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Research Paper

Chemical profile and antibacterial activity of *Mentha viridis L.* essential oils and ethanolic extract

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Abstract. *Mentha viridis* was the model plant for this study to estimate the antibacterial activity and define the essential metabolites' essential chemical compositions. This study was designed to estimate ethanol extract's chemical composition and essential oil isolated from leaves of commercial *M. viridis*. The essential primary and secondary metabolites were estimated and defined as: total protein (45.29 µg/ml), total alkaloids (0.116 mg/g), total flavonoids (0.9931 mg/g), and total phenolic compounds (0.2326 mg/g). The antibacterial activity of *M. viridis* ethanol extract was screened against two-gram negative bacterial strains, *Pseudomonas aeruginosa* and *Escherichia coli* (DH5-α). This work proved that both the ethanol extract and the essential oil extract of *M. viridis* leave have potent antibacterial activity against tested bacteria. There is a difference in clear zone diameter by comparing the control ethanol treatment to the ethanol extract. The total essential oil obtained by hydro-distillation technique and identified by Gas chromatography-mass spectrometry. The main compounds of the oil were Cyclohexanone, 5-methyl-2-(1-methylethylidene)- (33.46%), D-Carvone (32.30%), Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.alpha., 2.beta., 5.alpha.) (7.13%), Carveol (5.31%), Dihydrocarvyl acetate (5.30%). All these characterizations enable *M. viridis* to have antibacterial, antioxidant, anti-inflammatory and anticarcinogenic effects.

Keywords: Antibacterial activity, essential oils, ethanol extract, gas chromatography-mass spectrometry (GC-MS), *Mentha Viridis*.

INTRODUCTION

Mentha viridis is famous for its medicinal and aromatic properties for thousands of years. This herb belongs to the family *Lamiaceae*. Mint is growing mainly as essential field crops among vegetables. Also, mint is applied as perfumery, culinary preparation, flavoring foods, cosmetics, beauty and body care. Multiple medicinal herbal plants are also used for food, oil, and fiber plants. It is rich in volatile oil, which gives a pleasurable aroma (Peter, 2001; Kalemba and Kunicka, 2003; Balla *et al.*, 2017).

The species is eaten in the form of chutney as it is useful as a digestive and gastro-stimulant. Leaves are popularly used as a tea flavoring agent, while herbalists use the whole plant as a carminative. The dried and fresh plants and their essential oils are widely applied in chewing gum, food, cosmetics, toothpaste, confectionery and pharmaceutical industries. The essential oil of *M. viridis* showed vigorous insecticidal and mutagenic activity. They also have antimicrobial, antioxidant, anti-inflammatory, anticarcinogenic and analgesic effects

(Yonis and Beshir, 2004; Lawrence, 2006; Rita and Animesh, 2011; Shaikh *et al*., 2014; Balla *et al*., 2017).

The essential oils that are isolated from aromatic plants are very necessary in many applications. Many plants are applied for different industrial goals and pharmacological actions, such as drugs, perfumery, and food manufacturing. They also do not enhance the “antibiotic resistance”, a phenomenon caused by synthetic antibiotics' long-term use. However, because of increasing herbal product usage, intensive care should be given to their toxicity potency and drug-drug interactions (Braun and Cohen, 2014; Brown and Wright, 2016; Hassan *et al*., 2019).

The essential oils obtained from the *Mentha* genus have a high commercial value due to monoterpene menthol. This menthol is extensively used in food, cosmetics, personal care and pharmaceutical products (Gaurav, 2016). Besides, some bioactivities include anti-inflammatory, antioxidant, anticarcinogenic, analgesic, and antimicrobial effects. The pharmacological effects of *Mentha spp.* are mainly due to compound groups of phenolic and essential oil compounds. The essential phenolic compounds included in *Mentha* species are caffeic acid derivatives and flavonoids containing glycosides (such as apigenin, luteolin, naringenin and eriodictyol). However, reported results on the biological activity and chemical composition of *Mentha* have mostly focused on investigating its essential oils. *Mentha* plants' essential oils consisted of sesquiterpenoids and monoterpenoids whose content has variable percentage composition (Ouakouak *et al*., 2015; Balla *et al*., 2017; Hassan *et al*., 2019).

