

# STUDIES ON PHYTOCHEMICAL COMPOSITION OF EXTRACT OF *MURRAYA KOENIGII* LEAVES

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## ABSTRACT

The present study shows very interesting findings. The methanolic extract of leaves of *Murraya koenigii* was analysed through phytochemical screening which shows the presence of various phytochemicals like saponins, terpenoids, phenols, steroids, amino acids etc. Also the GC-MS analysis shows the presence of number of bioactive chemical constituents in the methanolic extract of *Murraya koenigii* leaves. These bioactive chemicals have various antimicrobial, antioxidants, Anticancer, Anti-inflammatory, Antipyretic and analgesic activities.

*Index Terms:* Bioactive, Methanolic Extract, Phytochemicals, Screening, GC-MS.

## INTRODUCTION

*Murraya koenigii*, family Rutaceae, commonly known as Curry leaf plant is a highly valued plant for its medicinal value and characteristic aroma, is a popular spice and condiment of India. *Murraya koenigii* also known as sweet neem or kadi patta is a tropical to sub-tropical tree in the family Rutaceae, which is native to India.

The leaves are widely used in many dishes in Indian subcontinent. Often used in curries, the leaves are generally called by the name "curry leaves". It is a small tree, growing 4–6 m tall, with a trunk up to 40 cm in diameter. The leaves of *Murraya koenigii* are also used as a herb in Ayurvedic and Siddha medicine in which they are believed to possess anti-disease properties,<sup>[1]</sup> but there is no high-quality clinical evidence for such effects.<sup>[1][2]</sup>

Phytochemical Screening refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds.

The importance of medicinal plant in drug development is known to human being and have used them for different diseases from the Stone Age [3]. Traditional treatment from wild plants used by tribes has always guided researchers to search for novel medications to develop healthy life for humans and animals [4]. In addition, some medicinal plants are still not investigated clearly so there is a need to evaluate them scientifically

## MATERIALS AND METHODS

### Collection of Sample

Fresh leaf of *Murraya koenigii* were collected from Amravati City, Dist- Amravati (Central region of India) in the month of January –2019.

### Processing of the sample

Fresh Leaves of plants were washed well using tap water and thrice using distilled water and it was dried in shade for a period of 7 days, at an ambient temperature of 28°C. After drying, plant Materials were cut into small pieces. The dried samples were grinded properly using a mortar and pestle and finally using a grinder, to obtain the powdered form and stored at room temperature till their use in the experiment [5]

### Preparation of extracts

Dried powdered material (25 gm) of sample was extracted with methanol in soxhlet apparatus. The temperature of heating mantle was adjusted to 65°C for methanolic extraction. The extract was concentrated by gradually evaporating the solvent in the same extractor. The concentrated extract was collected in sterile bottles and refrigerated until use [6]

### Phytochemical analysis (Qualitative analysis)

#### Test for proteins and amino acids

##### Ninhydrin Test:-

To the sample extract, few drops of Ninhydrin reagent were added. After mixing it well, the solution was boiled in water for 2-3 minutes. A bluish-blackish colour indicated the presence of proteins [8].

##### Test for saponins

Froth test for saponins was used. 1g of the sample was weighed into a conical flask in which 10 ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10 ml of sterile distilled water in a test tube. The test tube was stopper and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

##### Test for carbohydrates

Molisch's reagent was added to 2 ml of both extract. A little amount of concentrated sulphuric acid was added to it and allowed to form a layer. The mixture was shaken well, and allowed to stand for few more minutes, which was then diluted by adding 5 ml of distilled water. Purple precipitate ring showed the presence of carbohydrates [9], [10], [11].

**Test for tannins**

Ferric chloride test:- 0.5g of the dried powdered sample was boiled in 20 ml of water in a test tube & then filtered. A few drops of 0.1%  $\text{FeCl}_3$  was added & observed for brownish green-black or blue-black coloration. [7], [11].

**Test for terpenoids (Salkowski test)**

5 ml of extract was mixed in 2 ml of chloroform, and concentrated  $\text{H}_2\text{SO}_4$  (3 ml) was carefully added to form a layer. A reddish brown coloration at the inter face was formed to show positive results for the presence of terpenoids.

**Test for Phenols: Ferric chloride test:** Extracts was treated with 3-4 drops of ferric chloride solution. Formation of bluish black or dark green colour indicates the presence of phenols.

**Flavonoids Lead acetate Solution Test:** Test solution when treated with few drops of 10% lead acetate solution would results in the formation of yellow precipitate.

**Test for alkaloids**

0.5 gm of extract was stirred with 4 ml of 1% dilute hydrochloric acid. It was boiled and filtered.

**Dragendorff's test**

1 ml of the filtrate was treated with few drops of Dragendorff's reagent. Orange brown precipitate indicated the presence of alkaloids[12], [13].

**Test for Glycosides (Fehling's test)**

Fehling's soln A& B was diluted with distilled water and boiled for 1 min. To this clear blue solution 8 drops of plant extract was added after that it was mixed with 1 ml of fehling's Solution and boiled in a water bath for 5 min. The formation of brick red precipitation indicates the presence of glycosides.

