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Essential oil composition, antimicrobial and antioxidant properties of *Mosla chinensis* Maxim

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ABSTRACT

The essential oil of *Mosla chinensis* Maxim was analysed by gas chromatography/mass spectrometry, and its main components are carvacrol (57.08%), *p*-cymene (13.61%), thymol acetate (12.68%), thymol (6.67%), and γ-terpinene (2.46%). The essential oil exhibited great potential antimicrobial activity against all eight bacterial and nine fungal strains. Antioxidant activity was also tested, the essential oil showing significantly higher antioxidant activity than that of the methanol extract. In addition, the amounts of total phenol components in the plant methanol extract (47.3 ± 0.4 μg/mg) and the oil (80.7 ± 0.5 μg/mg) were determined. The results presented here indicate that the essential oil of *M. chinensis* has antimicrobial and antioxidant properties, and is therefore a potential source of antimicrobial and antioxidant agents for the food and pharmaceutical industries.

1. Introduction

Lipid peroxidation and microbial contamination are the two major causes of food deterioration during its processing and storage. It has been estimated that as many as 30% of people in industrialized countries suffer from a food-borne disease each year, and at least two million people died from diarrhoeal diseases worldwide in 2000 (WHO, 2002). Therefore, new methods must be established to ensure food safety. The safety of synthetic antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene (BHT), and propyl gallate, are now under scrutiny (Sasaki et al., 2002). Thus, the food industry is undertaking the rapid development and use of natural antioxidants and antimicrobial agents, especially those of plant origin, to replace synthetic food additives. One such possibility is the use of essential oils as antioxidant and antimicrobial additives. So far, a great number of plant essential oils and extracts have been tested for their antimicrobial and antioxidant activities. Lemongrass, rosemary, thyme, oregano, clove, sage, and basil are reported to possess stronger antioxidant and antimicrobial activities than those of synthetic antioxidants, and some of them are widely used in processed foods for lipid stabilization (Burt, 2004; Sacchetti et al., 2005). Thus, medicinal plants are a potential source of food additives in the food industry.

The genus *Mosla* (Labiatae) consists of about 22 species of annual herbs, often aromatic, widely distributed in India, Malaysia, Indonesia, China, Korea, and Japan. The flora of China contains 12 species of the genus *Mosla* and one variety (Wu & Li, 1977). Most plants of the genus *Mosla* are used as folk medicines in China.

Molsa chinensis is widely distributed in the south of China. The leaves of this plant are frequently used as a wild vegetable or as an additive in foods, to confer aroma and flavour. In traditional Chinese medicine, *M. chinensis* is used for the treatment of heat exhaustion, the common cold, fever, and gastrointestinal disturbances. It has been reported that *M. chinensis* possesses antibacterial, anti-inflammatory, antiviral, and antinociceptive activities (Chen, Lin, & Namba, 1989; Osawa et al., 1990; Yan et al., 2002).

To the best of our knowledge, the antimicrobial activity of *M. chinensis* against a wide range of food-associated microorganisms (bacteria, fungi, and yeast) and its potential antioxidative properties have not been studied. The aim of this study was to determine the chemical composition of the hydrodistilled essential oil of *M. chinensis* by gas chromatography (GC)/mass spectroscopy (MS), to evaluate its antimicrobial activity against food-associated microorganisms, and the antioxidative properties of the essential oil and the methanol extract.

2. Materials and methods

2.1. Plant material

M. chinensis plants were collected from Hunan province, China, during the vegetative stage (July, 2006). Voucher specimens (No. 2006070815) were deposited in the herbarium of the Institute of Medicinal Plant Development, Beijing, China.

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2.2. Extraction of essential oil

The air-dried leaves and stems of *M. chinensis* were ground and soaked in 25% salt solution for 3 h and then subjected to hydro-distillation for 3 h using the Clevenger apparatus. The distilled oil was dried over anhydrous sodium sulfate and stored in a freezer at −80 °C until analysed and 1 μl essential oil was diluted in 1 ml *n*-hexane for GC–MS measurement.

2.3. Preparation of the methanol extract

The powered air-dried aerial part of *M. chinensis* (100 g) was extracted exhaustively with methanol (HPLC grade) under refluxing for 3 times, 1 h each time. The filtered liquid was combined and concentrated under vacuum at 50 °C by using a rotary evaporator (Heidolph, Laborota 4000, Schwabach, Germany), yielding a crude extract (7.5 g).

