Phytochemical and Bioanalytical prospecting of *Murraya koenigii* leaves and exploring its Pharmaceutical properties

ABSTRACT

The 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, flavonoids, 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 1000µg/mL respectively. The ethyl acetate extract was effective only against *E. coli* (250µg/mL). While the acetone extract completely inhibited the bacteria *E. coli* (≥250µg/mL), it could exhibit the lethality only at high concentrations (≥1000µg/mL) for *S. aureus* and *S. typhi*. Moderate effects were shown by ethanol and chloroform extracts with the MICs ranging 750 - 1000µg/mL against the bacteria. The fungal strains *Aspergillus niger*, *Trichophyton rubrum* and *Candida albicans* were inhibited by methanol extract at concentrations of 1000, 1000 and 750µg/mL respectively. Acetone extract caused moderate (≥750µg/mL) and complete inhibition (≥250µg/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 - 1000µg/mL. The TLC and GCMS studies revealed the occurrences of 7 and 13 biochemical compounds in the *M. koenigii* leaf extracts.

INTRODUCTION

The plant *Murraya koenigii* (syn. *Bergera koenigii*, *Chalcas koenigi*i) 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 *Murraya koenigii* have 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 an herbal medicine in Ayurveda (Soetan, 2008).

Traditionally, the curry leaves have been used to make hair tonic (Arulselvan and Senthilkumar, 2007). The hypoglycemic effects of aqueous extract of *Murraya koenigii* have been demonstrated without any side effects and toxicity in many animal studies. (Kesari, Gupta and Watal, 2005). Arulselvan and Senthilkumar (2007) and Kesari, Gupta and Watal (2005) 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 *Murraya koenigii* in traditional medicine for the treatment of rheumatism, traumatic injury, spasms and snake bite (Pui-Hay et al., 1986; Kesari, Gupta and Watal, 2005). Fresh juice of the root is taken for the purpose of relieving the inflammation and pain associated with kidney (Ramsewak *et al*., 1999). Tachibana *et al* (2001) and Kumar *et al.* (2012) 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 (Tachibana *et al.*, 2001). The investigation carried out by Mylarappa, Ningappa and Srinivas (2008) 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 (Bonjar, Nik and Aghighi, 2004; Rao *et al*., 2007).

Another significant pharmaceutical application of *M. koenigii*, the anti-tumor activity, has been documented by various researchers (Adebajo *et al.,* 2006; Ito *et al.*, 2006; Muthumani *et al*., 2009). 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 tumericidal activity through inhibition of mitochondrial membrane potential and activation of caspase-9 and caspase-3.

Adebajo *et al.* (2006) 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 (2007), 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 (Ramsewak *et al.*, 1999; Tachibana *et al.*, 2001; Xie *et al.*, 2006; Iyer and Devi, 2008).

The immunomodulatory effect of *M. koenigii* was experimented in guinea pigs by Shah, Wakade and Juvekar (2008). 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 (Vasudevan and Parle, 2008) and insecticide effect on stored grain pest *Tribolium castaneum* (Herbst) (Gandhi, Pillai and Patel, 2010).

The anti-diarrheic and antimicrobial properties of *M. koenigii* have been investigated by some studies. Pui-Hay et al. (1986) and Keasri et al. (2007) from their studies have reported that the oral administration of curry leaf extracts could alleviate the dysentery and chronic diarrhea. Adebajo *et al.* (2006) 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, Knolker and Reddy (2008) and Dineshkumar, Annalava and Manjunatha (2010) 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 *Murraya koenigii*.

MATERIALS AND METHODS

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 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^{o}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^{o}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.

Phytochemical analysis of leaf extract

The aqueous extract of the curry leaf was subjected to phytochemical studies adopting the methods of **Aiyelaagbe and Osamudiamen (2009**) with slight modifications. The analysis focused on the tests for detection of eight compounds including tannins, saponins, steroids, flavonoids, terpenoids, cardiac glycosides, phlobatanins and anthraquinone.

Assay of antimicrobial activities of leaf extracts

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).

Inoculum preparation: The test bacterial cultures were prepared by inoculating each organism into sterile nutrient broth followed by incubation at 37^{o}C for 18-24 h. The density of the final inoculum was set at 10^{5}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^{o}C for 48 h.

Assay method: The antibacterial activities of plant extracts were tested by agar well diffusion method as described by Nain *et al.* (2011) 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 37ºC 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 28ºC 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.

