

Chemical composition and antibacterial activity of essential oil from the leaves of *Murraya koenigii* (L.) Spreng

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ABSTRACT

Chemical composition and antibacterial activity of the leaf essential oil of *Murraya koenigii* were investigated against clinically isolated bacterial strains. 14 compounds were identified by gas chromatography (GC) and GC-mass spectrometry accounting for about 98.1% of the total essential oil. α -pinene (49.3%), 2H-1-benzopyran (24.3%), 2-allyl-4-methylphenol (16.7%), and D-isomenthol (2.1%) were identified as the major chemical compounds. The essential oil produced mean zone of inhibition ranged between 14.0 and 7.6 mm. The essential oil showed antibacterial activity against all the bacterial strains tested with the minimum inhibitory concentration values of 125-500 μ g/ml. Although, the activity of the essential oil against the clinical isolates were much less than of ciprofloxacin, the standard drug used, the demonstrated antibacterial activities of *M. koenigii* leaf essential may support the folkloric uses of the plant.

KEY WORDS: Antibacterial activity, chemical composition, essential oil, *Murraya koenigii*

INTRODUCTION

Murraya koenigii (L.) Spreng (Rutaceae) is an aromatic small tree locally known as “Kariveppilai” by the people of Tamil Nadu. The plant originated in Terai regions of Uttar Pradesh, India and now widely found in all parts of India. It adorns every house yard of South India (Joseph and Peter, 1985). The plant is used in the Indian system of medicine to treat various ailments (Chopra *et al.*, 1996; Kesari *et al.*, 2005). The leaves and roots are bitter and cooling, used as anthelmintic, analgesic and to cure piles, to allay heat of the body, thirst, inflammation, and itching. The infusion of toasted leaves is used to stop vomiting. The green leaves are described to be eaten raw for the cure of dysentery (Kirtikar and Basu, 1991). Previous phytochemical investigations on this plant revealed two alkaloids 9-carbethoxy-3-methylcarbazole and 9-formyl-3-methylcarbazole and a known metabolite, 3-methylcarbazole (Chakrabarty *et al.*, 1997), and new carbazole alkaloid bismurrayafoline E (Nutan *et al.*, 1999). Xanthotoxin, Isobyakangelicol, phellopterin, gosferol, neobyakangelicol, byakangelicol, byakangelicin, and isogosferol were reported as minor furocoumarins of *M. koenigii* seeds (Adebajo and Reisch, 2000). The antihyperglycemic activity of *M. koenigii*

leaves in diabetic rats (Yadav *et al.*, 2002; Kesari *et al.*, 2007). A benzoisofuranone derivative and carbazole alkaloids were isolated from *M. koenigii* stem bark, and the compounds showed antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Aspergillus Niger*, and *Candida albicans* (Rahman and Gray, 2005). 10 carbazole alkaloids such as koenine, koenimbine, koenigine, koenidine, mahanimbine, mahanine, pyrayafoline-D, euchrestine-B, murrayafoline-1, and mahabinine-A isolated from the leaves of *M. koenigii* (Ito *et al.*, 2006), and mahanine pyrayafoline-D and murrayafoline-1 showed significant cytotoxicity against HL-60 cells. Mahanimbine and koenigine obtained from the leaves of *M. koenigii* showed radical scavenging activity (Rao *et al.*, 2007).

In the present investigation, the chemical composition and antibacterial activity of the leaf essential oil of *M. koenigii* are reported. In the previous reports, the essential oil of the leaves of *M. koenigii* was reported to possess antimicrobial (Goutam and Purohit, 1974), antifungal (Deshmukh *et al.*, 1986), and pesticidal (Pathak *et al.*, 1997) activities. However, this is the first report for the antibacterial activity of *M. koenigii* leaf essential oil on clinically isolated bacterial strains.

MATERIALS AND METHODS

Plant Material and Extraction of the Essential Oil

Healthy and well-grown fresh leaves of *M. koenigii* were collected from Sivapuri (11°24'24 N 079°42'59 E) village, Chidambaram and immediately brought to the laboratory using polythene bags. The fresh leaves were subjected to hydro distillation using Clevenger-type of apparatus for 4 h. The essential oil was dried over anhydrous sodium sulfate, and the essential oil was stored in the amber colored vial at 4°C until further analysis and antibacterial assay.

Gas Chromatography (GC) and GC-Mass Spectrometry (MS) Analysis

GC analysis was carried out using Varian 3800 GC equipped with a mass selective detector coupled to front injector type 1079. The chromatograph was fitted with VF 5 MS capillary column (Low bleed 5% phenyl, 95% dimethyl polysiloxane 30 m × 0.25 mm i.d., film thickness 0.25 µm). The injector temperature was set at 300°C, and the oven temperature was initially at 70°C then programmed to 200°C at the rate of 5°C/min, and held at 200°C for 10 min. Then, the temperature was increased to 300°C at the rate of 10°C/min, finally held at 300°C for 5 min. Helium was used as a carrier gas with the flow rate of 1.0 ml/min. The sample was injected in the split mode in the ratio of 1:100. The percentage of composition of the essential oil was calculated by the GC peak areas.

