Chemical Composition and Biological Activities of the Essential Oil of *Mentha suaveolens* Ehrh.

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Hydrodistilled oils of the fresh aerial parts of *Mentha suaveolens* Ehrh. cultivated in Egypt were prepared from samples collected along the four seasons. The percentage yields of these essential oils were 0.50%, 0.52%, 0.60%, and 0.47% of the dry weight for winter, spring, summer, and autumn samples. GC/MS analyses of all samples revealed a qualitative and quantitative variability in the oil composition. The total number of compounds identified was 46 among which 15 were common in all samples. The oxygenated compounds constituted about 45%, 46%, 63%, and 44% of the total composition of the oils for winter, spring, summer, and autumn samples, respectively. Carvone was the major constituent in spring, summer, and autumn samples (about 31%, 56%, and 35%, respectively), while limonene (ca. 26%) was the major constituent of the winter sample followed by carvone (ca. 25%). The essential oil of the highest yield (full-flowering summer sample), with the highest oxygenated constituents and carvone contents, was screened for certain biological activities. It exhibited analgesic and acute anti-inflammatory activities (75% and 82% relative to indomethacin). It also showed a potent *in vivo* antioxidant activity (96% relative to vitamin E). In addition, it exerted moderate cytotoxic, hepatoprotective, and *in vitro* antioxidant activities. Moreover, the oil had a potent antifungal activity against *Candida albicans* (MIC = 4 μg/ml), *Saccharomyces cerevisiae* (MIC = 5.2 μg/ml), and *Aspergillus niger* (MIC = 6.8 μg/ml).

Key words: Mentha suaveolens, Essential Oil, Seasonal Variations

Introduction

The genus *Mentha* of the Lamiaceae family is represented by about 19 species and 13 natural hybrids. *Mentha suaveolens* Ehrh. is native to Africa, Temperate Asia, and Europe (Abbaszadeh *et al.*, 2009).

Polymorphism has particularly been observed in hybrid populations of *Mentha* species which conserves novel genotypes. Studies of the essential oil content and composition have proved to be useful in the identification of the parentage of hybrids as they are characteristic for each mint, and the relationships among mints could be deduced from these patterns, although the composition may vary in the course of a year. Based on the monoterpene prevailing in their essential oil, mints have been classified into linalool, menthol, and carvone chemotypes, respectively (Šarić-Kundalić *et al.*, 2009, and literature cited therein).

According to the available literature, the constituents of the essential oils from *M. suaveolens* and its different chemotypes have been investigated by gas chromatrography/mass spectroscopy (GC/MS) analysis. The major constituent was either piperitone oxide (Hendriks and Van Os, 1976; Holeman *et al.*, 1985; Oumzil *et al.*, 2002), piperitenone oxide (Holeman *et al.*, 1985; Oumzil *et al.*, 2002; Koliopoulos *et al.*, 2010; Sutour *et al.*, 2010), piperitenone (Sutour *et al.*, 2010), dihydrocarvone (Hendriks and Van Os, 1976), menthol (Velasco-Negueruela *et al.*, 1996), pulegone (Velasco-Negueruela *et al.*, 1996; Oumzil *et al.*, 2002; Sutour *et al.*, 2008), or *cis-cis-p*-menthenolide (Sutour *et al.*, 2008).

Few reports were found dealing with the biological activities of the essential oil of *M. suaveolens*. Oumzil *et al.* (2002) reported that it had antimicrobial activity, pulegone being the most effective constituent against the tested microorganisms. It also exhibited a fair antibacterial activity

against *Staphylococcus aureus* and *Bacillus cereus* (Sutour *et al.*, 2008). Additionally, acetylcholinesterase (AChE) inhibitory capacity and free radical scavenging activity were reported for the oil (Ferreira *et al.*, 2006).

