

# Phytochemical and Bioanalytical studies on *Murraya koenigii* leaves and exploring its pharmaceutical properties

## ABSTRACT

The curry leaf plant *Murraya koenigii* is a widely consumed edible plant in Indian subcontinent for its nutritional benefits. Different parts of this plant have been proved to exhibit various pharmaceutical activities. The phytochemical analysis of aqueous leaf extract of *M. koenigii* indicted the presence of tannins, saponins, steroids, terpenoids and cardiac glycosides. The antimicrobial properties of five solvent extracts of *M. koenigii* leaves were tested against six pathogenic strains of microbes using agar well diffusion method. The MICs of methanol extract for the bacteria *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* were 250, 750 and 1000 $\mu$ g/mL respectively. While the acetone extract completely inhibited the bacteria *E. coli* ( $\geq 250\mu$ g/mL), it could exhibit the lethality only at high concentrations ( $\geq 1000\mu$ g/mL) for *S. aureus* and *S. typhi*. Moderate effects were shown by ethanol and chloroform extracts with the MICs ranging 750 - 1000 $\mu$ g/mL against the bacteria. The ethyl acetate extract was effective only against *E. coli* (250 $\mu$ g/mL). The methanol extract inhibited all the fungi with the MICs of  $\geq 1000\mu$ g/mL against both *Aspergillus niger* and *Trichophyton rubrum* and  $\geq 750\mu$ g/mL against *Candida albicans*. Acetone extract caused moderate ( $\geq 750\mu$ g/mL) and complete inhibition ( $\geq 250\mu$ g/mL) respectively of *A. niger* and *C. albicans*. The ethyl acetate, ethanol and chloroform extracts were effective only against *C. albicans* with the MICs ranging 500 - 1000 $\mu$ g/mL. The TLC and GC-MS studies revealed the occurrences of 7 and 13 secondary metabolites respectively in the *M. koenigii* leaf extracts encompassing three compounds with proven bioactive potentials.

**Key words:** *Murraya koenigii*, Curry leaves, Antimicrobial, Phytochemical, Bioactive

## 1. INTRODUCTION

The plant *Murraya koenigii* (syn. *Bergera koenigii*, *Chalcas koenigii*) is a tropical to sub-tropical small tree classified under the family Rutaceae. This plant is native to India and appears quite ornamental due to its compound leaves which are known as ‘curry leaves’ or ‘sweet neem leaves’. These leaves are often added as a spice, flavoring and seasoning ingredient in most of the curries and fried dishes of Indian subcontinent. They are also available dried, though the aroma is much inferior. These fruits are also very nutritious and endowed with many medicinal properties.

There has been a renewed interest in recent years on the application of functional foods for treating various ailments and infectious diseases of human. The secondary metabolites of the plants have been recognized and explored as an excellent source of phyto-medicines in pharmaceutical industries. By virtue of possessing essential phyto-constituents, the plant *M. koenigii* has been used in traditional or folk medicine for the treatment of several diseases. Consumption of various parts of this plant have been proved to offer beneficial pharmacological effects. The leaves of *M. koenigii* are frequently used as a source of herbal medicine in Ayurveda [1].

Traditionally, the curry leaves have been used to make hair tonic [2]. The hypoglycemic effects of aqueous extract of *M. koenigii* have been demonstrated without any side effects and toxicity in many animal studies [3]. Arulselvan and Senthilkumar [2] and Kesari *et al.* [3] from their independent studies have reported the reduction in blood glucose level, urea, uric acid, glycosylated hemoglobin and creatinine on a 30-day treatment in the streptozotocin induced diabetic rats with the aqueous leaf extracts.

Many studies have recorded the application of various parts of *M. koenigii* in traditional medicine for the treatment of rheumatism, traumatic injury, spasms and snake bite [4,3]. Fresh juice of the root is taken for the purpose of relieving the inflammation and pain associated with kidney [5]. Tachibana *et al.* [6] and Kumar *et al.* [7] from their studies with animal models reported that the carbazole alkaloids of the *M. koenigii* leaves exhibit anti-inflammatory and analgesic activities.

