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Original article

GC-MS analysis and *in vitro* antibacterial potential of volatile chemical constituents from leaves of *Murraya koenigii* L. Spreng

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Abstract

Murraya koenigii L. Spreng, commonly known as curry leaves, is usually employed for their flavoring properties in the food due to its distinct aroma. It is a source of essential oil that has been studied extensively for its therapeutic potentials. The volatile constituents were obtained from leaves of *M. koenigii* by hydro-distillation method. It was then evaluated for its antibacterial potential on four different strains of bacteria, *i.e.*, *Micrococcus luteus*, *Bacillus subtilis*, *Escherichia coli*, and *Pasteurella multocida* by agar well-diffusion method. The volatile constituents were then subjected to GC-MS analysis for their chemical composition. The volatile constituents showed the appreciable antibacterial spectrum against all four strains. The GC-MS analysis of volatile constituents revealed the presence of 4 unidentified and 7 identified major components. The unidentified and identified components constitutes 0.1932% and 99.1089%, respectively. The identified compounds were β-pinene (3.4155%), β-caryophyllene (15.8231%), sabinene (59.7176%), α-pinene (10.9345%), β-phellandrene (5.2556%), γ-terpinene (2.7974%) and myrcene (1.8632%). The findings clearly suggest that the volatile chemical constituents obtained from *M. koenigii* has pronounced antibacterial activity against the strains used and the components responsible for it were scrutinized.

Key words: *Murraya koenigii* L. Spreng, antibacterial, volatile constituents, composition, essential oil

1. Introduction

Treating ailments with the aroma and aromatic materials from plants, are regarded as an alternative system of medicine. The plant extracts and volatile chemical constituents from non-woody parts of the plant possess healing qualities. These volatile aromatic components of the plants are primarily volatile oils and are usually extracted by employing different distillation techniques. The extracted volatile oils are principally terpenoid compounds. These terpenoids are responsible for the medicinal properties, culinary purposes and fragrant uses of these aromatic plants (Dorman and Deans, 2000; Dorman, 1999).

These plant derived oils are extensively studied for their various therapeutic potentials (Özbek *et al.*, 2003) for, *e.g.*, *Colebrooke aoppositifolia* oil has antifungal, anthelmintic activity, the essential oil from *Melaleuca alternifolia* (tea tree) is commercially used as an antibacterial agent in antiacne products, the essential oil of *Foeniculum vulgare* has hepatoprotective and antimicrobial activity, *etc.* (Ishtiaq *et al.*, 2016; Ali *et al.*, 2011; Cox *et al.*, 2000; Anwar *et al.*, 2009). Particularly, the antimicrobial properties of these volatile oils and extracts have broaden their use from raw food processing and preservation to the pharmaceutical products and natural remedies for health related problems (Reynolds, 1996). The antimicrobial properties remain of the great interest for all these researchers due to increase in the burden of infectious diseases. A good number of studies have been carried out with the focus on the essential oils antiseptic properties exclusively on a specific micro-organism. The data presented by those researchers was of significant impact but due to the diversity of the micro-organisms and difference in the methodologies, alternatives are being prepared either by modifying the procedure or by changing the microbial strain, *etc.* (Janssen *et al.*, 1987; Hammer *et al.*, 1999).

The present study is about the volatile constituents obtained from curry leaves, *Murraya koenigii* L. Spreng (Rutaceae). The plant is commonly used for its flavoring properties in food in Pakistan. The oil of the plant material is also recorded to have appreciable antidiabetic and promising antioxidant potential due to the phytochemical constituents, *i.e.*, carbohydrates, tannins, alkaloids, steroids, triterpenoids and flavonoids present in it (Kusuma *et al.*, 2011). Besides this, different extracts of the plant possesses anti-ulcer and anthelmintic activity. The plant also found to reduce oxidative stress and have antilipid peroxidative, cytotoxic, chemoprotective and hepatoprotective effect (Dineshkumar *et al.*, 2010; Tachibana *et al.*, 2001; Sathaye *et al.*, 2011; Handral *et al.*, 2012).

