

Chemical Composition and *In vitro* Antifungal and Antioxidant Activities of Essential Oil from *Murraya koenigii* (L.) Spreng. Leaves

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Abstract

The chemical composition and the *in vitro* antifungal and antioxidant activity of essential oil of *Murraya koenigii* (L.) Spreng leaves have been studied. The yield of *M. koenigii* leaf essential oil (MKLEO) recorded as 0.52% which is higher than those reported earlier. Altogether 43 compounds were identified by GC-MS analysis representing 99.79% of the total composition of the oil, among which 3-carene, β -pinene, α -pinene, linalool, α -eudesmol, p-cymene, γ -terpinene, α -amorphene, allo-ocimene, sabinene, γ -terpinene, linalyl acetate, myrcene, β -eudesmol, carvone, limonene, β -elemene, α -terpineol were major constituents. Antifungal activity of MKLEO was tested against ten pathogenic fungi and it was found effective in a dose dependent manner. Furthermore, MKLEO was found to exhibit superior radical scavenging potency and reducing power with IC₅₀ and RP₅₀ values close to those of the standards.

Keywords: *Murraya koenigii*, Leaves, Essential oil, GC-MS, Antifungal, Antioxidant.

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Introduction

The essential oils are secondary metabolites produced by plants as a part of their defence mechanism are characterized by a complex mixture of several compounds belonging to different classes viz., hydrocarbons, phenols, terpenes, alcohols, aldehydes, ketones, esters, ethers and others [1-3]. Essential oils derived from aromatic plants apart from their use for flavouring or cosmetic purposes [4], have been widely used in the treatment of various diseases owing to their pharmacological properties such as antimicrobial, anti-inflammatory, antioxidant and several other biological activities [5-8]. In view of the interest harmful impacts of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), etc. on human health, natural antioxidants are gaining importance in modern therapeutics. Use of such synthetic antioxidants is prohibited as it leads to liver damage [9]. Furthermore, use of essential oils in food processing and packaging represent a valid alternative to prevent autoxidation and prolong shelf life of food products [10].

Murraya koenigii (L.) Spreng commonly known as Curry leaf is a shrub or a small tree under the family Rutaceae, growing up to 6 m height. The plant is native to India, Pakistan, Sri Lanka, Bangladesh and the Andaman Islands. It is also cultivated widely in South-East Asia and some parts of the United States and Australia. In tropical Africa it is planted in many countries, including Nigeria, Kenya, Tanzania and most of the Indian Ocean Islands, where Indian immigrants settled [11]. Leaves of the plant are widely use in Indian cookery for centuries and have a versatile role in traditional medicine [12].

Out of the 14 species globally reported under the *Murraya* genus, only two i.e. *M. koenigii* (L.) Spreng and *M. paniculata* (L.) Jack is found in India; of which, the former is most popular on account of its diverse medicinal properties and its use as a flavouring agent in different curries and foods since the ancient times [13]. In traditional system of medicine, the plant is regarded as analgesic, cooling, alexiteric, antiemetic, anthelmintic, antidiarrhoeal, febrifuge, carminative, purgative, stomachic and stimulant and used to alleviate body temperature, blood disorders, diarrhoea, dysentery, eruption, inflammation, itching, kidney pain, leukoderma, piles, snakebite, thirst, vomiting and blood purification. The leaves of the plant are used traditionally in the Indian Ayurveda system to treat diabetes [14].

Phytochemical investigations have led to the isolation and characterization of several chemical constituents from every part of the plant. The phytoconstituents present in *M. koenigii* leaves include phenols, steroids, saponins, quinones, alkaloids, flavonoids, tannins, carbohydrates, proteins, and volatile oils [15]. Almost all parts of the plant contain carbazole alkaloids well known for their various pharmacological activities, including anti-HIV, anticancer, antibacterial and antifungal activities [16,17]. Bark contains carbazole alkaloids namely mukoenine-A, B and C and murrastifoline-F, bis-2-hydroxy-3-methyl carbazole, bismahanine, bi koeniquinone-A, bismurrayaquinone-A, murrayacine, murrayazolidine, murrayazoline, mahanimbine, girinimbine, koenioline and xynthyletin [18]. Leaves contains koenimbine, O- methyl murrayamine, O- methyl mahanine, isomahanine, bismahanine, bispyrayafoline, glycozoline, 1-formyl-3 methoxy- 6-methyl carbazole, 6, 7-dimethoxy-1-hydroxy-3-methyl carbazole,