The whole plant of the *M. koenigii* serve as a rich source of vitamin A, folic acid and calcium, which contribute to the anti-oxidative property of the plant [6]. The investigation

carried out by Mylarappa *et al.* [8] on the free radical scavenging activities of alcohol:water extract of *M. koenigii* leaves proved the reduction of cytochrome-c, ferric and ferrous ions and inhibition of sugar oxidation and sulfate:ascorbate-induced fragmentation of DNA. The phytochemicals produced by the leaves as secondary metabolites such as alkaloids, tannins, flavonoids, terpenoids phenols, saponins and aromatic compounds attribute not only for the antioxidant activities but also for the defense mechanism of the plant against the microbes, predators and accidental injury [9,10].

Another significant pharmaceutical application of *M. koenigii*, the anti-tumor activity, has been documented by various researchers [11-13]. These studies have advocated the carbazole alkaloids identified from the stem barks and roots of *M. koenigii* are, mahanine, pyrayafoline-D and murrafoline-I could be used as the chemo preventive agents for tumor treatment. On testing with the CEM-SS human T-lymphoblastic leukemic cells, these compounds exhibited the tumoricidal activity through inhibition of mitochondrial membrane potential and activation of caspase-9 and caspase-3.

Adebajo *et al.* [11] have reported the hepato-protective effect of methanolic extract of *M. koenigii* leaf by way of causing reduction in the serum cholesterol, ALT and AST bilirubin, glucose and urea. A purified protein from the seeds of *M. koenigii* have been isolated by Shee and Sharma [14], which demonstrated trypsin inhibitory activity in bovine serum. Research studies on the anti-hypercholesterolemic properties of curry leaves signified the management of blood cholesterol and glucose level in type-2 diabetes induced mice models [5,6,15,16].

The immunomodulatory effect of *M. koenigii* was experimented in guinea pigs by Shah *et al.* [17]. The carbon clearance test and nitric oxide release test carried out with the methanolic leaf extract in this study demonstrated remarkable increase in phagocytic index, humoral and cell mediated immune response to ovalbumin antigen. Other interesting studies with the curry leaf extracts revealed the anti-amnestic potential i.e., memory improvement in mice [18] and insecticide effect on stored grain pest *Tribolium castaneum* (Herbst) [19].

The anti-diarrheic and antimicrobial properties of *M. koenigii* have been investigated by some studies. Pui-Hay *et al.* [4] and Kesari *et al.* [3] from their studies have reported that the oral administration of curry leaf extracts could alleviate the dysentery and chronic diarrhea.

Adebajo *et al.* [11] have validated the anti-protozoal activities of root and arial parts of *M. koenigii* and concluded that carbazole alkaloids contribute to the lethal activity against *Trichomonas gallinae*. Recently, Dineshkumar *et al.* [20] and Nalli *et al.* [21] have reported that the curry leaves are endowed with antiviral and antimicrobial properties, which needs further studies.

Although there are many reports concerning the systemic pharmacological effects of various parts of *M. koenigii* are available, extensive studies on the antimicrobial activities of curry leaves are scarce in the literature. Therefore, the present study was carried out to explore the phytochemical constituents, assay the antimicrobial properties and analyze the bioactive compounds of leaves of *M. koenigii*.

## 2. MATERIALS AND METHODS

### 2.1 Plant material and preparation of extracts

Leafy branches of *Murraya koenigii* were collected from the retail shops located in the vegetable market of Chennai city, India (Fig. 1). In the laboratory, the leaves were removed and washed thoroughly in sterile distilled water and surface sterilization was done using 70% ethanol for further processing.



**Fig. 1. Curry leaf (*Murraya koenigii*) plant**

Aqueous extract of the **curry leaves** was prepared by crushing and grinding the fresh leaves using mortar and pestle. Five grams of this homogenate was mixed in 25 mL of distilled water and heated at 50-60°C. This suspension was then filtered using Whatmann filter paper no.1. The filtrate was centrifuged at 2500 rpm for 10 minutes and the sediment was stored in sterile bottles at 5°C until use.