No information was found in the available literature on the seasonal fluctuation of the yield and composition of the essential oil of *M. suaveolens*. Also, the number of reports on the biological activities of the oil is limited. Therefore, it was interesting to study the effect of seasonal variation on the chemical composition of the essential oil from *M. suaveolens* Ehrh. cultivated in Egypt, in an effort to establish the best conditions for oil production. The essential oil was screened for analgesic, anti-inflammatory, hepatoprotective, and antioxidant activities. An *in vitro* study of the antioxidant and cytotoxic activities as well as antimicrobial screening of the essential oil were also carried out.

Material and Methods

Plant material

Fresh aerial parts of *M. suaveolens* Ehrh. were collected during the years 2009 and 2010 from plants cultivated in the Experimental Station of Medicinal and Aromatic Plants, Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Giza, Egypt. The plant was kindly authenticated by Dr. Gemma L. C. Bramley, Curator of the Lamiaceae collections, Herbarium Department, Library, Art & Archives, Royal Botanic Gardens, Kew, Richmond, Surrey, United Kingdom. A voucher specimen (M-20/313) was deposited at the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Giza, Egypt.

Preparation of the essential oil

Samples of fresh aerial parts (1 kg, each) were collected at three-months intervals along four seasons, *viz*. in January (winter), April (spring), July (summer), and October (autumn). They were subjected to hydrodistillation and the oils obtained dried over anhydrous sodium sulfate. The percentage yields were calculated on a dry weight basis, and the oils were kept in a refrigerator for further analysis. The specific gravities and refractive indices were determined according to the Egyptian Pharmacopoeia (CAPA, 1984) procedures. All stated values were the average of three determinations.

GC/MS analysis

The oils were subjected to gas chromatography/mass spectroscopy (GC/MS) analyses on an Agilent (Santa Clara, CA, USA) GC/MS system, model 6890, fitted with an Agilent mass spectroscopic detector (MSD), model 5937, as well as a 30 m long, cross-linked 5% phenylpolysiloxane fused silica column (HP5-MS capillary column; i.d., 0.25 mm; film thickness, 0.25 μm; Hewlett Packard, Palo Alto, CA, USA). The initial temperature was 60 °C, kept isothermal for 3 min, and then increased to 260 °C at 8 °C; the final temperature was kept isothermal for 15 min. The ion source temperature was 230 °C, and the quadrupole temperature was 150 °C. The carrier gas was helium adjusted at a flow rate of 0.1 ml/min. Ionization energy was 70 eV, and scan range was m/z 40–500 at 3.62 mu/scan.

Identification of the oil components

Library search for identification of the oil components was carried out using a Wiley and Nist (6th ed.) 275 L GC/MS data base. A series of authentic *n*-alkanes (C_8–C_{22}; Poly Science Inc., Niles, MI, USA) was subjected to GLC analysis under the same experimental conditions. The retention indices (Kovats indices, KI) of the oil compounds were computed by logarithmic interpolation between bracketing alkanes (Jennings and Shibamoto, 1980). Identification of the individual compounds was confirmed by comparison of their retention indices and MS fragmentation patterns with published data (Adams, 2004).

Analgesic activity

The analgesic activity of the essential oil (summer sample) was evaluated and compared with that of the standard drug indomethacin, using the acetic acid-induced writhing test as described by Koster *et al.* (1959). Swiss male albino mice (20–25 g) were divided into 3 groups each of 6 animals. The first group received 1 ml saline orally and was kept as a negative control. The second group received the essential oil orally in a dose of 100 mg/kg body weight (BW). The last group was orally administered 20 mg/kg BW indomethacin

to serve as a positive control. After 30 min, writhing was induced by intraperitoneal injection of 0.6% acetic acid (0.2 ml/mouse). The total number of writhes during a 30-min period starting 5 min after acetic acid injection was counted by placing each mouse in an individual plastic observation chamber.

These, as well as the following *in vivo* experiments, had been approved by the Official Ethics Committee, National Research Center, Dokki, Giza, Egypt (Ref. E:1–231), and were conducted according to the Ethics Committee's guidelines.