GC-MS analysis of essential oil was performed using Varian 3800 GC equipped with Varian 1200 L single quadrupole MS. GC conditions were same as reported for GC analysis and the same column was used. The MS was operated in the electron impact mode at 70 eV. Ion source and transfer line temperature were kept at 300°C. The MS was obtained by centroid scan of the mass range from 40 to 800 amu. Identification of components of the essential oil was done by matching their recorded spectra with the data bank MS of Wiley library provided by the instrument software.

Antimicrobial Activity

Microbial strains

The antibacterial activity of the essential oil of *M. koenigii* was investigated against five Gram-positive bacteria viz., *B. subtilis*, *Bacillus cereus*, *Micrococcus luteus*, *S. aureus* and *Staphylococcus epidermis* and five Gram-negative bacteria viz., *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Shigella flexneri*, and *Shigella dysenteriae*. The clinical isolates were received from the Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai

University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.

Disc diffusion assay

Antibacterial activity of *M. koenigii* leaf essential oil was tested against the above Gram-positive and Gram-negative bacteria by disc diffusion method (Vandan Berghe and Vlietinck, 1991; Cappuccino and Sherman, 1998). These bacteria were grown in Mueller-Hinton Agar medium (pH 7.3). 20 ml of agar medium were poured into the plates to obtain uniform depth and allowed to solidify. The standard inoculum suspension (10⁶ CFU/ml) was streaked over the surface of the media using a sterile cotton swab to ensure the confluent growth of the organism. The 6 mm diameter discs were prepared with Whatman No. 1 paper and used for the study. 10 µl of essential oil was diluted with two volumes of 5% dimethyl sulfoxide (DMSO) and impregnated on the filter paper discs, and placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the inoculated agar surface. Ciprofloxacin (5 µg/disc) was used as positive reference standard to determine the sensitivity of the tested strains, and 5% DMSO was used as a blind control. Finally, the inoculated plates were incubated at 37°C for 24 h and observed the inhibition zones including the diameter of the disc (mm). All the experiments were done in triplicate.

Determination of minimum inhibitory concentration (MIC)

The MIC values were determined for the bacterial strains which were sensitive to the essential oils in disc diffusion assay. The MIC of the essential oil was tested in Mueller-Hinton broth by broth macro dilution method (Ericsson and Sherris, 1971). The inoculation of the bacterial strains was prepared from 12 h old broth cultures and suspensions were adjusted to standard turbidity (10⁶ CFU/ml). The leaf essential oil of *M. koenigii* was dissolved in 5% DMSO to obtain 1000 µg/ml stock solution. 0.5 ml of stock solution was incorporated into 0.5 ml of Mueller-Hinton broth to get a concentration of 500, 250, 125, 62.5, and 31.25 µg/ml. 50 µl of standard suspension of the test organism was transferred to each test tube. The control tube contained only organism and was devoid of *M. koenigii* essential oil. The culture tubes were incubated at 37°C for 24 h. The lowest concentrations which did not show any growth of tested organism after the macroscopic evaluation was determined as MIC.

RESULTS AND DISCUSSION

The fresh leaves of *M. koenigii* yielded 0.34% (v/w) of essential oil. Gas chromatogram of the essential oil

is shown in Figure 1. Table 1 shows the compounds identified in the leaf essential oil of *M. koenigii* in order of elution from VF 5 MS column. 14 compounds were identified by GC-MS and they are tricyclene, α -Pinene, camphene, *trans*-isolimonene, α -Gurjunene, *trans*-caryophyllene, β -Elemene, elemicin, *trans*- β -Farhesene, D-isomenthol, epsilon-cabinene, 2H-1-benzopyran, 2-allyl-4-methylphenol, and α -Guaiene, representing 98.1% of the essential oil, whereas α -pinene (49.3%), 2H-1-benzopyran (24.3%), 2-allyl-4-methylphenol (16.7%), and D-isomenthol (2.1%) were identified as the major chemical compounds.

The chemical composition of *M. koenigii* varied from region to region. Studies on the major components of *M. koenigii* oil carried out so far have indicated that β -caryophyllene, β -phellandrene, and α -pinene were the versatile components present in this plant essential oil. It is worth mentioning that there is variation in the chemical composition of *M. koenigii* oils reported from India (Hiremath *et al.*, 1997; Mallavarapu *et al.*, 1999; Raina *et al.*, 2002) and other parts of the world (MacLeod and Pieris, 1982; Wong and Tie, 1993). The composition of any plant essential oil is influenced by the presence of several factors such as local climatic, seasonal, and experimental conditions (Daferera *et al.*, 2000) thereby altering the biological activities studied (Vardar-Ünlü *et al.*, 2003).