For the purpose of preparing solvent extracts, the curry leaves were completely dried under shade and powdered using a blender. Five grams of this leaf powder was mixed with 100 mL of methanol in a conical flask and kept in rotary shaker at 150 rpm for 24h. Later, it was filtered in a conical flask and kept at 30°C to facilitate the evaporation of the solvent until the final volume reaches one-fourth of the original volume. Similar method was followed for preparing the ethanol, acetone, ethyl acetate and chloroform leaf extracts. Stock solutions of these solvent extracts were prepared at a concentration of 10 mg/mL using Dimethyl Sulfoxide (DMSO) and stored at 5°C until further studies.

## 2.2 Phytochemical analysis of leaf extract

The aqueous extract of the curry leaf was subjected to phytochemical studies adopting the methods of Aiyelaagbe and Osamudiamen [22] with slight modifications. The analysis focused on the tests for detection of seven compounds including tannins, saponins, steroids, terpenoids, cardiac glycosides, phlobatannins and anthraquinone.

## 2.3 Assay of antimicrobial activities of leaf extracts

### 2.3.1 Indicator organisms

The antimicrobial properties of the solvent extracts were explored through screening their activities against six standard strains of microbial pathogens obtained from IMTECH (Chandigarh, India). The indicator microbes included (MTCC strain numbers are given in parentheses) three bacteria such as *Staphylococcus aureus* (96), *Escherichia coli* (433) and *Salmonella typhi* (3917) and three fungi such as *Aspergillus niger* (1344), *Candida albicans* (3017) and *Trichophyton rubrum* (296).

### 2.3.2 Inoculum preparation

The test bacterial cultures were prepared by inoculating each organism into sterile nutrient broth followed by incubation at 37°C for 18-24 h. The density of the final inoculum was set at 10<sup>5</sup>CFU/mL corresponding to the McFarland standard solution no.0.5 by checking the turbidity at 600 nm. The fungal cultures for the test were prepared by individually inoculating the standard strains on sterile Potato Dextrose Agar (PDA) medium and incubation at 28°C for 48 h.

### 2.3.3 Assay method

The antibacterial activities of plant extracts were tested by agar well diffusion method as described by Nain *et al.* [23] with slight modifications. A small portion (2 mL) of each bacterial inoculum was swabbed onto a sterile Mueller Hinton Agar (MHA) prepared in a petri dish. Using a sterile cork borer (6mm dia.) five wells were cut on the agar medium. Four different volumes comprising of respective concentrations of the solvent extract from the stock solution viz., 25µL (250µg), 50µL (500µg), 75 µL (750µg) and 100 µL (1000µg) were loaded individually into these wells. The fifth well was loaded with 0.1 mL of sterile DMSO to serve as the control. For each bacterial culture a total of five such plates were prepared encompassing all the five solvent extracts. These plates were then incubated at 37°C and examined after 24h. Anti-bacterial activity of the solvent leaf extract was determined by the appearance of zone of growth inhibition around the well. A zone diameter of  $\geq 10$  mm was considered positive for the inhibition of bacterial growth.

For the purpose of testing the antifungal activity of each solvent extract, an aliquot of a well grown fungal culture was mixed with the sterile molten PDA and poured into a petri dish. After the solidification of agar, five wells were cut and solvent extract was loaded in the similar manner followed for antibacterial assay. The plates were maintained at 28°C for 24-48h and the antifungal activity of the extract was determined through recording the zone of growth inhibition.

The antimicrobial assay for each solvent extract was carried out in triplicates under strict aseptic conditions and the average of zone diameters was considered as final for evaluation.

## 2.4 Bioanalytical studies on the leaf extract

The methanol extract of *M. koenigii* was subjected to analytical studies in order to decipher the bioactive compounds contained in it.

### 2.4.1 Thin layer chromatography (TLC)

A sample (10mg/mL) of dry powder of methanol leaf extract was spotted on the silica gel plate. It was run with mobile phase solvent consisting of chloroform:methanol prepared with different solvent ratios (9:1, 8:2 and 7:3). The secondary metabolites of the leaf extract got separated from the inoculum as different spots on the stationary phase based on their molecular weight and the affinity towards the mobile phase. Subsequent to the separation, the spots of separated compounds were detected in iodine chamber and identified based on their corresponding frontal ratio (R<sub>f</sub>) values.