This work's target was to define the chemical composition of the essential metabolites from the ethanol extract of *M. viridis.* Besides analyzing the essential oils by a GC-MS system to determine the essential oils and the chemical compositions for their antibacterial activity

MATERIALS AND METHODS

Plant and bacterial strains

Mentha viridis (L.) is the commercial mint in Egypt (known as Spearmint). It was commercially supplied and taxonomically identified in the ecology and taxonomy lab, Faculty of Science, Helwan University.

Pseudomonas aeruginosa and *Escherichia coli* (DH5-α) tested bacteria are morphologically and biochemically identified according to Bergey's manual in Microbiology lab, Faculty of Science, Helwan University. They were stored in glycerol stocks.

Mint ethanol extract characterization

Some essential metabolites were detected in the ethanol extract of mint-like flavonoids and phenolic compounds.

Total phenolic compounds

Total phenolic contents were determined from the ethanol extract of mint according to the method described by Kujala *et al.* (2000), using Folin–Ciocalteu reagent (Sigma-Aldrich) and gallic acid as a standard. Briefly, 0.5 ml of filtered extract was added to the test tube containing 2.5 ml Folin–Ciocalteu reagent (diluted with ethanol 1:1), 2 ml Na_2CO_3 (7.5%) and mixed well. After 15 min incubation at room temperature, the absorbance of mixtures was measured spectrophotometrically at 765 nm by a Jenway 6405 UV-Vis spectrophotometer.

Total flavonoids

Total flavonoids content of *M. viridis* extract was determined using aluminum chloride colorimetric assay (Zhishen *et al*., 1999). 0.5 ml of the extract was added to 150 µl of 5% $NaNO_3$ and allowed to stand for 6 minutes. Then 150 µl of 10% $AlCl_3$ solution was added and allowed to stand for 6 minutes, after which 200 µl solution of 1 M NaOH was added then the mixture was completed to 5 ml with methanol and mixed well. All chemicals used were highly purified and obtained from (Sigma-Aldrich). After incubation for 15 min, the absorbance was measured spectrophotometrically against a blank at 510 nm.

Both total phenolic compounds and total flavonoids content were calculated according to the following equation:

Concentration (mg/g) = ((R-B) * dilution factor*factor) / 1000 (1)

**R: samples reading at spectrophotometer, B: blank reading at spectrophotometer*

Total Alkaloids

Alkaloids were determined using Harborne (1973) method as follows: 5 grams of the sample were weighed into a 250 ml beaker, and add 200 ml of 10% acetic acid in ethanol then covered for 4 h. After that, concentrate the filter on a water bath to one-quarter of the original volume. The concentrated ammonium hydroxide (10%) (Sigma-Aldrich) was added dropwise to the extract until the precipitation was complete. The total solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed as mg/g.

Total protein

The total protein was extracted from mint leaves according

Table 1. Means of some essential metabolites in *M. viridis*.

Total metabolite	Phenolics (mg/g)	Flavonoids (mg/g)	Alkaloids (mg/g)	Proteins (µg/ml)
Concentration	0.2326 ± 0.00011	0.9931 ± 0.00012	0.116 ± 0.0011	45.29 ± 0.012

to Bradford (1976) as follows: Weight 0.5 g of *M. viridis* leaves were weighted and grind well with 0.5 ml of [2x] buffer. Then vortex 10 min and centrifuge for 15 min at 21952 g at 4°C. The supernatant contained the total protein content of plant species. Finally, the protein concentration was estimated according to Bradford (1976) as follows: 0.1ml of supernatant was pipette into a test tube, and 5 ml of protein reagent was added, mixed, and measured by Spectrophotometer at wavelength 595 nm. The concentration of protein was determined from the protein standard curve. The concentration was calculated according to the following equation:

$$X\ (\text{conc.}) = (Y\ (\text{abs.}) - 0.030) / 0.007 \quad (2)$$

Oil extract

The oil of the examined *M. viridis* leaves was obtained by a hydro-distillation technique using Clevenger's apparatus. One-hundred grams of plant materials were placed in a two-liter round bottom flask, and dH_2O was added and mixed vigorously. The contents were boiled gently for four hours until the volatile oil has been distilled. The volatile crude oil of the plant was transferred via a pipette to a separate brown glass bottle. The excess water was removed via a capillary tube, and the pure oil was decanted into a brown glass bottle and kept in the refrigerator until needed for analysis.