The leaf oil possess antimicrobial activity and it is also studied by various researchers on different bacterial strains (Nutan *et al.*, 1998; Rahman and Gray, 2005; Ningappa *et al.*, 2010). In the present study, the antibacterial activity of essential oil was evaluated against *Micrococcus luteus*, *Bacillus subtilis*, *Escherichia coli* and

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Pasteurella multocida which is so far to our knowledge has not been performed. The composition of the essential oil from leaves was also identified by GC-MS analysis.

2. Material and Methods

2.1 Chemicals

All the chemicals used were of analytical grade. Nutrient agar (Merck, Germany) for culture media and ciprofloxacin discs CFX 5µg (Bio-Rad) were used to carry out the study.

2.2 Plant material

The fresh leaves were collected from the garden of PCSIR Laboratories Lahore, Pakistan. The plant was identified and authenticated by Dr. Uzma Hanif, Lecturer Government College University, Lahore, Pakistan. The voucher sample (LCWU. Herb. Bot. 2351) was deposited in herbarium of Lahore College for Women University, Lahore, Pakistan. The gas chromatography-mass spectrometry (GC-MS) analysis was carried out by using Agilent 7890GC/5975MS system, Germany.

2.3 Extraction of volatile chemical constituents

Fresh leaves (½ kg) were cut into small pieces and subjected to extraction of volatile constituents by hydro-distillation, using Reverse Dean - Stark method (Naz *et al.*, 2010).

2.3.1 Reverse Dean-Stark method

A round bottom flask of $5dm^3$ capacity was taken and heated on isomentle. More than half of the flask was filled with water so that the material is immersed into it. A reverse dean - stark attachment was fixed on the mouth of the flask and a coiled condenser was attached on its top. Due to the production of steam in the flask, the pockets of the plant material which contained essential oil was opened. The steam produced causes the rupturing and release of oil from these pockets. The oil produced was carried away by the steam, rising out of the flask. The steam with oil was rising up in the condenser. The hot vapors condensed into liquid and the liquid dropped into the reverse dean - stark apparatus. The oil having low density than water produced so floated on the water. This forms a layer on the water surface in the reverse dean-stark apparatus. This layer on the top of the water pushed the water in the bottom through the side arm back into the flask for recirculation. Hence, the water under the oily layer was used again and again keeping the level of water in the flask at constant rate. This water changes into steam and than into water on condensation.This interconversion of steam into water carried out oil from the plant material, and thus affected the extraction and separation of oil from the leaves of *M. koenigii*.

The oil was separated from water by a separating funnel. The yield of the oil was 2.53%. For the dehydration of oil, sodium sulfate was added into it. The oil is then stored in amber color bottle to prevent denaturation.

2.4 Microorganisms

Four different strains were used for testing antibacterial activity included; *Micrococcus luteus*, *Bacillus subtilis*, *Escherichia coli*, and *Pasteurella multocida*. The test organisms used in this study were obtained from Microbiology Lab. of Lahore College for Women University Lahore, Pakistan. The bacterial strains were numbered as Specimen No. LCWU: ML. 2286M, 2287B, 2288E and 2289P. The bacteria were cultured on nutrient agar slants. The cultures were conserved periodically by subculturing and preserved at 4ºC prior to use them.

2.5 Screening of antibacterial activity

The antibacterial efficacy of the volatile constituents were estimated by agar well-diffusion method (Irshad *et al.*, 2012).

Different concentrations of volatile oil of *M. koenigii* were prepared in the 20% DMSO (four concentrations 1%, 25%, 75%, and 100%) by serial dilution method. The microbial starter cultures were seeded into respective medium by gentle mixing 0.1 ml of the 24 hours fresh cultures with 35 ml sterile molten agar in sterile petriplates. After the hardening of agar, four wells of about 7mm depth were made using borer aseptically. The wells were filled with 0.1 ml of volatile constituents. The assay plates were incubated at 37ºC for 24 h. The diameter of zone of inhibition around each of the well was considered as measurement of antibacterial activity. Each test was carried out in triplicates and average diameter was calculated.