#### 2.4.2 Gas chromatography Mass Spectrometry (GC-MS)

Identification of volatile compounds present in the methanol leaf extract was carried out using a Perkin Elmer Gas Chromatography Claurus 500 system interfaced to a Mass Spectrometer (GC/MS) equipped with Elite-1 fused silica capillary column composed of 100% Dimethyl poly siloxane with an electron ionization input of 70eV. Relative percentage amount of each component was calculated by comparing its average peak area to the total areas and confirmed with reference to the standard retention times.

### 3. RESULTS

#### 3.1 Phytochemistry of leaf extract

The aqueous extract of *M. koenigii* showed positivity for the possession of five compounds out of the seven secondary metabolites tested for. Based on their reaction with the test reagent, their presence was recorded as strongly positive (++++), moderately positive (+++), weakly positive (++) and few (+). Among the detected phytochemicals, the terpenoids and steroids were observed to be of higher proportion than that of tannins followed by saponins and cardiac glycosides (Table 1).

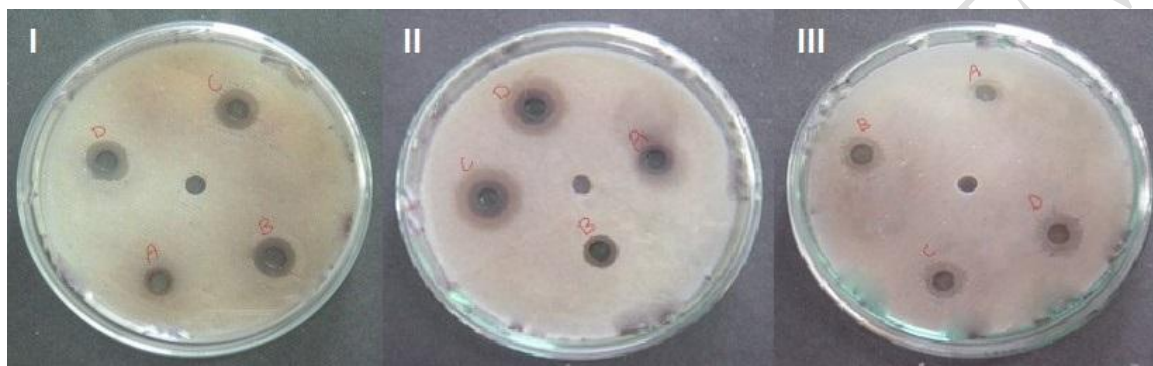
**Table 1. Occurrences of phytochemicals in the aqueous leaf extract of *Murraya koenigii***

S. No.	Phytochemicals tested	Results
1.	Tannins	+++
2.	Saponins	++
3.	Phlobatannins	-
4.	Terpenoids	++++
5.	Steroids	++++
6.	Cardiac Glycosides	+
7.	Anthraquinone	-

+: positive; -: negative

#### 3.2 Assay of antimicrobial activities of leaf extracts

The responses of all the three indicator bacteria to the solvent leaf extracts of *M. koenigii* are presented in table 2. Among the solvent extracts the methanol portion exhibited superior antibacterial activity on all the three indicator bacteria viz., *S. aureus*, *E. coli* and *S. typhi* with corresponding MIC values ( $\mu\text{g/mL}$ ) of 250, 750 and 1000 (Fig. 2). Although the acetone extract was highly effective ( $\text{MIC} \geq 250\mu\text{g/mL}$ ) against *E. coli* and it could inhibit the other two bacteria only at the maximum concentration ( $\text{MIC} \geq 1000\mu\text{g/mL}$ ). While the ethanol and chloroform extracts were moderately lethal with ( $\text{MIC } 750\text{-}1000\mu\text{g/mL}$ ) against three and two bacteria respectively, the ethyl acetate extract was only against *E. coli* ( $\text{MIC} \geq 250\mu\text{g/mL}$ ).