Acute anti-inflammatory activity

The acute anti-inflammatory activity of the essential oil (summer sample) was evaluated and compared with that of the standard indomethacin using the carrageenan-induced rat paw oedema test according to the method of Winter *et al.* (1962). Adult male albino rats, weighing 130–140 g, were divided into 3 groups each of 6 animals. The first group received 1 ml saline orally and was kept as a negative control. The second group was given the essential oil orally in a dose of 100 mg/kg BW. The last group was orally administered 20 mg/kg BW indomethacin and served as a positive control. One h later, inflammation was induced by subplantar injection of 0.1 ml of 1% carrageenan solution in saline in the right hind paw while 0.1 ml saline was injected in the left hind paw. Four h after oral administration of the tested sample, the rats were euthanized by an overdose of chloroform, both hind paws were excised and separately weighed to calculate the weight of the oedema. The percentage of oedema (inflammation) was calculated according to the following equation:

$$\%\ \text{oedema} = \frac{\text{weight of the right paw} - \text{weight of the left paw}}{\text{weight of the left paw}} \cdot 100.$$

Furthermore, the percentage of inhibition in the mean of the treated group in comparison with the control non-treated group was estimated and calculated according to the following equation:

$$\%\ \text{inhibition} = \frac{\text{paw oedema of control} - \text{paw oedema of treated group}}{\text{paw oedema of control}} \cdot 100.$$

Antioxidant activity

The *in vivo* antioxidant activity of the essential oil (summer sample) was investigated by determination of the glutathione (GSH) level in the blood of alloxan-induced diabetic rats according to Beutler *et al.* (1963) using α-tocopherol (vitamin E) as a standard drug. Adult male albino rats, weighing 130–140 g, were divided into 4 groups (6 animals per group). The first group was kept as a negative control while diabetes mellitus was induced in the other 3 groups, according to the method described by Eliasson and Samet (1969), by a single intraperitoneal injection of alloxan (150 mg/kg BW), followed by overnight fasting. Blood samples were obtained from the retro-orbital venous plexus of each rat, and blood glucose levels were measured to confirm induction of diabetes according to the method of Trinder (1969) using bioMérieux kits. The first group of diabetic rats was kept untreated. The second group was given the essential oil in a dose of 100 mg/kg BW intraperitoneally. The third group was administered 7.5 mg/kg BW vitamin E and served as a positive control. The blood glutathione level was calculated by the following equation:

$$\text{GSH (mass\%)} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \cdot \frac{37.5}{1000} \cdot \frac{2.5}{0.1} \cdot 100.$$

Hepatoprotective activity

The hepatoprotective activity of the essential oil (summer sample) was evaluated by determination of the activities of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) in the blood of liver-damaged rats. Liver damage in male albino rats (body weight, 100–130 g) was induced according to the method of Klassan and Plaa (1969) by intraperitoneal injection of 5 ml/kg BW of 25% carbon tetrachloride (CCl_4) in liquid paraffin. Seventy-two h after administration of CCl_4, blood samples were withdrawn for determination of the enzyme activities. The rats were divided into 3 groups each of 6 animals. The first group of rats received a daily oral dose of 1 ml saline for 7 d before liver damage was induced. Administration of 1 ml saline was continued after induction of liver damage for another 7 d. This group served as a negative control. The second group of the rats was pretreated with a daily oral dose of 100 mg/

kg BW of the essential oil for 7 d. Administration of the essential oil was continued after induction of liver damage for another 7 d. The last group of rats was pretreated with a daily dose (25 mg/kg BW) of silymarin for 7 d before liver damage was induced. Silymarin administration was continued for another 7 d after induction of liver damage. This group served as a positive control. Blood was obtained from the retro-orbital venous plexus through the eye canthus of anaesthetized rats. The levels of AST, ALT (Thewfweld, 1974), and ALP (Kind and King, 1954) were estimated in blood samples collected from each group at zero time, 7 d after receiving the test sample, 72 h after induction of liver damage, and 7 d after treatment with the test sample.