**RESULTS AND DISCUSSION**

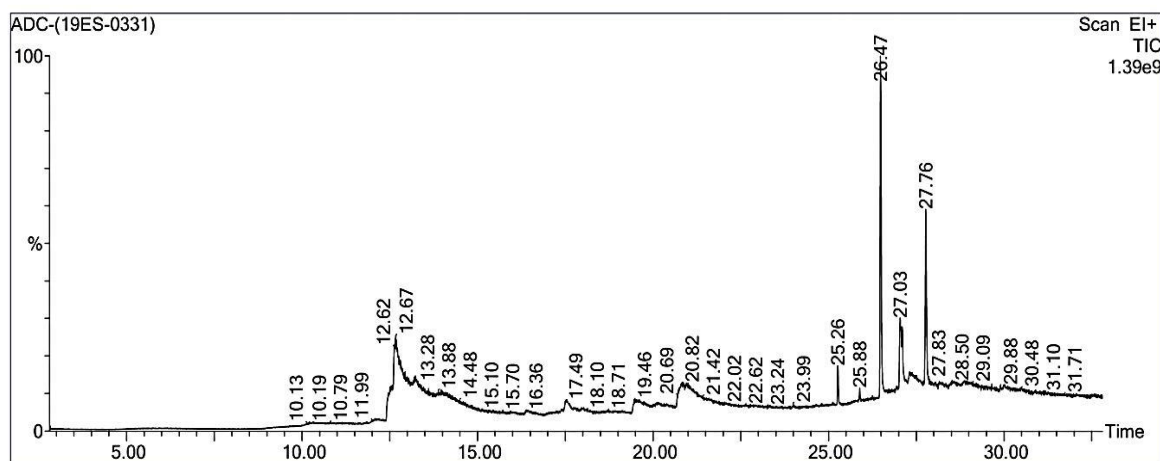
The leaves of *Murraya koenigii* were rich in phytochemical activity, as shown in Table 1.

**Table 1**  
**Phytochemical Screening of *Murraya koenigii* Leaves**

Sr. No.	Test Perform	Result
1	Test for Protein and Amino acids Ninhydrin Test	-
2	Test for Saponins	++
3	Test for carbohydrates Fehling's Test	+
4	Test for tannins Gelatin test	-
5	Test for terpenoids (Salkowski test)	-
6	Test for Phenols	++
7	Test for Flavonoids Lead acetate solution test	-
8	Test for alkaloids Dragendorff's test	++
9	Test for Glycosides, Fehlings Test	+

Interpretation on mass spectrum GC-MS was concluded using the data base of National Institute Standard and Technology (NIST). The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 $\mu\text{m}$  df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1 $\mu\text{L}$  of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min<sup>-1</sup>; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

Figure 1 shows the Gas Chromatography – Mass Spectrometry result of methanolic extract of *Murraya koenigii* leaves.



Gas chromatogram of methanolic leaves extract of *Murraya koenigii*

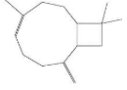
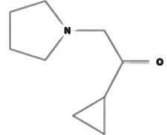
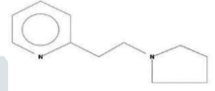
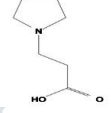

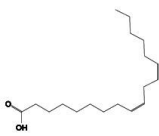


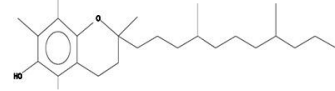
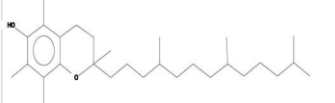

Figure 1

GC-MS of *Murraya koenigii* leaves Extract

The major bioactive chemical constituents present in methanolic extract of *Murraya koenigii* leaves are listed in table 2 along with their chemical structure.

Table 2

Chemical Constituents of *Murraya koenigii* Leaves By GC-MS Analysis

Sr. No.	Retention Time	Chemical Constituent	Molecular Weight	% Peak Area	Structure
1	12.667	Bicyclo[7.2.0] undec-4-ene, 4,11,11-trimethyl 8-methylene-, [1r-(1r,4z,9s)]	204	27.006	
2	12.932	Ethanone,1- Cyclopropyl-2 (1-Pyrrolidinyl)-	153	8.190	
3	13.223	2-(2-Pyrrolidin- 1-yl-ethyl)-Pyridine	176	17.501	
4	13.958	Pyrrolidin-1- Propionic acid	143	8.080	
5	20.826	11-tridecen-1-ol	198	4.297	
6	20.946	9,12- Octadecadienoic acid (z,z)-	280	4.336	
7	25.257	Heptacosane, 1-Chloro	414	1.374	
8	26.473	Heptacosane	380	14.295	
9	27.033	DL-Alphatocopherol	430	3.951	
10	27.093	Vitamin E	430	2.836	
11	27.763	Tritetracontane	604	8.134	

**CONCLUSION**

Phytochemical evolution is to confirm the presence of various chemical constituents present in plant. The result of phytochemical screening listed in Table no. 1 due to higher polarity of methanolic extract revealed the presence of maximum phytochemical composition specially alkaloids, tanins, terpenoids, saponins, phenols, carbohydrates etc. These phytoconstituents independently responsible for the broad range of medicinal properties. GC-MS chromatogram analysis of the methanolic extract of *Murraya Koenigii* is shown in Fig. 1 indicates the presence of various phytochemical constituents on comparison of the mass spectra of the constituents with the NIST (2008) libraries. Some major chemical constituents listed in table no. 2 with their structure. There are various phytoconstituents which contribute to the medicinal activities like antimicrobial, antioxidants, Anticancer, Anti-inflammatory, Antipyretic and analgesic.

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