For negative control, a filter paper saturated with 20% DMSO was used to check possible susceptibility of this solvent against the bacteria, while ciprofloxacin discs served as positive control.

2.6 GC-MS analysis

The GC-MS analysis of volatile constituents of leaves of *M. koenigii*, was performed using Agilent GC-MS, equipped with DB-5 MS split and split-less mode column model DB-5 MS dimensions (30 nm X 0.25 mm), diameter of 0.25 µm. The operation mode was conducted at 70eV. Helium was the carrier gas maintained at a pressure of 11.66 psi and a flow rate of 1.00 ml/min. The injector was operated in the temperature range of 45-350°C. The oven temperature was programmed to increase as follows; 50°C at 6°C/min to 200°C (5 min) at 6 °C/min to 325°C (10 min).The temperature was kept constant for 5 min in the beginning of the procedure and at the end of sample run. The essential oil was filtered through 0.45 µm filter using filtration syringe. The analysis was carried out utilizing split-less mode, injecting 2.00 µl of the analyte sample at 50°C (Peter *et al.*, 2012).

A mass range of 35-500 atomic mass unit (amu) was scanned and analyzed with the help of GC-MS lab-solution software that contained in it NIST-417 LIB, for identification and characterization of sample. The name, molecular formula of the components was ascertained and by using homologous series of compounds, the retention indices for each compound was assessed.

2.7 Statistical analysis

The results are presented as the mean ± SEM. The results were calculated using Microsoft Excel 2013.

3. Results

3.1 Antibacterial activity

The volatile oil from the leaves of *M. koenigii* showed comparable *in vitro* antibacterial activity on all four strains of the bacteria included in this study, at different concentrations shown in Table 1. The antibacterial activity was estimated by the measurement of the diameter of zone of growth inhibitions with the help of vernier caliper.

Table 1: Results of antibacterial activity of essential oil from leaves of *M. koenigii* compared with ciprofloxacin, tested on four different bacterial strains

Conc. of volatile constituents (%)	Zone of inhibition (cm)			
	Micrococcus luteus	*Bacillus subtilis*	*Escherichia coli*	*P. multocida*
DMSO 20%	0.50±0.577	0.55±0.451	0.57±0.120	0.39±0.577
Ciprofloxacin (5µg)	4.18±0.088	5.54±0.144	5.91±0.105	4.80±0.038
EO 1 %	–	–	2.16±0.067	1.46±0.033
EO 25 %	1.93±0.088	2.00±0.058	2.50±0.033	2.46±0.033
EO 75 %	–	–	–	2.80±0.058
EO 100 %	–	–	–	–

The results are expressed as mean ± SEM. 20% DMSO solution was used as negative control, 5% ciprofloxacin was used a positive control, EO 1%, EO 25%, EO 75%, EO 100% are concentrations of essential oil of *M. koenigii*. The results of different concentrations of volatile oil were compared with ciprofloxacin (5 µg).

3.2 GC-MS analysis

The GC-MS analysis of essential oil of *M. koenigii* was performed to elucidate its composition. The GC-MS spectra obtained, revealed eleven sharp peaks shown in Figure 1. The identified and unidentified components along with their percentage composition and retention times are presented in Table 2. The unidentified and identified components constitute 0.1932% and 99.1089%, respectively.

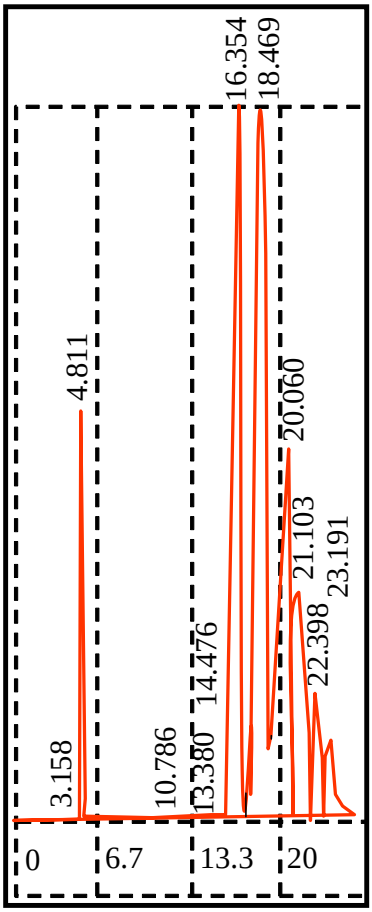

Figure 1: GC-MS fingerprint of essential oil from leaves of *M. koneigii*

Table 2: Results of GC-MS analysis of identified and unidentified components of essential oil from *M. koneigii* leaves