2.4. Essential oil analysis

The essential oil was analysed with a Hewlett Packard 6890 GC–MS and the Hewlett Packard 5973 MSD apparatus. The GC unit was equipped with a methyl silicone HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The oven temperature was programmed from 40 °C (2 min) to 260 °C at 8 °C/min. Carrier gas was Helium at a flow rate of 0.8 ml/min (constant flow). The injector temperature was 250 °C, and the oil sample was injected using split mode injection (1:25). The ion source temperature of the MS unit was 260 °C, and mass spectra were recorded at an ionizing voltage of 70 eV. The linear retention indices for all the compounds were determined by co-injection of the sample with a solution containing the homogous series of C_8–C_{20} *n*-alkanes (Van Den Dool & Kratz, 1963). The compounds of essential oil were identified on the basis of comparison of their retention indices and mass spectra with publish data (Adams, 2001) and computer matching with National Institute of Standards and Technology (NIST, 3.0) libraries provided with computer controlling the GC–MS system. The retention indices were calculated for all volatile constituents using a homogous series of *n*-alkanes C_8–C_{20}.

2.5. Antimicrobial activity

2.5.1. Microbial strains

The essential oil was tested against a panel of bacteria, which included laboratory control strains obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA) and the Agricultural Culture Collection of China (ACCC; Beijing, China): *Enterococcus faecalis* (ACCC 10180), *Listeria monocytogenes* (ACCC 11120), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Staphylococcus albus* (ATCC 8032), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 19430), and fungal microorganisms *Aspergillus niger* (ATCC 02512), *Aspergillus fumigatus* (ACCC 30367), *Aspergillus flavus* (ATCC 9643), *Aspergillus terreus* (ATCC 42025), *Chae tomium indicum* (ATCC 36704), *Chaetomium globosum* (ATCC 36703), *Mucor racemosus* (ACCC 30522), *Candida albicans* (ATCC 10231), and *Candida rugosa* (ATCC 10571).

2.5.2. Antimicrobial screening

The disk diffusion method was used to determine the antibacterial activity of the essential oil (Sokmen et al., 2004). Briefly, using 100 μl of a suspension containing 10^8 colony-forming units (CFU)/ml of bacteria, 10^6 CFU/ml of yeast, or 10^4 spore/ml of fungus was spread on nutrient agar (NA), saboraud dextrose agar (SDA), or potato dextrose agar (PDA), respectively. The disks (6 mm in diameter) were impregnated with 5 μl of 10 mg/ml essential oil (dissolved in dimethylsulfoxide [DMSO]) and placed on the inoculated agar. Negative controls were prepared using DMSO. Gentamicin (30 μg/disc) and nystatin (30 μg/disc) were used as the positive reference standards. The inoculated plates were incubated at 37 °C for 24 h for the bacterial strains, at 27 °C for 48 h for the yeasts, and at 27 °C for 72 h for the fungi. The antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms.

2.5.3. Determinations of the minimum inhibitory concentration (MIC)

The MICs of the samples against the test bacterial strains and yeasts were determined by the micro-well dilution method (Sokmen et al., 2004). Inocula of the microorganisms were prepared from 12 h cultures and the suspensions were adjusted to 10^8 CFU/ml of bacteria or 10^6 CFU/ml of yeast. The essential oil was dissolved in 10% DMSO and serial twofold dilutions of the oil were prepared in a 96-well plate, ranging from 7.8 μg/ml to 1000 μg/ml. The MIC was defined as the lowest concentration of the essential oil at which the microorganism did not demonstrate visible growth. Microorganism growth was indicated by turbidity. The MICs of the standards (gentamicin, nystatin) were also determined in parallel experiments, to control for the sensitivity of the microorganisms.

The MIC value for each of fungus was evaluated based on the agar dilution method, as described previously (Sokmen et al., 2004). The oil was added aseptically to sterile melted PDA medium containing DMSO (0.5%, v/v; Sigma Chemical Co.) at the appropriate volume to produce a concentration range of 7.8–1000 μg/ml. The resulting PDA agar solutions were immediately poured into Petri dishes after vortexing. The plates were spot inoculated with 5 μl (10^4 spore/ml) of each fungus. Nystatin was used as the reference antifungal drug. The inoculated plates were incubated at 27 °C for 72 h. At the end of the incubation period, the plates were evaluated for the presence or absence of microbial growth. The MIC value was determined as the lowest concentration of the essential oil at which an absence of growth was recorded.

