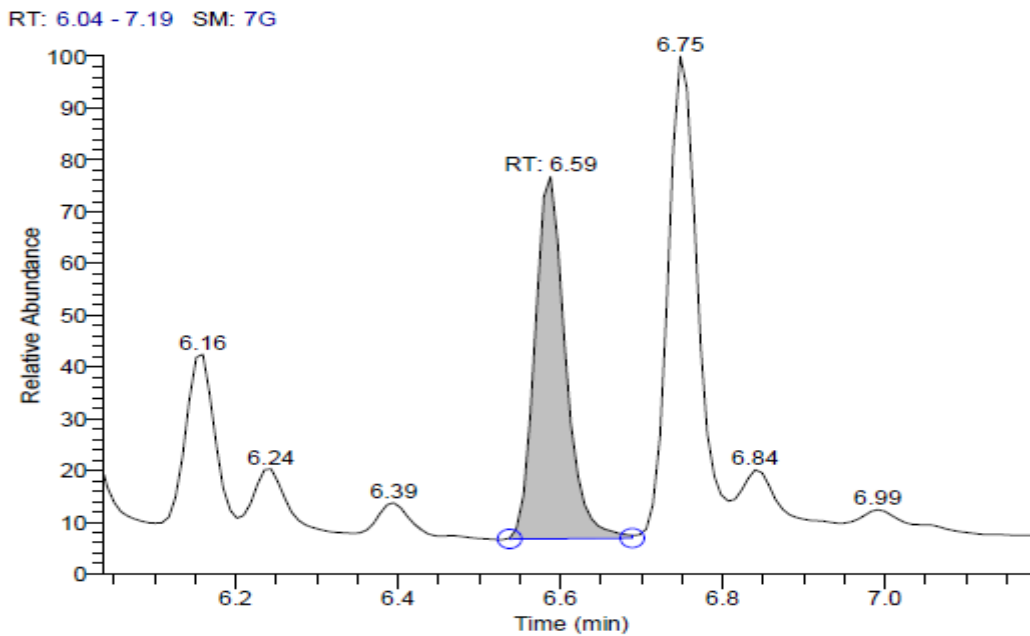
Library Search Results Table

Compound Name	RT	Molecular Formula	Cas #
Cyclopentanol, 3-methyl-	4.47	C6H12O	18729-48-1
Cyclopentanol, 2-methyl-, trans-	4.47	C6H12O	25144-04-1