**Fig. 2. Susceptibility response of Bacterial cultures to curry leaf solvent extracts**

**I) *Staphylococcus aureus* II) *Escherichia coli* III) *Salmonella typhi***

**Table 2. Results of assay of antibacterial activity of solvent leaf extracts of *M koenigii***

Sl. No	Solvent used	Effect of different concentrations of leaf extract on indicator bacteria											
		<i>Staphylococcus aureus</i>				<i>Escherichia coli</i>				<i>Salmonella typhi</i>			
		250 $\mu\text{g/}$ ml	500 $\mu\text{g/}$ ml	750 $\mu\text{g/}$ ml	1000 $\mu\text{g/}$ ml	250 $\mu\text{g/}$ ml	500 $\mu\text{g/}$ ml	750 $\mu\text{g/}$ ml	1000 $\mu\text{g/}$ ml	250 $\mu\text{g/}$ ml	500 $\mu\text{g/}$ ml	750 $\mu\text{g/}$ ml	1000 $\mu\text{g/}$ ml
1	Methanol	+	+	+	+	-	-	+	+	-	-	-	+
2	Ethyl acetate	-	-	-	-	+	+	+	+	-	-	-	-
3	Acetone	-	-	-	+	+	+	+	+	-	-	-	+
4	Ethanol	-	-	-	+	-	-	+	+	-	-	-	+
5	Chloroform	-	-	-	-	-	-	-	+	-	-	+	+

+: zone of inhibition; -: absence of zone

Assay of antifungal activities of solvent extracts indicated that the methanol extract was better than the other extracts by inhibiting all the three indicator fungi despite being effective against *A. niger*

and *T. rubrum* at high concentrations (MIC  $\geq 1000\mu\text{g/mL}$ ) and against *C. albicans* at moderate concentration (MIC  $\geq 750\mu\text{g/mL}$ ). This was followed by the acetone extract which showed moderate and superior activities respectively against *A. niger* (MIC  $\geq 750\mu\text{g/mL}$ ) and *C. albicans* (MIC  $\geq 250\mu\text{g/mL}$ ). Least inhibitory effects (500 - 1000 $\mu\text{g/mL}$ ) were caused by ethyl acetate, ethanol and chloroform extracts as they were lethal only against *C. albicans* (Table 3).

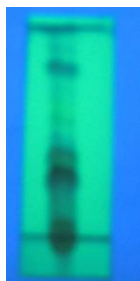
**Table 3. Results of assay of antifungal activity of solvent leaf extracts of *M koenigii***

Sl. No	Solvent used	Effect of different concentrations of leaf extract on indicator fungi											
		<i>Aspergillus niger</i>				<i>Trichophyton rubrum</i>				<i>Candida albicans</i>			
		250 $\mu\text{g/}$ ml	500 $\mu\text{g/}$ ml	750 $\mu\text{g/}$ ml	1000 $\mu\text{g/}$ ml	250 $\mu\text{g/}$ ml	500 $\mu\text{g/}$ ml	750 $\mu\text{g/}$ ml	1000 $\mu\text{g/}$ ml	250 $\mu\text{g/}$ ml	500 $\mu\text{g/}$ ml	750 $\mu\text{g/}$ ml	1000 $\mu\text{g/}$ ml
1	Methanol	-	-	-	+	-	-	-	+	-	-	+	+
2	Ethyl acetate	-	-	-	-	-	-	-	-	-	+	+	+
3	Acetone	-	-	+	+	-	-	-	-	+	+	+	+
4	Ethanol	-	-	-	-	-	-	-	-	-	-	-	+
5	Chloroform	-	-	-	-	-	-	-	-	-	-	+	+

+: zone of inhibition; -: absence of zone

### 3.3 Separation and identification of bioactive compounds of curry leaves

Bioanalytical studies using TLC and GC-MS facilitated the separation and identification specific secondary metabolites, which could act as bioactive compounds, of leaves of *M. koenigii*. The chromatogram of TLC performed with the methanol leaf extract showed seven compounds separated as distinct bands with R<sub>f</sub> values ranging from 0.28 to 0.91 on the silica gel sheet (Fig. 3; Table 4).



