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Chemical Composition and Antimicrobial Activity of Essential Oil of *Mentha viridis*

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Abstract: The study was aimed to investigate essential oil chemical composition and antimicrobial activities of essential oils extracted from leaves of *Mentha viridis*. The oil was extracted by hydrodistillation method and analyzed by Gas chromatography–mass spectrometry (GC–MS), to determine the chemical composition of the volatile fraction and identify their chemo-types. The essential oil of *M. viridis* leaves were tested against four standard bacterial species: two Gram-positive bacteria viz, *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), two Gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and fungal strains viz, *Candida albicans* (ATCC 7596) using the agar plate diffusion method. GC-MS analysis revealed that *M. viridis* was constituted by D-Carvone (64.63%) as a major component followed by D-Limonene (12.27%), (-)-8-p-Menthen- 2-yl, acetate, trans (2.59%), Cyclohexanol, 2-methyl - 5- (1-methylethenyl) (2.36%), Eucalyptol (2.28%), 3-Hexadecyne (1.82%), Caryophyllene (1.72%), Beta–myrcene (1.43%), Trans-Carveyl acetate (1.37%), (-). Beta-Bourbonene (1.08%), and other traces compounds. Antimicrobial activity of essential oil of *M. viridis* dissolved in methanol (1:10), showed high activity against the Gram-negative bacteria (*E. coli* & *P. aeruginosa*) (17 & 16 mm). It also showed against Gram positive bacteria (*B. subtilis* & *S. aureus*) (16 & 15 mm) and against (*C. albicans*) (16 mm). This study conducted for essential oil of *M. viridis* leaves proved to have potent activities against antimicrobial activity *in vitro*.

Keywords: *In-vitro,* Antimicrobial Activity, Gas Chromatography–Mass Spectrometry (GC-MS), Essential Oils, *Mentha viridis* (Leaves)

1. Introduction

Herbs are plant valued for their medicinal and aromatic properties and often grown and harvested for these unique properties. In most parts of the world, herbs are grown mainly as field crops or on small scale as catch crop among vegetables. The knowledge on herbs has been handed down from generation to generation thousands of years [1] (Brown, 1995). Herbs are used as natural source for treatment of various diseases. Also herbs are used for flavoring foods, culinary preparation, perfumery, cosmetics, beauty and body care. Many medicinal herbs are also food, oil and fiber plant [2] (Peter, 2001).

Herbs are rich in volatile oil which gives pleasurable aroma. In addition, herbs may contain alkaloids and glycoside which have great pharmaceutical effect. Essential oils have been extensively investigated for their activity against a number of storage fungi, plant and human pathogens, bacteria, insect, pests and other harmful microorganisms. Almost all essential oil of herbs and spices (individual or combination) are highly inhibitory to selected pathogenic and spoil-age microorganisms [3] (Kalemba and

Kunicka, 2003).

Mentha species (commonly known as mint or pudina) is a well-known genus (family: Lamiaceae) for medicinal and aromatic value. The genus *Mentha* includes 25–30 species that grow in the temperate regions of Eurasia, Australia and South Africa [4] (Dorman *et al*., 2003).

Mentha spicata L. (spearmint) is a creeping rhizomatous, glabrous and perennial herb with a strong aromatic odor. The oil of *M. spicata* is rich in carvone and presents a characteristic spearmint odor [5] (Jirovetz *et al*., 2002).

The species has been found useful as digestive and gastro-stimulant this is eaten in the form of chutney. Leaves are popularly used as tea flavouring agent, while herbalist use whole plant as carminative [6] (Yonis and Beshir, 2004). The fresh and dried plants and their essential oils are widely used in food, cosmetic, confectionary, chewing gum, toothpaste and pharmaceutical industries [7] (Lawrence, 2006). The essential oil of *M. spicata* showed strong insecticidal and mutagenic activity [8] (Franzios *et al*., 1997).

Mentha plants are mainly used for treatment of disorders of gastrointestinal tract. They have also been reported to have antioxidant, anti-inflammatory, antimicrobial, analgesic and anticarcinogenic effects [9, 10 & 11] (Shaikh *et al*., 2014; Rita and Animesh, 2011; McKay and Blumberg, 2006). The pharmacological effects of *Mentha* plants are chiefly bound to the presence of two main compound groups: phenolic and essential oil compounds. The main phenolics in reported *Mentha* plants include derivatives of caffeic acid and glycosidic forms of the flavonoids luteolin, apigenin, eriodictyol and naringenin. However, previous studies on the chemical composition and biological activity of *Mentha* have mainly focused on the essential oils. *Mentha* plants essential oils are mainly composed of monoterpenes and sesquiterpenes, which content and composition varies [12 & 13] (Kumar *et al*., 2011; Maffei *et al*., 2006).