GC-MS analysis for mint essential oils

Mass spectra were recorded using Shimadzu GCMS-QP2010 (Tokyo, Japan) equipped with Rtx-5MS fused bonded column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) (Restek, USA) equipped with a split–splitless injector. The initial column temperature was kept at 45°C for 2 min (isothermal) and programmed to 300°C at a rate of 5°C/min and kept constant at 300°C for 5 min (isothermal). The injector temperature was 250°C. Helium carrier gas flow rate was 1.41 ml/min. All the mass spectra were recorded applying the following condition: (equipment current) filament emission current, 60 mA; ionization voltage, 70 eV; ion source, 200°C. Diluted samples (1% v/v) were injected with split mode (split ratio 1: 15) (Adams, 2001).

Mint ethanol extract

Alcoholic extracts were prepared using ethanol solvent. The leaves of mint (50 g) were soaked in 250 ml of 99.9% ethanol at room temperature for 24 h. The extracts were separated from the other solid plant residues using filter paper, and then, it was centrifuged at 11200 g, and the supernatants were filtered using Whatman filter paper No.1 (Okigbo and Mmeka, 2008; Fonkeng *et al.*, 2015). The ethanolic extract was dried using a rotary evaporator to dryness, and the dried extract was dissolved in DMSO (dimethyl sulfoxide) and applied in plates using disc diffusion assay. The extract was then used for the detection of some essential metabolites such as flavonoids and phenolic compounds.

Antibacterial bioassay of mint ethanol extract

The ethanol extract of *M. viridis* leaves was tested against two bacterial species: two Gram-negative bacterial strains, *Escherichia coli* (DH5-α) and *Pseudomonas aeruginosa* bacteria. This assay was applied using the agar disk diffusion method according to CISI (2012). LB Agar plates are inoculated with a standardized inoculum of the two-test microorganism. Then, filter paper discs (about 6 mm in diameter) containing *M. viridis* ethanol extract are placed on the agar surface, and then incubated at 37°C overnight. The control bioassay was applied by inoculating only ethanol in wells.

Statistical analysis

Data collected were subjected to analysis of variance test in Minitab 19. Mean average, standard deviations were estimated.

RESULTS AND DISCUSSION

Characterization of some essential physiological metabolites in *M. viridis*

M. viridis contains high concentrations of some essential physiological compounds (phenolic compounds, flavonoids, alkaloids and proteins). The results of some physiological studies made in the present work are shown in Table 1. These essential metabolites were estimated as they are responsible for the antibacterial activity.

Characterization of mint essential oils

GC/MS analysis of essential oils extracted from *M. viridis*

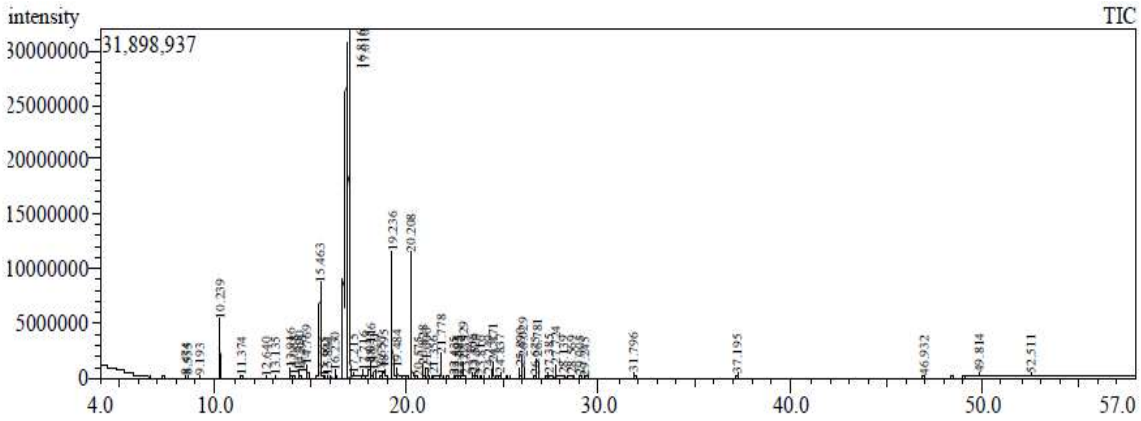

Figure 1. GC-MS Chromatogram of essential oil of *Mentha viridis*.