**Fig. 3. TLC chromatogram showing the bands of separated compounds of curry leaf extract**

**Table 4. Separation of compounds of methanol leaf extract of *Murraya koenigii* by TLC**

S. No.	Types of band	Rf value
1.	Band 1	0.28
2.	Band 2	0.37
3.	Band 3	0.45
4.	Band 4	0.55
5.	Band 5	0.65
6.	Band 6	0.80
7.	Band 7	0.91

The GC-MS analysis of methanol extract inferred the existence of volatile compounds in it (Fig 4). The chromatogram showed the separation of a total of 13 compounds at a wide range of retention times (Table 5). The peak values of each of these compounds were recorded and their identification was achieved in conform to the retention time and average peak area percentage of standard compounds on GC-MS chromatogram.

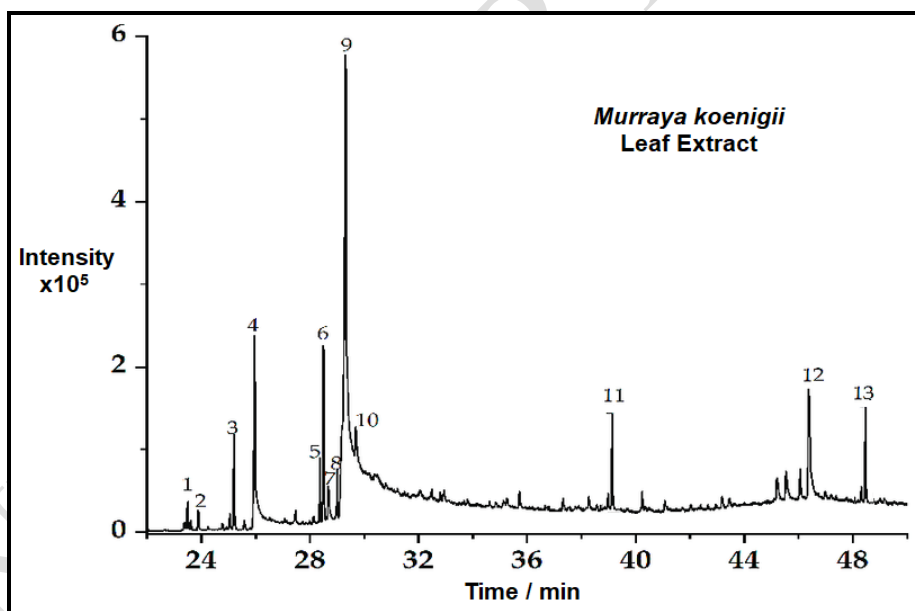


Fig. 4. GC-MS chromatogram showing the occurrences of compounds (peaks) of curry leaf extract

**Table 5. Bioactive compounds of *M. koenigii* leaves identified by GC-MS**

S. No	Retention time	Compound identified	Peak area %
1.	1.961	2-cyclopenten-1-one	0.94
2.	2.715	2-propanone	0.99
3.	3.284	Ethyl (dimethyl) isopropoxysilane propane	0.56
4.	5.201	4H-Pyran-4-one	5.79
5.	5.759	1-methyl-pyrrolidine-2-carboxylic acid	29.05
6.	5.949	1-methyl-2-pyrrolidineethanolpicidolic acid	41.93
7.	10.330	3,4-Dihydroxy-5-methyl-dihydrofuran-2-one	0.98
8.	11.843	Alpha -D-Glucopyranoside	5.41
9.	27.809	9,12-octadecadienoic acid(z,z)-	0.18
10.	29.192	Z-8,10-hexadecadienoic acid	6.66
11.	29.417	9,12-octadecadien-1-ol	0.98
12.	29.898	9,12-octadecadienoic acid(z,z)-cis-7	1.53
13.	32.907	Z,E-7,11-Hexadecadien-1-yl acetate.	1.17

#### 4. DISCUSSION

The traditional system of medicine employing the herbs and their products as pharmaceuticals has been proved to be an efficient method of treating the diseases without any adverse effects. Since *Murraya koenigii*, the curry leaf plant, is regarded for its nutritional and health benefits, the present study was carried out to explore its phytochemistry and antimicrobial properties through a methodical scientific approach. In order to understand the pharmacological effects of this plant, contemporary researchers have investigated different plant parts such as stem bark [13], fruits [24], root [5], etc. The present study employed the extracts of leaves of this plant as most of the research studies advocate that the curry leaves encompass superior medicinal properties [25-27].