Bioanalytical studies on the leaf extract

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

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). Subsequent to the separation, the spots of separated compounds were detected in iodine chamber and identified based on their corresponding Rf values.

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.

RESULTS

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. Among the detected phytochemicals, the rate of occurrences of terpenoids and steroids was higher 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.	Phlobatamins	-
4.	Terpenoids	++++
5.	Steroids	++++
6.	Cardiac Glycosides	+
7.	Anthraquinone	-

+: positive; -: negative

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 (µg/mL**)** of 250, 750 and 1000 (Fig. 2). Although the acetone extract was highly effective (MIC ≥250µg/mL) against *E. coli* and it could inhibit the other two bacteria only at the maximum concentration (MIC ≥1000µg/mL). While the ethanol and chloroform

extracts were moderately lethal with (MIC 750-1000µg/mL) against three and two bacteria respectively, the ethyl acetate extract was only against *E. coli* (MIC ≥250µg/mL).

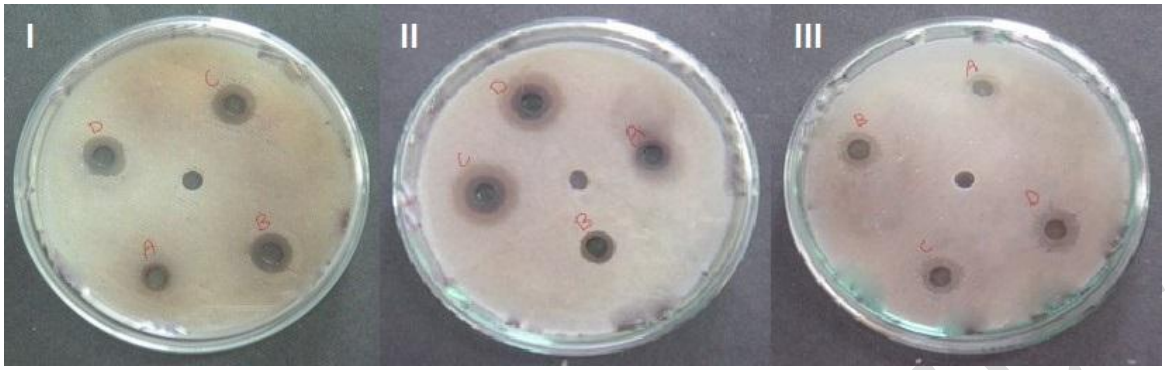

Fig. 2. Susceptibility response of Bacterial cultures to curry leaf solvent extracts I) *Staphylococcus aureus* II) *Escherichia coli* III) *Salmonella typhi*

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 ≥1000µg/mL) and against *C. albicans* at moderate concentration (MIC ≥750µg/mL). This was followed by the acetone extract which showed moderate and superior activities respectively against *A. niger* (MIC ≥750µg/mL) and *C. albicans* (MIC ≥250µg/mL). Least inhibitory effects were caused by ethyl acetate and chloroform extracts as they were lethal only against *C. albicans* (Table 3).

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											
		Staphylococcus aureus				*Escherichia coli*				*Salmonella typhi*			
		250 µg/ml	500 µg/ml	750 µg/ml	1000 µg/ml	250 µg/ml	500 µg/ml	750 µg/ml	1000 µg/ml	250 µg/ml	500 µg/ml	750 µg/ml	1000 µg/ml
1	Methanol	+	+	+	+	-	-	+	+	-	-	-	+
2	Ethyl acetate	_	_	_	_	+	+	+	+	-	-	-	-
3	Acetone	-	-	-	+	+	+	+	+	-	-	-	+
4	Ethanol	-	-	-	+	-	-	+	+	-	-	-	+
5	Chloroform	-	-	-	-	-	-	-	+	-	-	+	+

+: zone of inhibition; -: absence of zone

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											
		Aspergillus niger				*Trichophyton rubrum*				*Candida albicuns*			
		250 µg/ml	500 µg/ml	750 µg/ml	1000 µg/ml	250 µg/ml	500 µg/ml	750 µg/ml	1000 µg/ml	250 µg/ml	500 µg/ml	750 µg/ml	1000 µg/ml
1	Methanol	-	-	-	+	-	-	-	+	-	-	+	+
2	Ethyl acetate	–	–	–	–	–	–	–	–	–	+	+	+
3	Acetone	–	–	+	+	–	–	–	–	+	+	+	+
4	Ethanol	–	–	–	–	–	–	–	–	–	–	–	+
5	Chloroform	–	–	–	–	–	–	–	–	–	–	+	+

+: zone of inhibition; -: absence of zone

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 Rf values ranging from 0.28 to 0.91 on the silica gel sheet (Fig. 3; Table 4).