Free radical scavenging activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The free radical scavenging activity of the essential oil (summer sample) was assessed using the DPPH assay according to Takao *et al.* (1994) as modified by Delazar *et al.* (2004). DPPH (4 mg) was dissolved in methanol (50 ml) to obtain a concentration of 80 μg/ml. Serial dilutions of the essential oil were prepared in methanol (20–400 μg/ml). Diluted solutions (1 ml each) were mixed with equal volumes of DPPH and allowed to stand for 30 min at room temperature. The control sample was prepared by mixing 1 ml of DPPH with 1 ml methanol. The absorbance of each sample was recorded at 517 nm. The experiment was performed in triplicate, and the average absorbance for each concentration was recorded. The same procedure was followed for ascorbic acid and silymarin which were used as positive controls. The IC_{50} value was calculated as the concentration (μg/ml) of test sample causing 50% quenching.

Cytotoxicity assay

The cytotoxicity of the essential oil (summer sample) was measured using the sulforhodamine B assay (SRB) (Skehan *et al.*, 1990) on four human carcinoma cell lines: liver (HEPG2), colon (HCT116), larynx (HEP2), and breast (MCF7). The IC_{50} (dose which reduces the survival to 50%) values were calculated and compared to those of the reference drug doxorubicin.

Antimicrobial activities

The antimicrobial screening of the essential oil was performed by the agar disc diffusion method (Bauer *et al.*, 1966). Standard discs impregnated with tetracycline (5 μg/disc) and amphotericin B (5 μg/disc) served as postive controls for antibacterial and antifungal activities, respectively. Filter discs impregnated with 10 μl of dimethyl sulfoxide (DMSO) were used as a negative control. The essential oil was dissolved in DMSO at a concentration of 100 mg/ml. Aliquots of 50 μl (equivalent to 30 μg of the essential oil) were aseptically added to the cups of the inoculated plates. *Staphylococcus aureus* (ATCC12600), *Streptococcus faecalis* (ATCC19433), *Bacillus subtilis* (ATCC6051), *Escherichia coli* (ATCC11775), *Neisseria gonorrhoeae* (ATCC19424), *Pseudomonas aeruginosa* (ATCC10145), *Candida albicans* (ATCC26555), *Saccharomyces cerevisiae* (ATCC2180-1A), and *Aspergillus niger* (Laboratory Collection) were used.

Minimum inhibitory concentrations (the lowest concentration that causes 100% inhibition, MIC_{100}) were determined for *Candida albicans*, *Saccharomyces cerevisiae*, and *Aspergillus niger* using the broth microdilution method (Wu *et al.*, 2003).

Statistical analysis

The data obtained are presented as means ± standard error (S.E.), and the significance of difference between test and control groups was statistically analysed using student's t-test (Snedecor and Cochran, 1982). *P* values of 0.05 or less were considered criteria for significance.

Reference drugs and kits

Alloxan, carrageenan, ascorbic acid, doxorubicin, and DPPH were from Sigma (St. Louis, MO, USA), amphotericin B from Bristol-Myers Squibb (Baar, Switzerland), indomethacin from EIPICO Pharmaceutical Co. (6 October City, Egypt), silymarin and tetracycline from Sedico Pharmaceutical Co. (6 October City, Egypt), α-tocopherol from Pharco Pharmaceutical Co. (Alexandria, Egypt), the glutathione kit was from WAK-Chemie Medical (Steinbach, Germany), and transaminase and alkaline phosphatase kits, and blood glucose level kit were from bioMérieux Co. (Craponne, France).

Results and Discussion

The percentage yields of the essential oils obtained by hydrodistillation of the aerial parts of *M. suaveolens* Ehrh., collected at three-months intervals along the four seasons, were 0.50%, 0.52%, 0.60%, and 0.47% for winter, spring, summer, and autumn samples, respectively, on a dry weight basis. Their specific gravities were 0.500, 0.685, 0.615, and 0.517 g/cm^3 at 25 °C, respectively, and refractive indices recorded at 20 °C were 1.491, 1.492, 1.487, and 1.488, respectively. All oil samples exhibited nearly the same physical characters being oily, colourless, with spearmint-like odour, and readily soluble in 70% ethanol.