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koenigine, koenine, koenidine and (-) mahanine, mahanimbine, isomahanimbine, koenimbidine, murrayacine, Isomahanimbicine, euchrestine B, mahanine, mahanimbicine, mahanimbine, bismurrayafoline E, mahanimbicine, bicyclomahanimbicine, cyclomahanimbine, bicyclomahanimbine, mahanimbidine, mukonicine, 1-formyl-3-methoxy-6-methyl carbazole and 6, 7- dimethoxy-1- hydroxy-3 methyl carbazole [19-22]. The leaves of *Murraya koenigii* also consist of protein, carbohydrates, fibre, minerals, carotene, vitamin C, Nicotinic acid [22]. Bioactive carbazole alkaloids including murrayanol, murrayagetin, marmesin-1''-O-rutinoside, mukoline, mukolidine, girinimbine and koenoline from roots; mahanimbine, koenimbine, isomahanine murrayanol, mahanimbine, murrayazolidine, girinimbine, koenimbine and mahanine from fruits and iskurryam, koenimbine, koenine, mahanimbine, girinimbine, koenimbine, mahanine and isomahanine alongwith indicolactone, anisoalctone and 2'',3''epoxyindicolactone and minor furocoumarins such as xanthotoxin, isobyaknagelicol, byakangelicol, isogosferol. isoheraclenin, isoimperatonin, oxypeucedanin, isopimpinellin and bergaptanwere have been extracted from the seeds [23]. The most important chemical constituents responsible for its intense characteristic aroma are p-gurjunene, p-caryophyllene, p-elemene and o-phellandrene [24]. Essential oils from *M. koenigii* serves as an important part in soap making ingredients, lotions, massage oils, diffusers, potpourri, scent, air fresheners, body fragrance, perfume oils, aromatherapy products, bath oils, towel scenting, spa's, incense, facial steams, hair treatments, and more [25,26]. Chemical constituents of *M. koenigii* leaves reported to exhibit anticancer, hepatoprotective, antipyretic, antioxidant, anti-obesity, antimutagenic, antimicrobial, antifungal, insecticidal, antibacterial effect [13]. Previous studies recorded high antioxidant activity in leaves which is mainly attributed to the presence of chemical constituents namely mahanimbine, murrayanol and mahanine [27,28]. These constituents have also been found responsible for antibacterial and antifungal activities of the leaves [19]. The work presented was aimed to investigate the chemical composition of the essential oil from *Murraya koenigii* leaves collected from Dehradun, Uttarakhand (India) and to evaluate antifungal and antioxidant activities of the essential oil.

Materials and methods

Plant material

Fresh leaves of *Murraya koenigii* used for the study were collected from suburbs of Dehradun, located in the Doon Valley on the foot hills of the Himalayas and the capital city of the state of Uttarakhand, India during the onset of monsoon and were authenticated by Systematic Botany Section of Botany Division, FRI. A voucher specimen of the collected material is preserved in the Chemistry Division for future reference.

Extraction of essential oil

M. koenigii leaves (200 g) were taken into round bottom flask and soaked in distilled water in the ratio of 1:3 and then hydro distilled for 10 h using a Clevenger type apparatus. The distillate was extracted with diethyl ether (3 × 50 ml); the ethereal layer was dried over anhydrous sodium sulphate and the diethyl ether was removed on a gently heated water bath (300°C). The yield of an essential oil was calculated on air dried basis. For accuracy the experiments were repeated three times. The process was further repeated several times to isolate sufficient quantity of the essential oil for the analysis. The percentage yield of *M. koenigii* leaf essential oil (MKLEO) was calculated with respect to the quantity of leaf material used for essential oil extraction.