Table 2. Chemical composition of essential oil of *M. viridis.*

No.	Compound	RT-min	Area %
1	alpha.-Pinene	8.474	0.09
2	beta.-Pinene	8.555	0.08
3	6-Methylheptan-3-ol	9.193	0.08
4	Eucalyptol	10.239	2.00
5	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	11.374	0.07
6	Pentanoic acid, 2-methylbutyl ester	12.640	0.04
7	3-Octanol, acetate	13.135	0.06
8	Cyclohexane, 1-methyl-4-(1-methylethenyl)-, trans-	13.916	0.43
9	Cyclohexanone, 5-methyl-2-(1-methylethyl)-	14.068	0.07
10	Bornyl chloride	14.493	0.23
11	Cyclohexanone, 5-methyl-2-(1-methylethenyl)-, trans-	14.769	0.71
12	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.alpha.,2.beta.,5.alpha.)-	15.463	7.13
13	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-	15.665	0.10
14	2-Cyclohexen-1-one, 2-(2-methyl-2-propenyl)-	15.844	0.08
15	Cyclohexanone, 5-methyl-2-(1-methylethylidene)-	16.816	33.46
16	D-Carvone	17.010	32.30
17	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	17.215	0.28
18	Isopulegol acetate	17.716	0.28
19	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	18.035	0.29
20	2,5,5,8a-Tetramethyl-3,4,4a,5,6,8a-hexahydro-2H-chromene	18.116	0.58
21	Bicyclo[4.1.0]heptane, 3,7,7-trimethyl-, [1S-(1.alpha.,3.beta.,6.alpha.)]-	18.657	0.13
22	Dihydrocarvyl acetate	19.236	5.30
23	Carveol	20.208	5.31
24	.alfa.-Copaene	20.575	0.06
25	(-)-.beta.-Bourbonene	20.828	0.55
26	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta	21.000	0.34
27	Ylangene	21.356	0.17
28	Caryophyllene	21.778	1.00
29	(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	22.435	0.09
30	p-Mentha-1,8-dien-7-yl acetate	22.595	0.11
31	Humulene	22.685	0.10
32	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decane-rel-	22.929	0.76
33	(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	23.035	0.08
34	.beta.-copaene	23.406	0.15
35	trans-.beta.-Ionone	23.524	0.30

Table 2. Contd.

36	Guaia-1(10),11-diene	23.816	0.14
37	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1	24.249	0.13
38	cis-Calamenene	24.471	0.67
39	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1.alpha.,4a	24.837	0.16
40	4a,5-Dimethyl-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalen-1-ol	25.890	0.47
41	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)-	26.029	0.96
42	Humulene epoxide I	26.675	0.07
43	Copaene	26.781	0.86
44	isoledene	27.724	0.62
45	(3R,4aS,8aS)-8a-Methyl-5-methylene-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydrona	28.139	0.14
46	Muurol-5-en-4-one <cis-14-nor->	28.569	0.09
47	(E)-2-((8R,8aS)-8,8a-Dimethyl-3,4,6,7,8,8a-hexahydronaphthalen-2(1H)-ylidene)prop	29.005	0.09
48	2-Pentadecanone, 6,10,14-trimethyl-	31.796	0.30
49	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	37.195	0.24
50	Tetratetracontane	49.814	0.15