GC/MS analyses of the samples (Table I) revealed a qualitative and quantitative variability in the oil composition. The overall chromatographic profile of only the summer sample was dominated by oxygenated compounds, while oil samples from the other seasons were dominated by hydrocarbons. Carvone was the major constituent in the spring, summer, and autumn samples (30.56%, 55.74%, and 34.80%, respectively), while limonene (25.98%) was the major constituent of the winter sample followed by carvone (24.72%). Limonene was the only monoterpene hydrocarbon detected in winter, spring, and summer samples. It was the major component in the autumn sample. The major sesquiterpene hydrocarbon in winter, spring, and summer samples was *trans*-*β*-caryophyllene (7.84%, 8.51%, and 2.72%, respectively) while in the autumn sample, it was *β*-bourbonene (5.18%). The highest percentage

Table I. Chemical composition of the essential oil of *M. suaveolens* cultivated in Egypt at different seasons.

Peak	Kovats index	Identified compound	Content (%)			
			Winter	Spring	Summer	Autumn
1	928	*α*-Pinene	-	-	-	6.48
2	963	Sabinene	-	-	-	4.01
3	969	*β*-Pinene	-	-	-	0.67
4	1021	Limonene	25.98	22.59	26.78	29.18
5	1088	Linalool	0.07	-	0.26	-
6	1150	Borneol	-	1.14	1.38	-
7	1168	*cis*-Dihydrocarvone	-	1.28	0.25	-
8	1189	*trans*-Carveol	-	1.82	0.53	-
9	1193	*cis*-Carveol	-	-	0.44	-
10	1235	Carvone	24.72	30.56	55.74	34.80
11	1264	Carvone oxide	-	-	0.37	-
12	1275	*neo*-Dihydrocarveol acetate	1.73	-	-	-
13	1296	Dihydrocarveol acetate	9.98	0.83	0.33	0.22
14	1305	*trans*-Carvyl acetate	-	2.03	0.78	-
15	1333	*cis*-Carvyl acetate	1.44	0.37	0.47	0.91
16	1348	*α*-Copaene	0.33	0.37	-	-
17	1357	*β*-Bourbonene	4.15	3.82	1.57	5.18
18	1380	*cis*-Jasmone	0.83	0.76	0.37	1.79
19	1397	*trans*-*β*-Caryophyllene	7.84	8.51	2.72	2.50
20	1407	*β*-Cedrene	-	-	0.41	-
21	1423	*β*-Copaene	0.17	0.27	0.35	1.74
22	1435	*α*-Humulene	3.01	3.96	1.19	-
23	1447	*γ*-Muurolene	1.82	1.49	0.40	3.01
24	1465	Germacrene D	5.50	8.50	2.06	-
25	1483	Bicyclogemacrene	1.50	2.07	0.58	0.95
26	1502	*γ*-Cadinene	0.22	0.23	0.13	-
27	1511	*trans*-Calamenene	1.65	1.52	0.58	1.87
28	1527	*α*-Cadinene	0.26	0.16	0.09	-
29	1533	Di-epi-*α*-cedrene epoxide	0.27	0.16	-	-
30	1540	1,5-Epoxy salvial-4(14)-ene	-	-	-	0.24
31	1567	Germacrene D-4-ol	-	-	0.59	-
32	1571	Spathulenol	1.39	1.22	-	2.20
33	1594	Globulol	0.01	0.08	-	-

Table I continued.