GC-MS analysis of MKLEO

Essential oils are a complex mixture mainly of mono- and sesquiterpenes containing hundreds of components and thus it is not possible to isolate pure constituents by column chromatography. However, the chemical components of the essential oil can be qualitatively and quantitatively characterized by sophisticated Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) techniques. The GC-MS analysis of MKLEO was carried out with Gas Chromatography-Mass spectrometry (GC-MS) system (Agilent 7697B) fitted with a DB-5 ms capillary column (30 m × 250 µm × 0.25 µm, Agilent 122-S002). The injector, GC-MS interface, ion source, and MS Quadrupole temperature were maintained at maintained at 220°C and the transfer line to be held at 220°C. The oven temperature for volatile oil was programmed at 40°C (1 min), 40 to 220°C (3°C/min), 220 °C (20 min). Oil was diluted to 1% (w/v) in hexane and 1 µL injected. Detection was performed by mass spectrometer in the EI mode (ionization energy of 70 eV, ion source temperature of 180°C, emission current of 220 µA). Acquisition was made in full scanning mode (mass range 50-900 m/z; 3 scans/second). Maximum ionization time was 25 miliseconds. Helium flow rate through the column was 1 mL min⁻¹ with a 30:1 split. Identification of the individual oil components was accomplished by comparison of retention times with standard substances and by matching mass spectral data with MS libraries (NIST and Wiley 275.1) using a computer search and literature [29].

Evaluation of Antifungal Activity of MKLEO

Tested fungi

Antifungal activity of MKLEO was evaluated against altogether 10 pathogenic fungi viz., *Alternaria alternata* (AA), *Aspergillus flavus* (AF), *Aspergillus niger* (AN), *Aspergillus parasiticus* (AP), *Fusarium oxysporum* (FO), *Fusarium moniliforme* (FM), *Mucor mucedo* (MM), *Penicillium notatum* (PN), *Penicillium funiculosum* (PF), and *Trichoderma viride* (TV). These fungi were isolated from the infected saplings and spoiled foods by Standard Blotter Method [30] and identified based on growth characteristic, mycelial morphology, spore

morphology and other important characters using standard protocol [31,32]. Pure cultures of each of the selected fungal species were made separately and maintained on potato dextrose agar (PDA). These pure cultures were used for antifungal assay.

Antifungal activity assay

The antifungal activity was determined using the disc diffusion method [33]. Initially, the medium was prepared by dissolving potato dextrose agar (Hi Media) in distilled water and autoclaving at 121°C for 15 minutes. 20 ml of sterile PDA media was poured in sterilized petridishes (9 cm diameter) and allowed to solidify which were used for antifungal assay. Spore suspension was prepared in 0.9% saline water and adjusted to give a final concentration of $1-5 \times 10^5$ cfu/ml. The essential oil was diluted with Tween 40 to obtain the final concentrations of 1000, 750, 500, 250, 100, 50, 25 µg/ml, respectively. A plug of 1-week-old fungal culture (5 mm diameter) was placed on the centre of the sterilised plates containing PDA. About 10 µl of each concentration was injected to the sterile disc papers (6 mm diameter). Then the prepared discs were placed on the culture medium. Carbendazim (2 mg/ml) and Tween 40 were served as positive and negative control respectively. The plates were then incubated at 30°C for 4-5 days, and colony diameter was measured and recorded after 5 days. The growth inhibition of each fungal strain was calculated as the percentage inhibition of a radial growth relative to the control as:

$$\text{Inhibition (\%)} = [(1-A/B) \times 100]$$

Where A=mean diameter of fungal colony in treatment (mm); B=mean diameter of fungal colony in control (mm). All experiments were performed in triplicate.

Determination of MIC

The minimum inhibitory concentration (MIC) was determined through the broth dilution method [34,35]. Fungi were first grown in the potato dextrose broth for 24 h and then the inoculums were diluted for five times (10-5 dilution) to control its vigorous growth. Then each test tube was added with 1.8 ml of potato dextrose broth and different concentrations MKEO followed by inoculation of 0.2 ml of respective fungi and kept at 28°C for 48 h. The tubes were examined for visual turbidity. Lowest concentrations of the extracts showing no turbidity (without microbial growth) were considered as the minimal inhibitory concentration.

Evaluation of antioxidant activity of MKLEO

The antioxidant activity of MKLEO was evaluated by means of the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging method [36-38]. Briefly, different amounts of the tested sample (50-250 µg/mL) were added to 5 mL of a 0.004% methanol solution of DPPH. Finally, the absorbance was read against a blank at 515 nm after 30 min of incubation in the dark. All the observations were taken as triplicate. BHT, catechin, gallic acid and ascorbic acid were used as the

standard antioxidants. Inhibition of free radicals by DPPH in per cent (IC%) was calculated using the following equation-

$$\text{IC\%} = [(A_o - A_s) / A_o] \times 100,$$

Where A_o and A_s are the absorbance values of the control and test sample, respectively. Per cent inhibition was plotted against concentrations and the equation for the line was used to obtain the IC50 value.