RT: Retention time

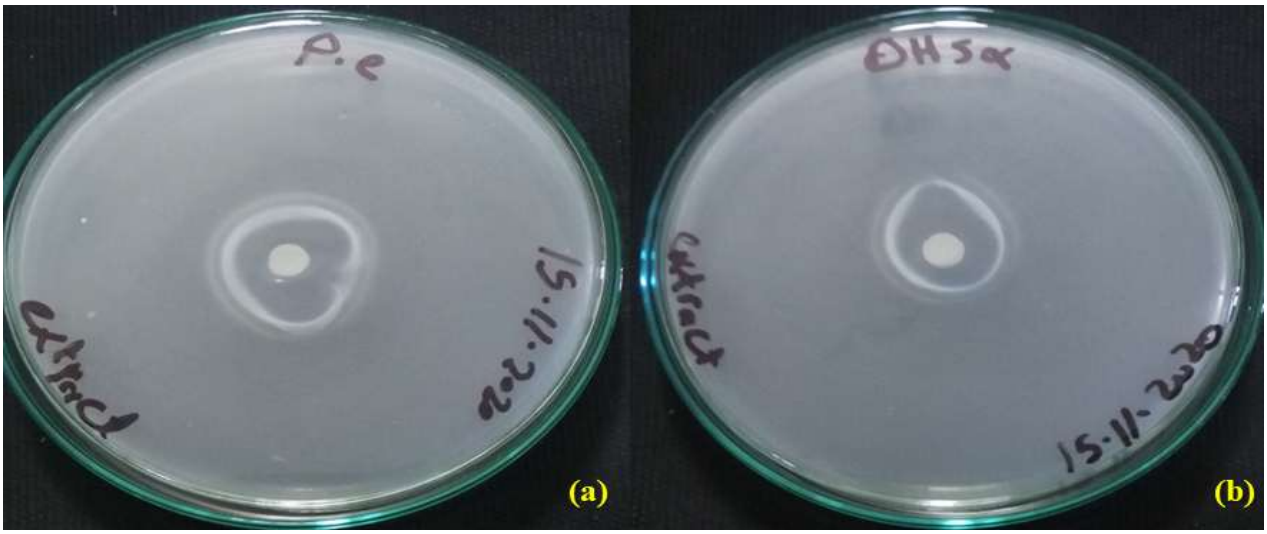

Figure 2. Clear zone of antibacterial activity of oil *M. viridis* against bacteria.

represented with total ion chromatogram in Figure 1 and results compiled in Table 2 revealed the identification of 50 compounds, representing (50%) of the total peak area of chromatogram. The major compounds were Cyclohexanone, 5-methyl-2-(1-methylethylidene)- (33.46%), D-Carvone (32.30%), Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.alpha.,2.beta.,5.alpha.)- (7.13%), Carveol (5.31%), Dihydrocarvyl acetate (5.30%).

The essential oil of *M. viridis* is useful and applied for medicinal and commercial purposes as it possesses a range of aroma chemicals used in perfumery, flavor and other allied industries. *M. viridis* is also applied pharmaceutically as an anti-inflammatory and as Hand sanitizer. Moreover, the essential oils' major constituents may be utilized as an essential tool in oil authentication. Balla *et al*. (2017) concluded that the antibacterial activity might likely be associated with a high methyl acetate concentration. Hassan *et al*. (2019) also found that the chemical composition of essential oil isolated from leaves of *M. viridis* has antimicrobial, antioxidant activity, and cytotoxicity.

Antibacterial bioassay of mint ethanol extract

The ethanol extract of *M. viridis* leaves was screened for antibacterial activity against two-gram negative bacterial strains (*Pseudomonas aeruginosa* and *Escherichia coli* (DH5-α)). The ethanol extract of *M. viridis* leaves showed high antibacterial activity against these two bacterial strains. Therefore, this result (Figure 2 and Table 3) showed that the extracts tested inhibited all microorganisms'

Table 3. Antibacterial activity of oil *M. viridis* against selected bacteria.

Tested bacteria used	Mean diameter of growth inhibition zone (mm)
Pseudomonas aeruginosa	2.2 ± 0.10
Escherichia coli (DH5-α)	1.7 ± 0.11

growth through microorganisms' sensitivities varied.

These results are compatible with Lixandru *et al*. (2010), who examined the oil extract of spearmint oil, which has considerable inhibition capacity against *E. coli*. This result also complied with Nakatani (1994) and Balla *et al*. (2017), who stated that, spearmint oil has obvious antibacterial activity against *E. coli*. Also, Zuzarte *et al*. (2011) examined the chemical composition of essential oils of *Lavandula viridis* as an antifungal against *Candida albicans*. *M. viridis* has no antibacterial activity and has antivenom activity as proved by Silva *et al.* (2019).

CONCLUSION

The ethanol extract of *M. viridis* showed fractionation of some essential metabolites like flavonoids and phenolic compounds. They showed antibacterial activity against two-gram negative bacterial strains, *Pseudomonas aeruginosa* and *Escherichia coli*. Also, total proteins and total alkaloids were estimated in mint leaves. The essential oil of *M. viridis* obtained by hydro-distillation was characterized using GC-MS. The essential medicinal constituents are the essential oils, such as Cyclohexanone, 5-methyl-2-(1-methylethylidene)-, D-Carvone, Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.alpha.,2.beta.,5.alpha.)-, Carveol (5.31%), Dihydrocarvyl acetate. It can be concluded that the active chemical compounds present in *M. viridis* could be applied in the treatment of various bacterial infections.

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