Peak	Kovats index	Identified compound	Content (%)			
			Winter	Spring	Summer	Autumn
34	1603	1,10-Di-epi-cubenol	0.86	0.65	0.34	1.04
35	1619	2*R*,5*E*-Caryophyll-5-en-12-al	0.21	0.13	-	-
36	1627	*epi*-α-Cadinol	1.70	1.22	0.60	1.51
37	1640	*epi*-α-Muurolol	1.18	0.85	0.31	0.50
38	1663	Bisabolone oxide	-	0.51	-	-
39	1715	Mint sulfide	0.18	-	-	-
40	1730	α-Bisabolol oxide	-	2.02	-	-
41	1960	Phytol	0.49	0.12	-	0.36
42	1965	Manoyl oxide	0.23	0.18	0.14	0.27
43	2100	Abietatriene	-	0.05	-	0.09
44	2400	*n*-Tetracosane	-	-	-	0.02
45	2500	*n*-Pentacosane	-	-	-	0.09
46	2600	*n*-Hexacosane	-	-	-	0.05
Number of identified compounds			28	32	28	25
% of identified compounds			97.72	99.47	99.76	99.68
(A) Hydrocarbons			52.61	53.54	36.86	56.08
Monoterpenes			25.98	22.59	26.78	40.34
Sesquiterpenes			24.98	29.38	9.50	13.62
Saturated aliphatic hydrocarbons			-	-	-	0.16
Aromatic hydrocarbons			1.65	1.57	0.58	1.96
(B) Oxygenated constituents			45.11	45.93	62.90	43.60
Monoterpenes			37.94	38.03	60.55	35.93
Sesquiterpenes			5.62	6.84	1.84	5.25
Other oxygenated constituents			1.55	1.06	0.51	2.42

of esters was in the winter sample among which dihydrocarveol acetate (9.98%) was the major constituent. The proportions of the compounds of the essential oils changed somewhat with the seasons; however, there was no significant difference in the content of the main component, carvone.

Previous investigations of the chemical composition of the essential oil from *M. suaveolens* from different populations and various geographical regions showed that the plants belong either to the menthol or carvone chemotype (Šarić-Kundalić *et al.*, 2009, and literature cited therein). The production of piperitenone, piperitone oxide, piperitenone oxide, menthol, and pulegone follows the menthol pathway (Hendriks and Van Os, 1976; Holeman *et al.*, 1985; Velasco-Negueruela *et al.*, 1996; Oumzil *et al.*, 2002; Sutour *et al.*, 2008, 2010; Koliopoulos *et al.*, 2010). Carvone, dihydrocarvone, and carveol characterize the carvone pathway (Hendriks Van Os, 1976; Šarić-Kundalić *et al.*, 2009; Kumar *et al.*, 2011). *M. suaveolens* Ehrh. cultivated in Egypt can be categorized as the carvone chemotype since this compound was a major constituent throughout the seasons.

The essential oil obtained in the highest yield (full-flowering summer sample), and having the highest contents of oxygenated constituents and carvone, was screened for a number of biological activities. It exhibited analgesic and acute anti-inflammatory activities [75.0% and 81.6% relative to a standard dose (20 mg/kg BW) of indomethacin] (Tables II and III).

The essential oil exerted a potent *in vivo* antioxidant activity (95.8% relative to α-tocopherol) (Table IV). The *in vitro* antioxidant (free radical scavenging) activity of the essential oil had an IC_{50} value of 200 μg/ml, in comparison to silymarin (45 μg/ml) and ascorbic acid (7.5 μg/ml).

The essential oil prevented the increase in the blood levels of AST, ALT, and ALP by 56.8%, 52.6%, and 53.5%, respectively (Table V). This protective effect was comparable to that of silymarin which prevented the rise in the level of these enzymes by 64.1%, 60.9% and 76.0%, respectively. Furthermore, administration of the essential oil for another 7 days after induction of liver damage led to a significant decrease in the enzyme levels which indicates stabilization

Table II. Analgesic activity of the essential oil of *M. suaveolens* in the acetic acid-induced mouse writhing test.