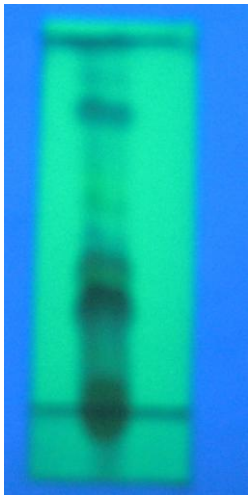

Fig. 3. TLC chromatogram showing the bands of separated compounds of curry leaf extract

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 these compounds were recorded and their identification was achieved by the comparative study of retention time and average peak area percentage of standard GC-MS chromatogram.

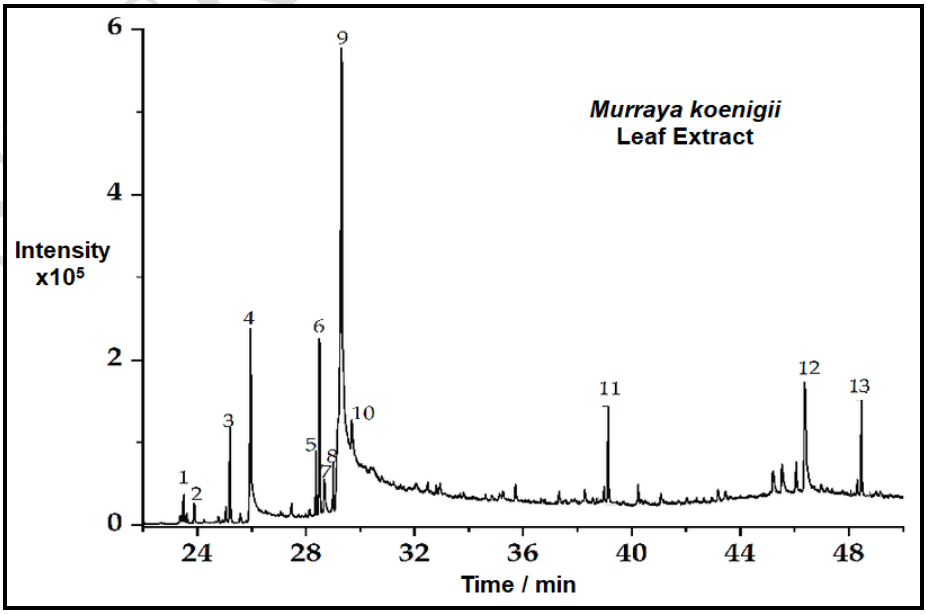

Fig. 4. GC-MS chromatogram showing the peaks of compounds of curry leaf extract

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-pyrrolidineethanolpidolic 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)-cic-7	1.53
13.	32.907	Z,E-7,11-Hexadecadien-1-yl acetate.	1.17

DISCUSSION

The traditional system of medicine employing the herbs and their products as pharmaceutics 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 this plant, contemporary researchers have investigated different plant parts such as stem bark (Muthumani *et al.*, 2009), fruits (Tembhurne and Sakarkar, 2009), root (Ramsewak *et al.*, 1999), 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 (Mishra et al., 2009; Singh, Tiwari and Prajapat, 2010; Akula al., 2016).

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 (Singh, Tiwari and Prajapat (2010) and Mathur *et al*., 2011; Akula *et al*., 2016). In conform to our finding, Mishra *et al.* (2009) 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. (2016) 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 (Sing, Tiwari and Prajapat, 2009; Bobbarala *et al.*, 2009*;* Shibabudeen *et al*., 2010). This is followed by ethanol and acetone (Mioshra *et al.*, 2009).

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 (Bobbarala, 2009; Kaveri Singh et al., 2010**).** 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 (Mathur, Dua and Prasad, 2010) and petroleum ether (Kumar *et al.*, 2012).