2.6. Antioxidant activity

2.6.1. DPPH assay

The hydrogen-atom- or electron-donating ability of the extracts and some pure compounds were measured by the bleaching of a purple-coloured methanol solution of diphenylpicrylhydrazyl (DPPH) (Sokmen et al., 2004). Aliquots (0.2 ml) of different concentrations of the oil or the plant extract in methanol were mixed with 3.8 ml of a 0.006% (w/v) methanol solution of DPPH. After 30 min incubation at room temperature, the absorbance was measured at 517 nm. The percentage inhibition (*I*%) of free radical DPPH was calculated with the following formula: $I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$, in which, A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample), and A_{sample} is the absorbance of the test sample. DPPH scavenging activity is presented by IC_{50} value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. The synthetic antioxidant reagent BHT was used as the positive control.

2.6.2. Reductive potential

The reductive potential of the oil and the extract were determined according to the method of Bounatirou et al. (2007). Different concentrations of the oil or the extract in methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $K_3Fe(CN)_6$ (2.5 ml, 1% w/v). The mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (10%, 2.5 ml) was added to the mixture, which was then centrifuged for 10 min at 3000*g*. The upper layer of the solution (2.5 ml) was mixed with distilled

water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. The extract or the oil concentration providing 0.5 of absorbance (EC_{50}) was calculated by plotting absorbance at 700 nm against the corresponding extract or the oil concentration. BHT was used as reference compound.

2.6.3. β-Carotene bleaching assay

This method evaluates the capacity of the extract to reduce the oxidative loss of β-carotene in a β-carotene–linoleic acid emulsion (Taga, Miller, & Pratt, 1984). β-carotene (10 mg) was dissolved in 10 ml of chloroform ($CHCl_3$). An aliquot (0.2 ml) of this solution was added into a boiling flask containing 20 mg of linoleic acid and 200 mg of Tween 40. The chloroform was removed using a rotary evaporator at 40 °C for 5 min. Distilled water (50 ml) was slowly added to the residue with vigorous agitation, to form an emulsion. The emulsion (5 ml) was added to a tube containing 0.2 ml of the essential oil or the extract solution. The absorbance was immediately measured at 470 nm and the test emulsion was incubated in a water bath at 50 °C for 5 min, when the absorbance was measured again. BHT was used as the positive control. In the negative control, the essential oil or the extract were substituted with an equal volume of ethanol. The antioxidant activity (%) of the oil was evaluated in terms of the bleaching of the β-carotene using the following formula: % Inhibition = $[(At–Ct)/(C_0–Ct)] \times 100$, where *At* and *Ct* are the absorbances measured for the test sample and control, respectively, after incubation for 50 min, and C_0 is the absorbance values for the control measured at zero time during the incubation. The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated from plotting antioxidant percentage against extract concentration.

2.6.4. Assay for total phenolic content

Total phenolic contents of the plant extract and the oil were determined as described (Tsai, Tsai, Chien, Lee, & Tsai, 2008), with Folin–Ciocalteu reagent and garlic acid used as a standard. An aliquot (0.2 ml) of the oil or the extract solution was added to a volumetric flask. Then, 46 ml distilled water and 1 ml Folin–Ciocalteu reagent was added, and the flask was shaken thoroughly. After 3 min, a 3 ml solution of Na_2CO_3 (7.5%) was added, and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm on a UV–VIS 755B spectrophotometer (Shanghai, China). The results were expressed as milligrams of gallic acid equivalents (GAEs) per gram of extract.

2.7. Statistical analysis

All experiments were performed in triplicate. The data were recorded as means ± standard deviations and were analysed with SPSS (version 12.0 for Windows, SPSS Inc.). Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Chemical composition of the essential oil