Animal group (dose)	Number of writhes (mean ± S.E.)	% of inhibition as compared to the control	% of potency as compared to the standard
Control (1 ml saline)	46.8 ± 1.2	0	0
Essential oil (100 mg/kg BW)	25.9 ± 0.9*	44.7	75
Indomethacin (20 mg/kg BW)	18.9 ± 0.3 *	59.6	100

* Statistically significant difference from zero time at $P < 0.01$.

Table III. Acute anti-inflammatory activity of the essential oil of *M. suaveolens* in the carrageenan-induced rat paw oedema test.

Animal group (dose)	% of oedema (mean ± S.E.)	% of inhibition as compared to the control	% of potency as compared to the standard
Control (1 ml saline)	59.7 ± 1.8	0	0
Essential oil (100 mg/kg BW)	29.2 ± 0.3*	51.1	81.6
Indomethacin (20 mg/kg BW)	22.3 ± 0.4*	62.6	100.0

* Statistically significant difference from zero time at $P < 0.01$.

Table IV. *In vivo* antioxidant activity of the essential oil of *M. suaveolens* in rats.

Animal group (dose)	Blood glutathione (mass%) (mean ± S.E.)	% of inhibition as compared to the control	% of potency as compared to the standard
Control non-diabetic (1 ml saline)	36.2 ± 1.4	0	0
Diabetic untreated (1 ml saline)	21.8 ± 0.6*	39.8	0
Diabetic + essential oil (100 mg/kg BW)	34.3 ± 0.8	5.2	95.8
Diabetic + α-tocopherol (7.5 mg/kg BW)	35.8 ± 1.1	1.1	100

* Statistically significant difference from the control group at $P < 0.01$.

of hepatocyte cell membranes and repair of liver damage caused by CCl_4 (Singab *et al.*, 2005). Since prevention of CCl_4-induced liver damage is considered indicative of general hepatoprotective activity of a drug (Wu and Norton, 1996), the present study has established that the essential oil of *M. suaveolens* holds promise for nutritional and pharmaceutical applications.

As established by the American National Cancer Institute (NCI), a crude extract is considered cytotoxic when the IC_{50} value in the preliminary assay of Suffness and Pezzuto (1990) is less than 30 μg/ml. In this respect, the essential oil of *M. suaveolens* could be considered moderately cytotoxic (IC_{50} = 10.60, 14.00, 9.15, and 18.20 μg/ml in liver, colon, larynx, and breast carcinoma cell lines, respectively) as compared to doxorubicin (IC_{50} = 3.73, 3.73, 3.73, and 2.97 μg/ml, respectively). The antimicrobial activity of the essential oil in the agar disc diffusion assay was moderate against bacteria and very potent against fungi (Table VI). Therefore, the minimum inhibitory concentration (MIC) was determined for the fungi in the broth microdilution assay. The essential oil exhibited a broadspectrum potent antifungal activity against *Candida albicans* (MIC = 4 μg/ml), *Saccharomyces cerevisiae* (MIC = 5.2 μg/ml), and *Aspergillus niger* (MIC = 6.8 μg/ml). A correlation between the inhibition zone diameter in the agar disc diffusion assay and MIC values in the broth microdilution assay was found. *Candida albicans* showed the maximum zone of inhibition and the minimum MIC value followed by *Saccharomyces cerevisiae* and *Asperigillus niger*. Previous results of an investigation of antifungal properties had shown that monoterpene hydrocarbons had lower antifungal activity than oxygenated terpenes (Soković *et al.*, 2009). Hydrocarbons tend to be relatively inactive regardless of their structural type, and this inactivity is closely related to their limited hydrogen binding capacity and water solubility (Soković *et al.*, 2009). The higher antifungal, as compared to antibacterial, potential of the essential oil of *M. suaveolens* could be explained by the presence of carvone, which possesses a very strong antifungal activity (Soković *et al.*, 2009). These results indicate that the essential oil may be used as a natural preservative in food against the well known causal agents of food-borne diseases and food spoilage, after conducting the necessary *in vivo* toxicity studies.