Spearmint is species of mint native to North Africa, Egypt and Morocco. It is an invasive species in Great Lakes region where it was first sighted in 1843. Spearmint has long tradition medicinal use. It was taken as a tea to treat general digestive problems. Spearmint is widely used in commercially manufactured product, cooking and medicine for its aromatic and flavorsome qualities [14] (http://www.mountainroseherbs.com/spearmint.php, 2010).

Therefore, the objectives of this study were to analyze chemical composition of hydrodistilled essential oils of *M. viridis* by a GC-MS system to determine the essential oils investigate their antimicrobial activity.

2. Materials and Methods

2.1. Plant Material

The leaves of *Mentha viridis* were purchased from local farm in Al-kadaro region (Khartoum, Sudan), between January and February 2016. The plant was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan. Leaves of *M. viridis* were air dried, under the shade and pulverized and stored prior to extraction. Shade with good ventilation and the ground finely in a mill and kept in the herbarium unit their uses for extract preparation.

2.2. Method of Extraction

The oil of the tested *M. viridis* leaves was obtained by hydrodistillation technique using Clevenger's apparatus. Hundred grams from plant materials were placed in a two liters round bottom flask and distilled water was added and mixed thoroughly. The contents of the flask were boiled gently for four hours until the volatile oil has been distilled. The crude volatile oil of plant was transferred by means of a pipette into a separate brown glass bottle. Anhydrous sodium sulphate was added agitated gently to absorb the water and the clear oil was decanted into brown glass bottle and kept in the refrigerator until needed for analysis.

GC/MS analysis was conducted using Shimadzu Q P2010 GC/MS (Japan) instrument equipped with reference libraries. The flow rate of helium as carrying gas was (1 ml/min). The temperature program consisted of 50 – 280°C, at rate of 8°C/min. MS were taken at ionization voltage 70 eV. Library search was carried out using Wiley GC/MS library. The individual identifications were made by the comparison of fragmentation patterns with those found in the library of the Mass spectrometer and literature [15] (Adam, 2001).

2.3. Test Microorganisms

The oil solution of *M. viridis* was tested against four standard bacteria species: two Gram-positive bacteria viz., *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), two Gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and one standard fungal strains viz, *Candida albicans* (ATCC 7596) using the agar plate diffusion method. The standard bacterial and fungal strains used in the study were obtained from the Department of Microbiology, Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan. The bacterial cultures were maintained on nutrient agar and incubated at 37°C for 18 h and then used for the antimicrobial test.

2.4. In vitro Testing of M. viridis for Antimicrobial Activity

The cup-plate agar diffusion method described in (Kavanagh, 1972) [16] was used adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension (between 10^8 and 10^9 CFU/ml) was thoroughly mixed with 100 ml of molten sterile Mueller Hinton agar which was maintained at 45°C. 20 ml aliquots of the inoculated Mueller Hinton agar were distributed into sterile Petri-dish plates. The agar was left to set and in all of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer and agar discs were removed. Alternate cups were

filled with 0.1 ml of the oil using an automatic microlitre pipette, and thereafter the oil was allowed to diffuse at room temperature for two hours. The plates were then incubated in an upright position at 37°C for 24 h. Two replicates were carried out against each of the tested microorganisms. After incubation the diameters of the resultant growth inhibition zones were measured and averaged. The mean values were tabulated.

2.5. Antifungal Testing

The same method used for bacterial was adopted. However, the growth media used in case of fungi was Sabouraud Dextrose Agar. The inoculated medium was incubated at 25°C-27°C for 24-48 h for *Candida albicans.*

2.6. Antibacterial and Antifungal Activity of Reference Drugs Against Standard Microorganisms

2.6.1. Antibacterial Activity of Reference Drugs Against Standard Microorganisms

In the present work, two antibacterial drugs (Ciprofloxacin and Gentamicin) were tested at different concentrations obtained by taking 0.1 g of powdered drug and dissolved in 100 ml sterile distilled water to give a concentration of 1000 µg/ml followed by serial dilutions to give concentrations of 40, 20, 10 and 5 µg/ml. These drugs were tested against reference bacteria i.e. *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*.

2.6.2. Antifungal Activity of Reference Drugs

The antifungal drugs were also tested at different concentrations obtained by taking 0.1 g of each powdered drug and dissolved in 100 ml sterile distilled water to give a concentration of 1000 µg/ml followed by serial dilutions to give concentrations of (12.5, 25 and 50 µg/ml) Clotrimazole against reference fungi *Candida albicans* (5, 10, 20 and 40 µg/ml) Nystatin against the same organisms.