The antimicrobial activity of the essential oil of *M. koenigii* was studied against different clinically isolated strains of both Gram-positive and Gram-negative bacteria. The potency of the essential oil was assayed by the presence or absence of inhibition zones (zone diameter), and MIC values and the results are given in Table 2. The data obtained from disc diffusion method indicated that the essential oil possess antibacterial activity against all the clinically isolated bacterial strains. Gram-positive bacteria were more sensitive to the oil than the Gram-negative bacteria. *S. aureus* was the most sensitive bacterial strain with the inhibition zone of 14.0 mm and MIC value of 125 μ g/ml. The results of MIC indicated that the oil inhibited all the bacterial strains tested with the MIC values of 125-500 μ g/ml. The activity of the essential oil may be due to the presence of active compounds camphene and α -pinene (Davidson and Naidu, 2000). Although, the activity of the essential oil against the clinical isolates were much less than of ciprofloxacin, the standard drug used, the demonstrated antibacterial activities of *M. koenigii* leaf essential may support the folkloric uses of the plant. The strong antibacterial activity of *M. koenigii* leaf essential oil thus suggests that the essential oil could be used in chemotherapy particularly for the local population which

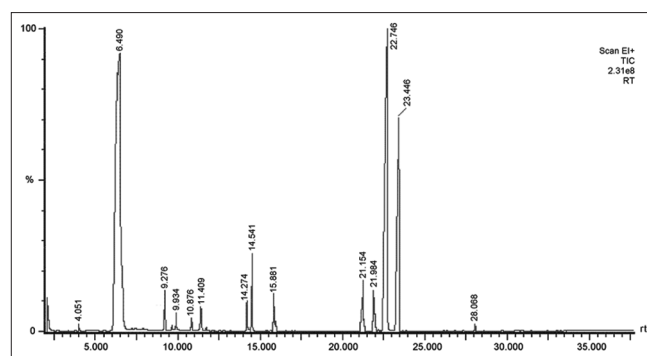


Figure 1: Gas chromatogram of the leaf essential oil of *Murraya koenigii*

Table 1: Chemical composition of the leaf essential oil of *M. koenigii*

Retention time (min)	Chemical constituents*	Composition (%)
4.051	Tricyclene	0.1
6.490	α -Pinene	49.3
9.276	Camphene	1.4
9.934	<i>trans</i> -Isolimonene	0.2
10.876	α -Gurjunene	0.1
11.409	<i>trans</i> -Caryophyllene	0.3
14.274	β -Elemene	0.2
14.541	Elemicin	1.0
15.881	<i>trans</i> - β -Farnesene	1.3
21.154	D-Isomenthol	2.1
21.984	γ -Cadinene	1.0
22.746	2H-1-Benzopyran	24.3
23.445	2-allyl-4-methylphenol	16.7
28.068	α -Guaiene	0.1
	Total	98.1

*Compounds listed in order of elution from VF 5 MS column.

M. koenigii: *Murraya koenigii*

Table 2: Antibacterial activity of the leaf essential oil of *M. koenigii*

Name of the microorganism	Mean zone of inhibition (mm)*		MIC of the essential oil (μ g/ml)
	Essential oil (10 μ l/disc)	Ciprofloxacin (5 μ g/disc)	
<i>Bacillus subtilis</i>	10.6 \pm 0.3	28.5 \pm 0.2	250
<i>Bacillus cereus</i>	12.0 \pm 0.5	24.5 \pm 0.7	250
<i>Micrococcus luteus</i>	11.6 \pm 0.3	27.3 \pm 0.6	125
<i>Staphylococcus aureus</i>	14.0 \pm 0.5	27.8 \pm 0.6	125
<i>Staphylococcus epidermis</i>	11.5 \pm 0.2	22.5 \pm 0.2	500
<i>Escherichia coli</i>	11.0 \pm 0.05	24.6 \pm 0.6	250
<i>Klebsiella pneumoniae</i>	9.0 \pm 0.5	22.0 \pm 0.5	500
<i>Proteus mirabilis</i>	8.3 \pm 0.3	22.1 \pm 0.4	500
<i>Shigella flexneri</i>	7.6 \pm 0.6	20.6 \pm 0.6	500
<i>Shigella dysenteriae</i>	11.3 \pm 0.3	21.6 \pm 0.6	250

*Mean zone of three assays; MIC: Minimum inhibitory concentration, NA: No activity, NT: Not tested, *M. koenigii*: *Murraya koenigii*

needs the cheap drug. Especially, against *S. aureus*. Because *S. aureus* has been most frequently cited as the cause of delayed wound healing and infection (Bowler *et al.*, 2001).

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