Table V. Effect of the essential oil of *M. suaveolens* on the levels of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) of liver-damaged rats.

Enzyme	Time	Level	Control	Oil (100 mg/kg BW)	Silymarin (25 mg/g BW)
AST [U/l]	Zero[a]	Mean ± S.E.[c]	29.4 ± 0.9	33.2 ± 1.2	32.3 ± 1.1
	7 d[a]	Mean ± S.E.[c]	28.6 ± 0.4	23.6 ± 1.1	30.6 ± 0.9
	72 h[b]	Mean ± S.E.[c]	136.9 ± 5.1	59.2 ± 2.3	49.2 ± 1.3
		% of change[d]	0	56.8	64.1
	7 d[b]	Mean ± S.E.[c]	151.7 ± 5.9*°	48.6 ± 1.9*	29.7 ± 0.6*
ALT [U/l]	Zero[a]	Mean ± S.E.[c]	31.6 ± 1.1	30.5 ± 0.8	27.8 ± 0.5
	7 d[a]	Mean ± S.E.[c]	30.9 ± 0.7	30.1 ± 1.1	26.8 ± 0.4
	72 h[b]	Mean ± S.E.[c]	143.9 ± 6.1	68.2 ± 2.5	56.2 ± 1.8
		% of change[d]	0	52.6	60.9
	7 d[b]	Mean ± S.E.[c]	149.2 ± 5.7*°	61.4 ± 2.3*	29.2 ± 0.8*
ALP [KAU/l]	Zero[a]	Mean ± S.E.[c]	6.8 ± 0.1	7.2 ± 0.1	7.3 ± 0.1
	7 d[a]	Mean ± S.E.[c]	7.1 ± 0.1	7.1 ± 0.1	6.9 ± 0.1
	72 h[b]	Mean ± S.E.[c]	67.9 ± 1.8	31.6 ± 0.8	16.3 ± 0.6
		% of change[d]	0	53.5	76
	7 d[b]	Mean ± S.E.[c]	73.4 ± 2.3*	27.4 ± 0.6*	6.9 ± 0.1*

[a] Enzyme level before induction of liver damage.
[b] Enzyme level after induction of liver damage.
[c] Enzyme level expressed as mean ± S.E.
[d] % of change calculated compared to the control group.
* Statistically significant from the control group at $P < 0.01$. °Statistically significant from 72 h after injection of CCl_4 at $P < 0.01$. KAU, King-Armstrong unit.

Table VI. Antimicrobial activity of the essential oil of *M. suaveolens* in the agar disc diffusion assay.

Tested microorganism	Diameter of inhibition zone [mm] (% of efficiency)		
	Oil (5 mg/disc)	Tetracycline (5 μg/disc)	Amphotericin B (5 μg/disc)
Gram-positive bacteria			
Staphylococcus aureus	12 (43)	28 (100)	-
Streptococcus faecalis	15 (48)	31 (100)	-
Bacillus subtilis	15 (47)	32 (100)	-
Gram-negative bacteria			
Escherichia coli	17 (57)	30 (100)	-
Neisseria gonorrhoeae	12 (35)	34 (100)	-
Pseudomonas aeruginosa	13 (42)	31 (100)	-
Fungi			
Candida albicans	24 (126)	-	19 (100)
Saccharomyces cerevisiae	21 (105)	-	20 (100)
Aspergillus niger	15 (94)	-	16 (100)

Conclusion

From the economical point of view, the aerial parts of *M. suaveolens* Ehrh. should preferably be collected in summer from plants cultivated in Egypt, when the highest yield of the oil is obtained. Moreover, the essential oil from such plant material has the highest percentage of oxygenated constituents and the highest carvone content. *M. suaveolens* Ehrh. cultivated in Egypt can be categorized as carvone-rich type, since this compound has a high relative level throughout the seasons. The different biological activities evaluated for the essential oil revealed significant efficacy and potency when compared to standard drugs. This suggests the incorporation of the oil in herbal formulations after necessary clinical trials.

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