3. Results and Discussion

The extracted yield of essential oil of the leaves of *M. viridis* was 3.5%. A total of 51 compounds were identified in the essential oils extracted from *M. viridis* collected in Khartoum region. The composition together with the percentage and retention time of *M. viridis*. The major components of *M. viridis* L. oil, which found in extracted sample were D-Carvone (64.63%), D-Limonene (12.27%), (-)-8-p-Menthen- 2-yl, acetate, trans (2.59%), Cyclohexanol, 2-methyl - 5- (1 methylethenyl) (2.36%), Eucalyptol (2.28%), 3-Hexadecyne (1.82%), Caryophyllene (1.72%), Beta–myrcene (1.43%), Trans-Carveyl acetate (1.37%), (-). Beta–Bourbonene (1.08%) are shown in Table 1 and 2.

Carvone-rich spearmint has been investigated earlier in India as well as other countries. Earlier study showed Carvone (59.6-72.4%) and limonene (10.7-24.8%) as major constituents of oil of *M. viridis* from the mid-hills of Himalayan region of India at different crop stages [17] (Verma *et al*., 2010). While M. viridis collected from different subtropical and temperate zones of north-west Himalayan region of India showed Carvone (49.6-76.6%) followed by limonene (9.5-22.3%), 1,8-cineole (1.3-2.6%) and transcarveol (0.3-1.5%) in its oils [18] (Chauhan *et al*., 2009).

The essential oil of *M. viridis* L. is useful for commercial purpose as it possesses a range of aroma chemicals used in perfumery, flavor, pharmaceutical and other allied industries. Moreover, the major constituents in the essential oils may be utilized as an important tool in oil authentication.

The essential oil of *M. viridis* leaves family (Lamiaceae) was screened for antimicrobial activity against four bacterial species: two Gram-positive bacteria viz., *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), two Gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and fungal strains viz, *Candida albicans* (ATCC 7596) using the agar plate diffusion method.

The oil of *M. viridis* dissolved in methanol (1:10) showed high activity (17 & 16 mm) against Gram negative bacteria (*E. coli* & *P. aeruginosa*) and (16 mm) against (*A. niger* & *C. albicans*). It also showed (16 & 15 mm) against Gram positive bacteria (*B. subtilis* & *S. aureus*).

Therefore, this result showed that the extracts tested inhibited the growth of all microorganisms though the sensitivities of microorganisms varied.

This result agreed with Lixandru, *et al*, (2010) [19] who stated that spearmint oil exhibited considerable inhibition capacity against *E coli*. This result was also complied with Nakatani and Nobuji (1994), who stated that, the spearmint oil has potent antibacterial activity against *E coli*.

The result of minimum inhibition concentration from Table 5 showed that 12.5 µg/ml was the lowest concentration at which all the tested microorganisms were inhibited. A comparison of observation given in Tables 4, 5 and 6, showed that the oil of *M. viridis* dissolved in methanol inhibited all bacteria higher than 40 µg/ml Ampicillin and except inhibited *S. aureus* higher than 5 µg/ml Ampicillin. It inhibited *E. coli* similar 10 µg/ml Gentamicin, and higher than 10 µg/ml Gentamicin. The oil of *M. viridis* of inhibited *C. albicans* with a higher than 25 µg/ml of Nystatin.

Table 1. *Chemical composition of essential oil of M. viridis.*

No.	Compound	RT(min)	KI	Area (%)	M
1	Beta-pinene	4.668	29729	0.05	136
2	Alpha-pinene	4.930	190082	0.35	136
3	Bicyclo (3.1.0) hexane, 4-methylene-1 –(1-methylethyl)	5.663	183566	0.33	136
4	Bicyclo (3.1.1) heptane, 6,6-dimethyl-2 –methylene- (1S)	5.746	364667	0.67	136
5	Beta – myrcene	5.951	783704	1.43	136
6	3-Octanol	6.039	153651	0.28	130