The hydro-distillation of dried aerial parts of *M. chinensis* gave yellowish essential oil (yields 1.8%, w/w). The chemical compositions are shown in Table 1, according to their elution order on a HP-5 MS capillary column, and their identification has been carried out by means of GC–MS analyses in combination with retention indices. Twenty five constituents accounting for 99.04% of total oil compositions were identified. The major components are carvacrol 57.08%, *p*-cymene 13.61%, Thymol acetate 12.68%, thymol 6.67%, γ-terpinene 2.46%, and 9-epi-(E)-caryophyllene 1.59%, comprising 94.09% of the essential oils. The chemical compositions of the essential oils of two *Mosla* species, *M. scabra* and *M. soochowensis,* have recently been reported. The major compounds in the *M. soochowensis* oil were methyleugenol (40.4–43.6%), nerolidene (10.5–11.0%), bornene (8.0–10.0%), dihydrocarvon (8.2–9.0%), and thujone (7.3–7.8%). In *M. scabra* oil, thujone (39.8%), caryophellene (8.9%), and eucalyptol (8.0%) were the major compounds. This indicates that the constituents of the essential oils of *Mosla* plant species differ greatly (Chen & Wu, 2005; Wu, Wu, Chen, Lin, & Huang, 2006). In species of *Origanum* and *Thymus*, *p*-cymene, γ-terpinene, carvacrol, and thymol are the major components which biologically and functionally closely associated, and *p*-cymene and γ-terpinene are the precursors of carvacrol and thymol (Burt, 2004). From this experiment, it revealed that the major components of *M. chinensis* essential oil are similar to species of *Origanum* and *Thymus*.

Table 1
Phytochemical compositions of the essential oils of *M. Chinensis*.

No	Components	*RI*	%	Method of identification
1	Tricyclene	928	0.04	GC, MS
2	1R-α-Pinene	935	0.17	GC, MS
3	1R-β-Pinene	978	0.04	GC, MS
4	Myrcene	992	0.81	GC, MS
5	α-Phellandrene	1006	0.06	GC, MS
6	3-Carene	1012	0.02	GC, MS
7	α-Terpinene	1018	0.95	GC, MS
8	*p*-Cymene	1027	13.61	GC, MS
9	Eucalyptol	1033	0.15	GC, MS
10	γ-Terpinene	1061	2.46	GC, MS
11	Terpinolene	1091	0.07	GC, MS
12	Isobormeol	1171	0.10	GC, MS
13	*p*-Menth-1-en-4-ol	1182	0.84	GC, MS
14	*p*-Menth-1-en-3-ol	1196	0.08	GC, MS
15	Carvacrol	1299	57.08	GC, MS
16	Thymol	1307	6.67	GC, MS
17	Thymol acetate	1359	12.68	GC, MS
18	Carvacrol acetate	1377	0.52	GC, MS
19	Caryophyllene	1431	0.28	GC, MS
20	Isocaryophyllene	1443	0.26	GC, MS
21	α-Humulene	1460	0.38	GC, MS
22	9-epi-(E)-Caryophyllene	1466	1.59	GC, MS
23	δ-Selinene	1497	0.13	GC, MS
24	δ-Cadinene	1524	0.05	GC, MS
25	α-Cadinene	1532	0.09	GC, MS
	Total		99.04	

RI: retention indices (Kovats index) on HP-5 MS column.
GC: identification by comparison of retention indices.
MS: identification by comparison of the mass spectrum with those of NIST library.

Several studies have shown that the principal constituents of *M. chinensis* oils are the phenolic compounds, thymol and its geometric isomer, and that the percentages of carvacrol and thymol vary from trace to 55% (Ji et al., 2004; Luo et al., 2006; Zheng, Yang, Gao, & Shen, 1998; Zheng, Zheng, Dai, Yang, & Shen, 2001). It has been reported that the composition of the essential oils from a particular species of plant can differ between harvesting seasons, extraction methods, and geographical sources, and that those from the different parts of the same plant can also differ widely (Burt, 2004). The production of thymol and carvacrol is believed to depend on some external variables, such as the soil, climatic conditions, harvesting time, or the amount of water to which the herb is exposed. These factors apparently favour the formation of one isomer over the other (Kimura, Yamaoka, & Kamisaka, 2006; Vassiliev, 1972).