Reducing power assay of MKEO

The reducing power of essential oil and various extracts was determined by the method reported earlier [39]. Varying concentrations of tested sample (50-250 µg/ml) were mixed with 2.5 ml of the phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixtures were incubated at 50°C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid was added to the mixtures, followed by centrifugation at 650.g for 10 min. The upper layer (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride ($FeCl_3$) and absorbance of the resulting solution were measured at 700 nm using spectrophotometer. All the readings were taken in triplicate and BHT, catechin, gallic acid and ascorbic acid were taken as the standard. The reducing power of samples was calculated by the following formula:

$$\text{RP (\%)} = [(A_o - A_s) / A_o] \times 100$$

Where A_o and A_s are the absorbance values of the control sample and the test sample, respectively. Percent (%) inhibition was plotted against concentration, and the equation for the line was used to obtain the RP50 value.

Statistical analysis

Experiments were performed in triplicate and results were expressed as means \pm standard deviations (SD). Statistical comparisons were made using one-way analysis of variance (ANOVA) by using SPSS 16.

Results and discussion

Yield and composition of essential oil

The yield of essential oil obtained through hydro distillation of *M. koenigii* leaves was 0.52% (v/w fresh material) higher than those reported earlier [40,41]. This suggested that *M. koenigii* plant located in the foothill of Himalayas possesses a higher content of essential oil. Further, the yield of oil also influenced by characteristic pedoclimatic conditions of the area where the source plant is grown, time and mode of harvesting and extraction efficiency [42] which prevent yield retarding factors like peroxidation, isomerization, or rearrangement of products due to temperature, light, and oxygen availability [43].

The GC-MS analysis of the essential oil obtained from *M. koenigii* leaves allowed the identification of altogether 43 compounds representing 99.79% of the total composition of

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the oil. The essential oil composition, with retention time and percentages are presented in **Table 1**.

Table 1. Chemical composition of essential oil from *M. koenigii* leaves.

Peak	Compounds	RT	Area (%)
1	α -Pinene	9.76	9.38
2	α -Thujene	10.37	1.12
3	3-Carene	10.9	18.52
4	Camphene	11.35	0.86
5	Linalool	12.56	5.42
6	Sabinene	13.25	2.55
7	p-Cymene	14.66	3.61
8	Limonene	15.8	1.58
9	1,8-Cineole	16.85	0.55
10	α -Terpinene	17.23	1.16
11	m-Cymene	17.87	0.45
12	β -Phellandrene	18.46	0.18
13	Eucalyptol	18.97	1.22
14	α -Muurolene	19.36	0.25
15	(Z)- β -Ocimene	20.51	1.38
16	(E)- β -Ocimene	24.23	1.16
17	γ -Terpinene	27.56	3.48
18	Myrcene	27.81	2.43
19	α - eudesmol	28.32	4.55
20	β -pinene	28.89	13.57
21	Geranyl acetate	29.74	0.57
22	Allo-Ocimene	29.92	2.75
23	α -Terpineol	30.48	1.48
24	Nerol	30.66	0.78
25	Carvone	30.91	1.68
26	Linalyl acetate	31.15	2.46
27	Lavandulyl acetate	31.63	0.56
28	Myrtenyl acetate	31.8	0.83
29	Neryl acetate	33.68	0.76
30	Geranyl acetate	35	0.58
31	β -Elemene	35.41	0.73
32	Z-Jasmone	35.79	0.86
33	α -Gurjunene	35.86	0.69

34	β -Caryophyllene	36.03	0.75
35	Germacrene D	36.18	0.62
36	β -Elemene	37.45	1.55
37	Cubenol	38.27	0.22
38	α -Amorphene	41.34	3.38
39	δ -Cadinene	42.3	0.56
40	Elemol	43.44	0.67
41	Viridiflorol	43.79	0.78
42	γ -Eudesmol	46.67	0.95
43	β - Eudesmol	47.3	2.16
Total			99.79