No.	Compound	RT(min)	KI	Area (%)	M
7	Beta –Ocimene	6.265	34360	0.06	136
8	D-Limonene	6.756	6727528	12.27	136
9	Eucalyptol	6.831	1247941	2.28	154
10	Trans-beta –Ocimene	6.891	77165	0.14	136
11	1,3,6-Octatriene, 3,7 –dimethyl – (Z)	7.111	72594	0.13	136
12	Gamma – Terpinene	7.375	24252	0.04	136
13	Cyclohexene, 1-methyl -4-(1 methyl ethylidene)	8.015	40955	0.07	136
14	3 – Nonanol	8.117	9667	0.02	144
15	1,6 – Octadien – 3 – ol, 3,7 – dimethyl	8.211	148552	0.27	154
16	Butanoic acid, 2- methyl, 3-methylbutyl ester	8.296	55929	0.10	172
17	4-Heptanol, 2,4,6 –trimethyl	8.694	107867	0.20	158
18	1,5,5 –trimethyl – 6 –methylene –cyclohexene	8.830	20560	0.04	136
19	3a, 6 - methano- 3ah- inden- 5 ol, octahydro, (3a.alpha,5.alpha, 6.alpha, 7a.alpha)	9.022	21599	0.04	152
20	1,4,9 – Decatriene, (Z)	9.086	31967	0.06	136
21	Bicycle (4.1.0)hept – 2-ene	9.662	62468	0.11	94
22	L.alpha - Terpineol	9.725	68180	0.12	154
23	P-Menth – 8 –en – 1- ol, stereoisomer	9.954	179698	0.33	154
24	Alpha.- Terpineol	10.230	78949	0.14	154
25	Cyclohexanol, 2-methyl - 5- (1-methyl ethenyl)	10.305	1292867	2.36	154
26	3-Hexadecyne	10.369	996739	1.82	222
27	2-Cyclohexen -1-ol, 2-methyl- 5-(1-methyl ethenyl), cis	10.446	36957	0.07	152
28	Cyclohexanone, 2-m2thyl – 5- (1-methyl ethenyl), trans	10.544	164466	0.30	152
29	Trans – Carveol	10.822	444930	0.81	152
30	Carveol	11.082	452769	0.83	152
31	Butanoic acid, 3-methyl, hexyl ester	11.198	38448	0.07	186
32	Cyclohexanone, 5-methyl-2-(1 methyl ethylidene)	11.280	105327	0.19	152
33	D- Carvone	11.399	35420806	64.63	150
34	2H-1-benzopyran,3,4,4a,5,6,8a-hexahydro-2, 5,5,8a tetramethyl, (2.alpha,4a.alpha,8a.alpha)	12.313	234641	0.43	194
35	2-Heptanone, 6-(3-acetyl-2-methyl-1-cyclo propen-1-yl),6-methyl	12.431	31975	0.06	222
36	Neodihydrocarveol	12.756	28278	0.05	154
37	(-)-8-p-Menthen- 2-yl, acetate, trans	13.034	1416968	2.59	196
38	Cyclohexane, 1-ethenyl- 1-methyl-2-(1 methyl ethenyl)-4-(1-methylethylidene)	13.269	38344	0.07	204
39	Trans-Carveyl acetate	13.725	749617	1.37	194
40	(-). Beta – Bourbonene	14.273	590831	1.08	204
41	Caryophyllene	14.969	944275	1.72	204
42	Beta – Copaene	15.136	91508	0.17	204
43	(E) – beta – Famesene	15.487	127863	0.23	204
44	Humulene	15.621	46076	0.08	204
45	Tricycle(4.4.0.0(2,8))decan-4-ol	15.681	65147	0.12	152
46	1,6-Cyclodecadiene, 1-methyl-5-methylene 8-(1-methylethyl), (S-(E, E))	15.871	56572	0.10	204
47	Gamma, muurolene	16.131	482831	0.88	204
48	1,5 – cyclodecadiene, 1,5 – dimethyl – 8-(1 methylethylidene)-, (E, E)	16.426	100538	0.18	204
49	Copaene	16.859	48564	0.09	204
50	Alpha – farnesene	24.244	22037	0.04	204
51	Phytol	24.852	62925	0.11	296
				100.00	

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

No.	Compound	RT (min)	KI	Area%	M
1	D- Carvone	11.399	35420806	64.63	150
2	D-Limonene	6.756	6727528	12.27	136
3	(-)-8-p-Menthen- 2-yl, acetate, trans	13.034	1416968	2.59	196
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15	2H-1-benzopyran,3,4,4a,5,6,8a-hexahydro-2,5,5,8a tetramethyl, (2.alpha,4a.alpha, 8a. alpha)	12.313	234641	0.43	194
16	Alpha-pinene	4.930	190082	0.35	136

No.	Compound	RT (min)	KI	Area%	M
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19	Cyclohexanone, 2-m2thyl – 5- (1-methyl ethenyl), trans	10.544	164466	0.30	152
20	3-Octanol	6.039	153651	0.28	130
21	1,6 – Octadien – 3 – ol, 3,7 – dimethyl	8.211	148552	0.27	154
22	4-Heptanol, 2,4,6 –trimethyl	8.694	107867	0.20	158