S.No.	Components	RT	Percentage of oil components (%)
1	Unidentified	03.158	0.0272
2	β-pinene	04.811	3.4155
3	Unidentified	10.786	0.0547
4	Unidentified	13.560	0.0521
5	Unidentified	14.467	0.0592
6	β-caryophyllene	16.554	15.8231
7	sabinene	18.469	59.7176
8	α-pinene	20.060	10.9345
9	β-phellandrene	21.103	5.2556
10	γ-terpinene	22.398	2.7974
11	Myrcene	23.191	1.8632

***RT:** Retention time

4. Discussion

Due to the emergence of resistance mechanisms in the bacteria, the researchers are working on discovering new therapies and ways to overcome this problem. Natural therapies are always preferred over synthetics. People trust more on herbal or traditional medicines for health care, because the allopathic treatments are more expensive and are often linked with serious adverse effects. A significant number of modern pharmaceuticals are based on or derived from medicinal plants. Herbal medicines are usually in the form of vegetable drugs or extract which primarily serve to treat various ailments and to maintain health (Noori *et al.*, 2015).

By this vision, the antibacterial activity of volatile chemical constituents from leaves of *M. koenigii* was estimated by Well diffusion method in four strains of bacteria, *i.e., Micrococcus luteus*, *Bacillus subtilis*, *Escherichia coli* and *Pasteurella multocida*. The 20% DMSO served as negative while ciprofloxacin disc (5 µg) served as positive control. The essential oil showed comparable antibacterial results. The oil showed its antibacterial activity against all four strains in various percentage compositions. The maximum antibacterial activity was observed against *Pasteurella multocida* at 75% concentration. At 25% concentration, the extract showed antibacterial activity against all four strains with maximum efficacy against *E. coli* and *Pasteurella multocida*. While the antibacterial

activity depicted at 1% concentration was negligible. None of the strains showed any antibacterial activity at 100% concentration which suggests that the essential oil was not effective in pure state (Table 1).

The GC-MS fingerprint (Figure 1) of the extracted oil sample showed the 11 sharp peaks and their retention time, was recorded. The fingerprint depicted 7 identified compounds that constitute 99.1089% of the oil while 4 peaks were of unidentified components that constitute 0.1932% of the oil. The identified compounds were β-pinene (3.4155%), β-caryophyllene (15.8231%), sabinene (59.7176%), α-pinene (10.9345%), β-phellandrene (5.2556%), γ-terpinene (2.7974%) and myrcene (1.8632%) (Table 2).

β-pinene, α-pinene and β-caryophyllene are recorded to have antibacterial action against gram positive bacterial strains (Knobloch *et al.,* 1989; Del-Vecgio-Vieira *et al.,* 2009). In addition to this, myrcene, sabinene and β-phellandrene are part of essential oils from different medicinal plants reported to have antibacterial spectrum (Deans and Svoboda, 1988, 1989; Alma *et al.,* 2003; Koutsoudaki *et al.,* 2005; Haznedaroglu *et al.,* 2001; Tatsadjieu *et al.,* 2003). All the identified components are terpenoids and these terpenoids are collectively responsible for the pronounced antibacterial action (Anwar *et al.,* 2009).

5. Conclusion

M. koenigii leaves essential oil holds mild antibacterial potential against the four different strains incorporated in this study as compared to ciprofloxacin. On the other hand, the study was to elucidate the chemical components of the essential oil. The GC-MS analysis provides the data about it, revealing the nature of chemical constituents present pertaining different therapeutic attributes.

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Conflict of interest

We declare that we have no conflict of interest.

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