It is evident from the data presented in table 1 that compounds including 3-carene (18.52%), β -pinene (13.57%), α -pinene (9.38%), linalool (5.42%), α -eudesmol (4.55%), p-cymene (3.61%), γ -terpinene (3.48%), α -amorphene (3.38%), allo-ocimene (2.75%), Sabinene (2.55%), γ -terpinene (2.48%), linalyl acetate (2.46%), myrcene (2.43%), β - eudesmol (2.16%), carvone (1.68%), limonene (1.58%), β -elemene (1.55%), α -terpineol (1.48%), (Z)- β -ocimene (1.38%), (E)- β -ocimene (1.16%), eucalyptol (1.22%) and α -thujene (1.12%) have been identified as the major chemical constituents present in the *M. koenigii* leaf essential oil. Essential oils of plants in the family Rutaceae are often composed of mono and sesquiterpenes. In the present investigation, the oil was dominated by constituents like α -pinene, 3 carene, β -pinene, α -amorphene, allo-ocimene, α -eudesmol, linalool, p-cymene, myrcene, and γ -terpinene while other components are limonene, (Z)- β -ocimene, (E)- β -ocimene, geranyl acetate, α -terpineol, carvone, linalyl acetate, β -eudesmol respectively. It is worth mentioning that composition of essential oils is influenced by several factors such as local climatic, seasonal, and processing or experimental conditions [44,45]. Presence or absence of specific compounds and their contents vary across the locations, species populations, agroclimatic conditions owing to anthropological, climatological, and ecological factors which has obvious impact on flavour characteristics [46]. In India, diverse flavour characteristics of curry leave from different regions has been previously determined and the differences in the chemical composition of essential oil of curry leaf plants of different origins is also evident in preceding reports [47-51].

Antifungal activity

The antifungal activity of *M. koenigii* leaves essential oil (MKLEO) determined against altogether ten pathogenic fungi by disc diffusion method. The growth inhibitory activities of

the essential oil against the tested fungi at different concentrations are summarized in Table 2.

Table 2. Antifungal activity of MKLEO (AA: *Alternaria alternata*; AF: *Aspergillus flavus*; AN: *Aspergillus niger*; AP: *Aspergillus parasiticus*; FO: *Fusarium oxysporum*; FM: *Fusarium moniliforme*; MM: *Mucor mucedo*; PN: *Penicillium notatum*; PF: *Penicillium funiculosum*; TV: *Trichoderma viride*).

Conc. (µg/ml) MKLEO	Antifungal activity (% inhibition) Mean ± SD									
	AA	AF	AN	AP	FO	FM	MM	PN	PF	TV
100	5.27 ± 0.83	3.25 ± 0.16	2.81 ± 0.21	3.50 ± 0.25	4.33 ± 1.06	4.52 ± 0.09	3.87 ± 0.46	2.55 ± 0.27	3.31 ± 1.13	3.75 ± 1.05
250	16.25 ± 1.25	12.37 ± 0.55	11.50 ± 0.63	12.97 ± 0.35	14.63 ± 1.27	13.85 ± 0.17	11.35 ± 0.37	10.39 ± 1.25	12.27 ± 1.47	12.87 ± 0.35
500	26.45 ± 0.43	25.93 ± 0.21	22.47 ± 0.21	23.83 ± 0.46	23.93 ± 1.06	24.23 ± 0.31	22.63 ± 0.57	21.13 ± 0.25	23.47 ± 1.13	24.17 ± 0.41
750	48.16 ± 0.25	46.55 ± 0.33	45.27 ± 1.25	45.77 ± 1.35	46.17 ± 0.37	47.63 ± 0.21	43.86 ± 1.37	43.16 ± 1.21	44.87 ± 0.55	45.29 ± 1.07
1000	67.85 ± 0.15	65.53 ± 0.21	65.21 ± 0.23	65.85 ± 1.15	66.23 ± 0.57	67.13 ± 0.25	64.51 ± 1.21	63.71 ± 0.25	64.75 ± 1.05	65.37 ± 1.07
2000	96.59 ± 0.23	95.15 ± 0.33	94.69 ± 1.27	95.63 ± 0.35	96.47 ± 0.37	95.93 ± 0.25	93.86 ± 1.37	93.15 ± 0.23	94.87 ± 0.39	95.37 ± 0.13
(+) Control	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
(-) Control	0	0	0	0	0	0	0	0	0	0