It is generally acknowledged that the nutritional and medicinal properties of any plant is contributed by the secondary metabolites, called phytochemicals, contained in it. Therefore, the present study intended to analyze the phytochemical constituents of the leaf extract of *M. koenigii*. The phytochemicals detected in the curry leaves by respective analytical studies of this study were tannins, saponins, terpenoids, steroids and cardiac glycosides (Table 1). While the

terpenoids and steroids were abundant, moderate presence of tannins and saponins were found. However, the cardiac glycosides were the least to be identified. Similar pattern of detection of phytochemicals have been reported by other researchers [26, 28]. In conform to our finding, Mishra *et al.* [25] from their studies on the antioxidant potentials of curry leaves have reported the occurrences of higher proportion total phenolics, flavonoids and tannins among the phytochemicals tested for. Akula *et al.* [27] demonstrated the antimicrobial activities of curry leaves and explained that the presence of coumarins, flavonoids, glycosides, phenol, saponins, steroids and tannins could attribute to this property.

Five extracts of leaves of *M. koenigii* were prepared using different solvents such as methanol, ethanol, acetone, ethyl acetate and chloroform. Among the solvents used for extraction of leaf components in the present study, the most frequently used solvent by the earlier workers has been methanol [26,29]. This is followed by ethanol and acetone [25].

The assay of antimicrobial activities of extracts of curry leaves was performed using agar gel diffusion method as it would be helpful not only detecting the activity but to determine the minimum inhibitory concentration (MIC) of the extract. Many earlier studies have adopted this method to determine the MIC values which is critical to determine efficacy of the extract [29, 26]. Our study prepared the extracts of curry leaves using common solvents such as methanol, ethanol, acetone, ethyl acetate and chloroform. Besides these solvents, some studies have attempted extracting the constituents using hexane [28] and petroleum ether [7].

The findings of antimicrobial assay of different solvent extracts of curry leaves revealed varying responses of the indicator organisms (Tables 1 & 2). Among the bacteria *S. aureus* showed the highest susceptibility ( $\geq 250$   $\mu\text{g/mL}$ ) to the methanol extract. It was inhibited by acetone and ethanol extracts only at high concentrations ( $\geq 1000$   $\mu\text{g/mL}$ ). The susceptibility patterns of the bacteria *E. coli* were highest to ethyl acetate and acetone ( $\geq 250$   $\mu\text{g/mL}$ ), moderate to methanol and ethanol ( $\geq 750$   $\mu\text{g/mL}$ ) and least to chloroform ( $\geq 1000$   $\mu\text{g/mL}$ ). The gram negative bacteria *S. typhi* was relatively resistant to the extracts as it was inhibited only at highest concentrations ( $\geq 1000$   $\mu\text{g/mL}$ ) of methanol, acetone and ethanol extracts and moderate concentration ( $\geq 750$   $\mu\text{g/mL}$ ) of chloroform. Antifungal activities of solvent extracts indicated that the yeast *C. albicans* was sensitive to all the extracts with maximum susceptibility to acetone ( $\geq 250$   $\mu\text{g/mL}$ ) followed by ethyl acetate ( $\geq 500$   $\mu\text{g/mL}$ ), methanol and chloroform ( $\geq 750$   $\mu\text{g/mL}$ ) and ethanol ( $\geq 1000$   $\mu\text{g/mL}$ ). The pathogenic fungi *T. rubrum* was found to be

highly resistant to all the solvents tested except the methanol ( $\geq 1000\mu\text{g/mL}$ ). The mold *A. niger* showed moderate and least susceptibilities to acetone ( $\geq 750\mu\text{g/mL}$ ) and methanol ( $\geq 1000\mu\text{g/mL}$ ) while resisting all the other extracts.