3.2. Antimicrobial activity

The disc diameters of the zones of inhibition and the MICs of the *M. chinensis* oil are shown in Table 2. These results indicate that the oil has strong antibacterial activity against all eight bacteria, and is

Table 2
Antimicrobial activity of the essential oil of *M. Chinensis*.[a]

Microorganisms	Essential oil		Gentamicin		Nystatin	
	DD[b]	MIC[c]	DD	MIC	DD	MIC
Gram-positive						
S. aureus	14.7 ± 0.58	62.5	24.3 ± 1.53	6.3		
S. albus	9.0 ± 1.00	250.0	24.0 ± 1.00	6.3		
E. faecalis	12.3 ± 1.53	125.0	28.0 ± 1.00	3.1		
L. monocytogenes	14.0 ± 0.00	62.5	29.3 ± 0.58	3.1		
Gram-negative						
E. coli	8.0 ± 1.00	250.0	22.0 ± 0.00	6.3		
K. pneumoniae	11.3 ± 0.58	125.0	18.0 ± 1.00	12.5		
P. aeruginosa	10.0 ± 0.00	125.0	19.0 ± 0.00	12.5		
S. typhimurium	9.0 ± 0.00	250.0	24.0 ± 0.00	6.3		
Fungi						
A. niger	14.0 ± 0.00	62.5			20.3 ± 1.53	31.3
A. fumigatus	12.0 ± 0.00	125.0			13.3 ± 1.53	62.5
A. flavus	15.0 ± 1.00	62.5			18.0 ± 0.00	31.3
A. terreus	14.3 ± 0.58	62.5			16.3 ± 0.58	31.3
C. indicum	12.0 ± 1.00	125.0			14.0 ± 1.00	62.5
C. globosum	16.3 ± 0.58	31.3			18.0 ± 1.00	31.3
M. racemosus	14.0 ± 1.00	62.5			19.0 ± 1.53	31.3
Yeast						
C. albicans	15.0 ± 1.00	62.5			24.0 ± 0.00	7.8
C. rugosa	16.0 ± 0.00	62.5			25.0 ± 1.00	7.8

[a] Results are means of three different experiments.
[b] Diameter of inhibition zone including disc diameter of 6 (mm).
[c] MIC, minimum inhibitory concentration (as μg/ml).

more effective against Gram-positive bacteria. Among the Gram-positive bacteria, *S. aureus* and *L. monocytogenes* were more sensitive to the essential oil. The oil also showed marked antifungal activities against two yeasts and seven moulds. The antimicrobial activities of the oil against bacteria and fungi were demonstrated to be dose dependent.

The antimicrobial activities of the *M. chinensis* oil can be attributed to the presence of high concentrations of carvacrol, which is known to occur at very high concentrations in many plant oils, including members of the Labiatae family, such as *Thymus, Coridothymus, Satureja, and Origanum* (Chorianopoulos et al., 2004; Sokmen et al., 2004; Bounatirou et al., 2007; Peñalver et al., 2005). The pharmacological actions of the essential oils are suggested to parallel to their carvacrol contents (Aydin et al., 2007). Carvacrol is considered to be biocidal, resulting in bacterial membrane perturbations. Furthermore, carvacrol might cross the cell membranes, thus penetrating into the interior of the cell and interacting with intracellular sites critical for antibacterial activities (Cristani et al., 2007; Ultee, Kets, & Smid, 1999). Another major component of the oil, *p*-cymene, which is precursor of carvacrol, is a very weak antibacterial, but it probably acts synergistically with carvacrol by expanding the membrane, which results in the destabilization of the membrane (Ultee, Bennink, & Moezelaar, 2002). It has also been suggested that minor components interact with the other components, affecting the antimicrobial activities of the oils. It is possible that the activity of the main components is regulated by the other minor molecules (Bounatirou et al., 2007).

3.3. Antioxidant activity

The principle of antioxidant activity is based on the availability of electrons to neutralize any free radicals. In this study, the antioxidant activity of *M. chinensis* oil and the methanol extract were evaluated by the reducing power assay, the β-carotene linoleate model system, and scavenging of DPPH free radicals.

3.3.1. Reductive potential

The reductive potential of a compound reflects its ability to act as an electron donor. The electron donor reacts with free radicals, converts them to more stable products, and finally terminates radical chain reactions. The reductive capacity of a compound is recognized as a significant indicator of its potential antioxidant activity (Bounatirou et al., 2007). The reducing capacity of the oil and the methanol extract are presented in Table 3. The extract and the essential oil of *M. chinensis* exhibited remarkable reduction activities. The essential oil (EC_{50} = 105.1 ± 0.9 μg/ml) showed higher reducing capacity than the methanol extract (EC_{50} = 313.5 ± 2.5 μg/ml). Also, the reducing power of the methanol extract and the oil were lower than that of synthetic antioxidant BHT (EC_{50} = 20.8 ± 0.1 μg/ml).