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 (≥250 µg/mL) to the methanol extract. It was inhibited by acetone and ethanol extracts only at high concentrations (≥1000 µg/mL). The susceptibility patterns of the bacteria *E. coli* were highest to ethyl acetate and acetone (≥250 µg/mL), moderate to methanol and ethanol (≥750 µg/mL) and least to chloroform (≥1000 µg/mL). The gram negative bacteria *S. typhi* was relatively resistant to the extracts as it was inhibited only at highest concentrations (≥1000 µg/mL) of methanol, acetone and ethanol extracts and moderate concentration (≥750 µg/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 (≥250µg/mL) followed by ethyl acetate (≥500µg/mL), methanol and chloroform (≥750µg/mL) and ethanol (≥1000µg/mL). The pathogenic fungi *T. rubrum* was found to be highly resistant to all the solvents tested except the methanol (≥1000µg/mL). The mold *A. niger* showed moderate and least susceptibilities to acetone (≥750µg/mL) and methanol (≥1000µg/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, Dua and Prasad (2010) 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.* (2012)** 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, Kumaravel and Alagusundaram (2011) 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 (Rahman and Gray, 2005). The research study by Mathur *et al*. (2011), 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 octadecedienoic acid, Ethyl α-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.

CONCLUSION

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 ane 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.

REFERENCES

1. Adebajo AC, Ayoola OF, Iwalewa EO, Akindahunsi AA, Omisore NO, Adewunmi CO, Adenowo TK. Anti-trichomonal, biochemical and toxicological activities of methanolic extract and some carbazole alkaloids isolated from the leaves of Murraya koenigii growing in Nigeria. Phytomedicine. 2006 Mar 13;13(4):246-54.
2. Aiyelaagbe OO, Osamudiamen PM. Phytochemical screening for active compounds in Mangifera indica leaves from Ibadan, Oyo State. Plant Sci Res. 2009;2(1):11-3.
3. Akula P, Sree AN, Santosh B, Sandeep B, Raviteja KB, Keerthi T. Evaluation of anti-microbial activity of leaf and bark extracts of Murraya koenigii (curry leaves). Journal of Pharmacognosy and Phytochemistry. 2016;5(3):101-5.
4. Arulselvan P, Subramanian SP. Beneficial effects of Murraya koenigii leaves on antioxidant defense system and ultrastructural changes of pancreatic β-cells in experimental diabetes in rats. Chemico-Biological Interactions. 2007 Jan 30;165(2):155-64.
5. Bobbarala V, Naidu KC. A search for alternatives to control phytopathogenic fungi Curvularia lunata. Asian J Exp Sci. 2009;23(3):1-6.
6. Bonjar GHS, Nik AK and Aghighi (2004), Antimicrobial and antifungal survey in plants used in indigenous herbal medicine of south east regions of Iran.J Biol Sci.

7. Dineshkumar B, Analava M, Manjunatha M. Antidiabetic and hypolipidaemic effects of few common plants extract in type 2 diabetic patients at Bengal. Dubai Diabetes and Endocrinology Journal. 2010 Mar 1;18(2):59-65.

8. Gandhi N, Pillai S and Patel P (2010), Efficacy of Pulverized Punica granatum (Lythraceae) and *Murraya koenigii* (Rutaceae) Leaves against Stored Grain Pest Tribolium castaneum (Coleoptera: Tenebrionidae), Int. J. Agric. Biol., Vol. 12, No. 4, 616–620

9. Hema R, Kumaravel S, Alagusundaram K. GC/MS determination of bioactive components of Murraya koenigii. Journal of American Science. 2011;7(1):80-3.

10. Ito C, Itoigawa M, Nakao K, Murata T, Tsuboi M, Kaneda N, Furukawa H. Induction of apoptosis by carbazole alkaloids isolated from Murraya koenigii. Phytomedicine. 2006 May 9;13(5):359-65.

11. Iyer D, Devi U. Phyto-pharmacology of Murraya koenigii (L.). Pharmacognosy Reviews. 2008;2(3):180.

12. Kesari AN, Rajeshkumar Gupta RK, Watal G(2005) "Hypoglycemic effects of Murraya koenigii on normal and alloxan-diabetic rabbits." J Ethnopharmacol 97(2): 247-251.

13. Knölker HJ, Reddy KR. Occurrence, Isolation, and Structure Elucidation. The Alkaloids: Chemistry and Biology. 2008 Jan 1;65:3-158.

14. Kumar V, Bandyopadhyay A, Sharma V, Suthar S, Tekale S. Comparative study of hypoglycemic and hypolipidemic potency of Murraya Koenigii for wound healing activity in type-2 diabetic rats. Int. J. Pharmacy & Biomed. Sei. 2012;2:150-61.