The results of antifungal activity assay clearly indicate that the essential oil has varying degree of antifungal activity against the all the tested pathogenic fungi in a dose-dependent manner (Table 1). The MIC values ranged from 25.5 to 75 µg/mL. From the results, it is also evident that the essential oil exhibit highest growth inhibition in all the tested fungi at test concentration of 2000 µg/ml and it is almost at par with positive control. The growth inhibition in all the tested fungi is moderate with 1000 µg/mL and 750 µg/mL treatment doses of the essential oil. Of the different treatment doses of essential oil, growth inhibitions in all tested fungi are much less as compared to positive control with treatment does of 500, 250 and 100 µg/mL. Among the tested fungi, maximum growth inhibition was recorded in *Alternaria alternata* with all the treatment concentrations of the essential oil. Growth inhibition in other tested fungi was also noticeable. The percentage growth inhibition in the tested fungi namely *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Mucor mucedo*, *Penicillium notatum*, *Penicillium funiculosum*, and *Trichoderma viride* with different treatment doses (100-2000 µg/ml) of *M. koenigii* leaf essential oil ranged between 5.27-96.59, 3.25-95.15, 2.81-94.69, 3.50-95.63, 4.33-96.47, 4.52-95.93, 3.87-93.86, 2.55-93.15, 3.31-94.87, and 3.75-95.37% respectively. In conclusion, *M. koenigii* leaf essential oil (MKLEO) found to be remarkably effective antifungal in accordance to the inhibition action against all tested pathogenic fungi. The fungi toxicity of MKLEO might be due to the presence of mono and sesquiterpenoids constituents [52]. Although some of these compounds feature low concentrations, they can have an effect on the overall efficiency of the antimicrobial activity of the essential oil through synergistic interaction with the other constituents [53,54].

Antioxidant activity

The antioxidant activity MKLEO were measured in terms of its free radical scavenging ability following DPPH radical scavenging protocol and reducing power assay. The results of radical scavenging potential in the form of their IC₅₀ values and reducing power as RP₅₀ are summarized in Table 3.

With respect to the results of antioxidant activity, it should be noted that IC₅₀ and RP₅₀ values were inversely related to the percentage of DPPH scavenging capacity and reducing power, i.e., the higher the scavenging and reducing power rate, the lower the IC₅₀ and RP₅₀ values respectively. The analysis of the results of antioxidant activity of the MKLEO demonstrated that there were significant differences in these values between the essential oil and the standard antioxidants. However, the difference in IC₅₀ and RP₅₀ values between standards and the essential oil evidently minor which demonstrates that the MKLEO exhibited superior antioxidant activity (Table 3). The free radical scavenging capacity and reducing of the MKLEO can be attributed to the presence of certain terpenoid and phenolic compounds well recognized for their antioxidant activity and synergistic effect of chemical constituents of the essential oil [55,56].

Table 3. Antioxidant activity of MKLEO and standards.

Sample/Standards	DPPH scavenging (IC ₅₀ µg/ml)	radical activity	Reducing ability (RP ₅₀ µg/ml)	power
MKLEO	56.83 ± 0.63		134.29 ± 1.21	
BHT	35.75 ± 0.43		112.35 ± 0.33	
Catechin	43.36 ± 0.71		141.37 ± 0.27	
Gallic acid	48.69 ± 1.12		148.53 ± 0.31	

Ascorbic acid	33.27 ± 0.37	110.73 ± 1.06
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Conclusion

The yield of essential oil from *M. koenigii* leaves was 0.52% higher than those reported earlier. The GC-MS analysis of the essential oil obtained from *M. koenigii* leaves allowed the identification of altogether 43 compounds representing 99.79% of the total composition of the oil, among which 3-carene, β -pinene, α -pinene, linalool, α -eudesmol, p-cymene, γ -terpinene, α -amorphene, allo-ocimene, sabinene, γ -terpinene, linalyl acetate, myrcene, β -eudesmol, carvone, limonene, β -elemene, α -terpineol, (Z)- β -ocimene, (E)- β -ocimene, eucalyptol and α -thujene have been identified as the major constituents. The essential oil (MKLEO) was found effective against all the ten pathogenic fungi tested in a dose dependent manner. Regarding the antioxidant activity, the MKLEO was found to exhibit superior radical scavenging potency and reducing power with IC50 and RP50 values close to those of the synthetic antioxidant (standards). The essential oil of *M. koenigii* has the potential to be used in the food, cosmetics, and pharmaceutical industries, since it exhibits antimicrobial and antioxidant properties. It is important to stress the importance of further studies, mainly for determining the mechanism of action of this essential oil as well as the action of its individual constituents.

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