3.3.2. Free-radical- scavenging activity

The effects of antioxidants in the DPPH-radical-scavenging test reflect the hydrogen-donating capacity of a compound. When the radical form of DPPH is scavenged by an antioxidant through the donation of a hydrogen to form a stable DPPH molecule, this leads to a colour change from purple to yellow, and a decrease in absorbance. The DPPH-radical-scavenging activity of the essential oil and the methanolic extract are shown in Table 3. Lower IC_{50} value indicates higher antioxidant activity. The oil (IC_{50} = 1230.4 ± 12.5 μg/ml) and the methanol extract (IC_{50} = 1482.5 ± 10.9 μg/ml) showed moderate DPPH-radical-scavenging activity, and were lower than that of synthetic antioxidant BHT (IC_{50} = 181.2 ± 7.5 μg/ml). The DPPH-radical-scavenging activity of the oil, the extract, and BHT decreased in the order of BHT > the oil > the methanol extract.

3.3.3. β-Carotene bleaching test

In the β-carotene bleaching test, β-carotene undergoes rapid discoloration in the absence of an antioxidant. This is because the coupled oxidation of β-carotene and linoleic acid generates free radicals. The linoleic acid free radical formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups attacks the highly unsaturated β-carotene molecule. As a result, β-carotene is oxidized and breaks down in part, subsequently losing its chromophore and characteristic orange colour, which is monitored spectrophotometrically (Shon, Kim, & Sung, 2003). The antioxidant activity of the essential oil and the extract were assayed in the β-carotene–linoleate model system and compared with that of BHT (Table 3). Both the oil and the extract prevented the bleaching of β-carotene. The EC_{50} values were compared with that of BHT. A lower EC_{50} value indicates greater antioxidant activity. The EC_{50} values of the essential oil and the methanol extract were found to be 588.2 ± 4.2 μg/ml and 789.4 ± 1.3 μg/ml, respectively. The β-carotene bleaching effects of BHT were found to be 1.88 and 2.53 times greater than the oil and the methanol extract, respectively.

3.3.4. Content of total phenolic

The amounts of total phenolics in the extract and the oil were determined spectrometrically according to the Folin–Ciocalteu

Table 3
IC_{50} or EC_{50} values (μg/ml), total phenolic contents of *M. chinensis* essential oil and methanol extract[a].

Sample	DPPH (IC_{50})	β-Carotene bleaching (EC_{50})	Reducing power (EC_{50})	Total phenol contents (μg GAE/mg)
Methanolic extract	1482.5 ± 10.9	789.4 ± 1.3	313.5 ± 2.5	47.3 ± 0.4
Essential oil	1230.4 ± 12.5	588.2 ± 4.2	105.1 ± 0.9	80.7 ± 0.5
BHT	181.2 ± 7.5	312.3 ± 2.8	20.8 ± 0.1	Ns[b]

[a] Values represent means ± standard deviations for triplicate experiments.
[b] Not studied.

method and calculated as garlic acid equivalents. The standard curve equation is, $y = 0.0054x + 0.0813$, $R^2 = 0.9971$. The amounts of total phenols found in the plant methanol extract and the oil were shown in Table 3. The total phenolic contents of the methanol extract and the essential oil are 47.3 ± 0.4 μg/mg and 80.7 ± 0.5 μg/mg, respectively. The results indicated that the oil has higher total phenolic compounds and antioxidant ability than the methanol extract. It revealed there is a relationship between the antioxidant ability and total phenol contents. Phenolic antioxidants are products of secondary metabolism in plants, and the antioxidant activity is mainly due to their redox properties and chemical structure, which can play an important role in chelating transitional metals, inhibiting lipoxygenase and scavenging free radicals (Decker, 1997). In the plant oils, oxygenated monoterpenes (especially the two well-known phenolic compounds, thymol and carvacrol) and monoterpene hydrocarbons are mainly responsible for the antioxidant potential (Ruberto & Baratta, 2000). According to our experiments, oxygenated monoterpenes and monoterpene hydrocarbons were the main components of the *M. chinensis* oil (Table 1). Therefore, the antioxidant activity of the essential oil can be attributed to the high contents of these compounds.

4. Conclusion

The results of the present work indicated that antioxidant activity of the essential oil of *M. chinensis* is higher than the methanol extract. The oil also exerted strong antimicrobial activities. The essential oil of *M. chinensis* may be an alternative additive in foods, pharmaceuticals and cosmetic preparations instead of more toxic synthetic antioxidants.

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