15. Mathur A, Prasad GBKS, Dua VK(2011), Anti-inflammatory activity of leaves extracts of Murraya koenigii, Int.J.Pharm.Biosciences, Vol2(1),pg:541-544.

16. Mathur A, Verma SK, Singh SK, Prasad GBKS, Dua VK (2011), Investigation of the antimicrobial, antioxidant and anti-inflammatory activity of compound isolated from *Murraya koenigii*, Volume: 2: Issue-1,pg: 477

17. Mishra J, Yousuf A, Singh RD, Aradhana (2009): Phtyochemical investigation and in-vitro antioxidant potential of leaves of Murraya koenigii. International Journal of Integrative Biology; 7(3): 171.

18. Muthumani PS. Ventatraman KV, Ramseshu R, Meera, Devi P, Kameswari B, Eswarapriya E (2009), Pharmacological studies of anticancer, antiinflammatory activities of Murraya koenigii (Linn) Spreng in experimental animals. J.Pharm. Sci.& Res.Vol.1(3),pg:137-141.

19. Mylarappa B, Ningappa DR, Srinivas L. Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (Murraya koenigii L) extracts. Food Chem. 2008; 106:2, 720-8.

20. Nain P, Kumar A, Sharma S, Nain J. In vitro evaluation of antimicrobial and antioxidant activities of methanolic extract of Jasminum humile leaves. Asian Pacific Journal of Tropical Medicine. 2011 Oct 1;4(10):804-7.

21. Pui-Hay BP, Yun-Cheng K, Kam-Hung N, Hung-Ta C, Qian L, Si-Xao Y, Pui- Hay BP, Yun- Cheng K, Kam- Hung N, Hung- Ta C, Qian L. A chemotaxonomic study of Murraya (Rutaceae) in China. Journal of Systematics and Evolution. 1986 Jun 18;24(3):186.

22. Rahman MM, Gray AI. A benzoisofuranone derivative and carbazole alkaloids from Murraya koenigii and their antimicrobial activity. Phytochemistry. 2005 Jul 1;66(13):1601-6.

23. Ramsewak RS, Nair MG, Strasburg GM, DeWitt DL, Nitiss JL. Biologically active carbazole alkaloids from Murraya k oenigii. Journal of agricultural and food chemistry. 1999 Feb 15;47(2):444-7.

24. Rao LJ, Ramalakshmi K, Borse BB, Raghavan B. Antioxidant and radical-scavenging carbazole alkaloids from the oleoresin of curry leaf (Murraya koenigii Spreng.). Food Chemistry. 2007 Jan 1;100(2):742-7.

25. Shah AS, Wakade AS and Juvekar (2008),"Immunomodulatory activity of methanolic extract of Murraya koenigii (L) Spreng. leaves.", Indian J Exp Biol 46(7), pg: 505-509.

26. Sharma P, Vidyasagar G, Bhandari A, Singh S, Bhadoriya U, Ghule S, Dubey N. A pharmacological evaluation of antidiarrhoeal activity of leaves extract of Murraya koenigii in experimentally induced diarrhoea in rats. Asian Pacific Journal of Tropical Disease. 2012 Mar 1;2(3):230-3.

27. Shee, C. and A. K. Sharma (2007), Purification and characterization of a trypsin inhibitor from seeds of Murraya koenigii, Enzyme Inhib Med Chem,Vol.22(1),pg: 115-120.

28. Singh K, Tiwari V, Prajapat R, Study of Antimicrobial activity of medicinal plants against various multiple drug resistant pathogens and their molecular characterization and its

bioinformatics analysis of antibiotic gene from genomic database with degenerate primer prediction. International Journal of Biological Technology, 1(2):15-19, (2010).

29. Tachibana Y, Kikuzaki H, Lajis NH, Nakatani N. Antioxidative activity of carbazoles from Murraya koenigii leaves. Journal of agricultural and food chemistry. 2001 Nov 19;49(11):5589-94.

30. Tembhurne SV, Sakarkar DM. Hypoglycemic effects of fruit juice of Murraya koenigii (L) in alloxan induced diabetic mice. International journal of pharmtech research. 2009; 1(4):1589-93.

31. Vasudevan M, Parle M. Antiamnesic potential of Murraya koenigii leaves. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2009 Mar;23(3):308-16.

32. Xie. J. T, W. T. Chang, et al. (2006), Curry leaf (Murraya koenigii Spreng.) reduces blood cholesterol and glucose levels in ob/ob mice, American Journal of Chinese_Medicine Vol.34(2) .pg:279-284.