Overall analysis of the antimicrobial assay inferred that the methanol extract possesses a superior potential of inhibiting the growth of all the indicator fungi and bacteria tested in the study (Tables 1 & 2). Mathur *et al.* [28] from their investigation on antibacterial activities of *M. koenigii* against the causative agents of bovine Mastitis demonstrated substantial lethal activities of methanol leaf extract. This extract inhibited the growth of both gram positive (*S. aureus*, *S. epidermidis*, *Streptococcus uberis*, *Corynebacterium gravis* and *Bacillus cereus*) and gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*,) even at lower concentrations (30mg/mL). The superior potential methanol extract could be due to its ability of interaction with the phytochemicals of the curry leaf and promotion of their drug-target binding capabilities. Our finding is in agreement with Sharma *et al.* [30] who demonstrated that the methanol leaf extract of *M. koenigii* showed a greater antibacterial, antifungal and antihelminthic activities compared to other solvent extracts.

The critical part of this study is the bioanalytical studies on the constituents of the leaves of *M. koenigii*. Since the methanol extract had shown greater antimicrobial potential than its counterparts, it was subjected to the analytical studies to identify the bioactive compounds. The screening study with the TLC indicated the presence of seven compounds on the chromatogram that were separated from the inoculum based on their molecular and affinity properties. Further advanced study with the GC-MS revealed the presence of 13 different compounds (Fig. 4; Table 5). In consonance with our study, Hema *et al.* [31] have deciphered the presence of 13 compounds from the methanol extract of curry leaves, among which three compounds is similar to that of the present study. In contrast with their finding, compounds such as 2-cyclopenten-1-one, Ethyl (dimethyl) isopropoxysilane propane, 4H-pyran-4-0, 3,4-dihydroxy-5-methyl-dihydrofuran-2-one, Z, E-7,11-Hexadecadien-1-yl acetate have been identified as novel compounds in the present study.

The main objective of this study is to explore the pharmaceutical properties of the leaves of *M. koenigii* in terms of demonstrating the antimicrobial activities. Some of the compounds as identified from the GC-MS analysis could be considered to be biologically active and attributable to the antibiotic activities of the curry leaves. As a testimony to our hypothesis,

some studies have demonstrated the antimicrobial activities of compounds isolated from *M. koenigii*. A study with the bioactive compounds isolated from the stem bark of *M. koenigii* demonstrated the antimicrobial activities of a dimeric carbazole alkaloid, 3,3'-[oxybis(methylene)]bis(9-methoxy-9H-carbazole) and three known steroids [32]. The research study by Mathur *et al.* [28], interestingly, published the data proximal to the findings of the present study. This research group established the anti-microbial, anti-oxidant and anti-inflammatory activities of nine compounds isolated from the methanol extracts of the leaves of *M. koenigii*, which included three compounds identified in the present study (9,12 octadecadienoic acid, Ethyl  $\alpha$ -D-glucopyranoside and 1-methyl-pyrrolidine-2-carboxylic acid).

The current study is an earnest attempt to investigate the phytochemistry and antibiogram of the leaf extracts of *M. koenigii*. The bioactive compounds deciphered in the pilot research work gains its significance as some of them have been proved to possess antimicrobial potentials by the contemporary studies conducted elsewhere. Further elaborate *in vitro* and *in vivo* studies with the purified compounds of curry leaves would help developing promising drugs with wide pharmaceutical applications.

## 5. CONCLUSIONS

The curry leaf plant *Murraya koenigii*, besides a source of rich nutrients, has been in use in the traditional medicine for treatment of various ailments. The phytochemical studies with the curry leaf extract indicated the presence of essential compounds with the abundance of terpenoids and steroids. The methanol could be considered as a suitable solvent to explore a wide spectrum antimicrobial properties of the curry leaf extract. Bioanalytical studies with TLC and GC-MS revealed the existence of seven and thirteen bioactive compounds respectively. Contemporary studies have demonstrated the antimicrobial potentials of some of the compounds identified in the present study. Purification bioactive compounds of curry leaves and carrying out further studies with a view of developing promising drugs have been suggested.

## COMPETING INTERESTS

The authors of this article declare that there are no competing interests.

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