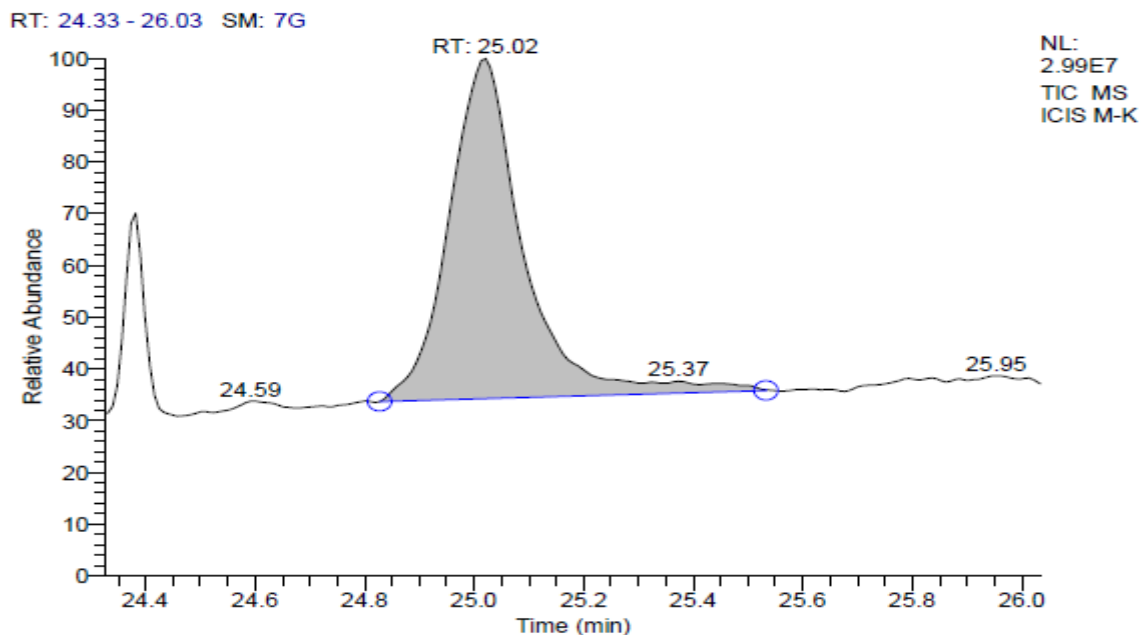
Library Search Results Table

Compound Name	RT	Molecular Formula
à-Pinene	6.16	C10H16



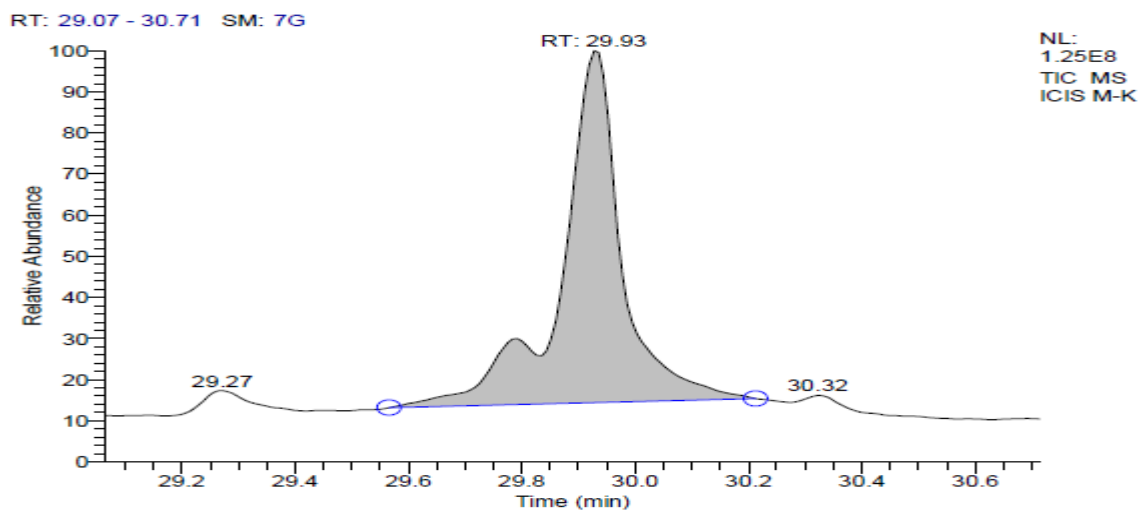
Library Search Results Table

Compound Name	RT	Molecular Formula
Oxalic acid, cyclohexyl butyl ester	6.59	C12H20O4



Library Search Results Table

Compound Name	RT	Molecular Formula	Cas #
ζ-Sitosterol	25.02	C29H50O	83-47-6
â-Sitosterol	25.02	C29H50O	83-46-5



Library Search Results Table

Compound Name	RT	Molecular Formula	Cas #
1-(3-Methoxy-2-nitrobenzyl)isoquinoline	29.93	C17H14N2O3	53055-08-6

Chemical Composition of *Murraya koenigii* leaves

Sr. No	Retention Time	Name of chemical constituent	Molecular Formula	Peak Area %
1	4.47	Cyclopentanol	C ₆ H ₁₂ O	1.07
2	6.16	α-Pinene	C ₁₀ H ₁₆	1.26
3	6.59	cyclohexyl butyl ester	C ₁₂ H ₂₀ O ₄	1.72
4	6.75	α-Phellandrene	C ₁₀ H ₁₆	2.74
5	15.45	(-)-Spathulenol	C ₁₅ H ₂₄ O	2.01
6	19.21	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	2.79
7	25.02	α -Sitosterol	C ₂₉ H ₅₀ O	9.37
8	26.62	13-Docosenamide	C ₂₂ H ₄₃ NO	4.82
9	26.78	Benzenemethanimine	C ₂₁ H ₂₇ N	4.82
10	29.93	1-(3-Methoxy-2-nitrobenzyl)isoquinoline	C ₁₇ H ₁₄ N ₂ O ₃	40.27
11	32.40	Tetratriacontane	C ₃₄ H ₇₀	4.09
12	32.69	Vitamin E	C ₂₉ H ₅₀ O ₂	5.94

The use of vitamin E in the treatment of some cancers is beneficial. Vitamin E and its derivatives promote tumor susceptibility of ionizing radiation during cancer treatment.

CONCLUSION

The presence of various phytochemical compounds in the *Murraya koenigii* justifies the use of whole plant for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting it to the biological activity will definitely give fruitful results. From the results, it could be concluded that *Murraya koenigii* contains various phytochemical compounds. Therefore, it is recommended as a plant of phytopharmaceutical importance.

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