Table 3. *Trace chemical composition of essential oil of M. viridis.*

No.	Compound	RT (min)	KI	Area%	M
1	Beta-pinene	4.668	29729	0.05	136
2	Beta –Ocimene	6.265	34360	0.06	136
3	Trans-beta –Ocimene	6.891	77165	0.14	136
4	1,3,6-Octatriene, 3,7 –dimethyl – (Z)	7.111	72594	0.13	136
5	Gamma – Terpinene	7.375	24252	0.04	136
6	Cyclohexene, 1-methyl -4-(1-methyl ethylidene)	8.015	40955	0.07	136
7	3 – Nonanol	8.117	9667	0.02	144
8	Butanoic acid, 2- methyl, 3-methylbutyl ester	8.296	55929	0.10	172
9	1,5,5 –trimethyl – 6 –methylene – cyclo- hexane	8.830	20560	0.04	136
10	3a, 6 - methano- 3ah- indenol, octahydro, (3a.alpha,5.alpha, 6.alpha, 7a.alpha)	9.022	21599	0.04	152
11	1,4,9 – Decatriene, (Z)	9.086	31967	0.06	136
12	Bicycle (4.1.0)hept – 2-ene	9.662	62468	0.11	94
13	L. alpha – Terpineol	9.725	68180	0.12	154
14	Alpha.- Terpineol	10.230	78949	0.14	154
15	2-Cyclohexen -1-ol, 2-methyl- 5-(1-methyl ethenyl), cis	10.446	36957	0.07	152
16	Butanoic acid, 3-methyl, hexyl ester	11.198	38448	0.07	186
17	Cyclohexanone, 5-methyl-2-(1 methyl ethylidene)	11.280	105327	0.19	152
18	2-Heptanone, 6-(3-acetyl-2-methyl-1-cyclo propen-1-yl),6-methyl	12.431	31975	0.06	222
19	Neodihydrocarveol	12.756	28278	0.05	154
20	Cyclohexane, 1-ethenyl- 1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)	13.269	38344	0.07	204
21	Beta – Copaene	15.136	91508	0.17	204
22	(E) – beta – Famesene	15.487	127863	0.23	204
23	Humulene	15.621	46076	0.08	204
24	Tricycle (4.4.0.0(2,8))decan-4-ol	15.681	65147	0.12	152
25	1,6-Cyclodecadiene, 1-methyl-5-methylene -8-(1-methylethyl), (S-(E, E)	15.871	56572	0.10	204
26	1,5 – cyclodecadiene, 1,5 – dimethyl – 8-(1-methylethylidene)-, (E, E)	16.426	100538	0.18	204
27	Copaene	16.859	48564	0.09	204
28	Alpha – farnesene	24.244	22037	0.04	204
29	Phytol	24.852	62925	0.11	296
	Total Identified Constituents			100.00	

Table 4. *Antimicrobial activity of oil M. viridis against the standard bacteria and fungi.*

Standard microorganisms	Mean Diameter of Growth Inhibition Zone (mm)
Tested Bacteria used	
Bacillus subtilis	15
Escherichia coli	17
Staphyococcus aureus	16
Pseudomonas aeruginosa	16
Tested fungi used	
Candida albicans	16

Table 5. *The antimicrobial activity of oil M. viridis against the standard bacteria and fungi at different concentrations.*

Standard microorganisms	Concentration (mg/ml)			
	Mean Diameter of Growth Inhibition Zone (mm)			
	100	50	25	12.5
Bacillus subtilis	15	14	12	11
Escherichia coli	17	15	14	12
Staphyococcus aureus	16	15	13	12
Pseudomonas aeruginosa	16	14	12	11
Tested fungi used				
Candida albicans	16	14	12	11

Key: Interpretation of results: MDIZ (mm): >15mm = Sensitive, 12-15mm= Intermediate, <12mm = Resistant, (−) = No inhibition. Concentration used 100 mg/ml at 0.1ml/cup.

Table 6. *Antibacterial activity of reference antibiotics against standard microorganisms.*

Drugs	Concentration (µg/ml)	Standard microorganisms used			
		Tested bacteria used			
		E. coli	*B. subtilis*	*Ps. aeruginosa*	*S. aureus*
Ampicillin	40	-	15	16	25
	20	-	14	13	20
	10	-	13	12	18
	5	-	12	-	15
Gentamicin	40	32	29	23	35
	20	30	22	22	33
	10	17	20	21	30
	5	-	17	19	28

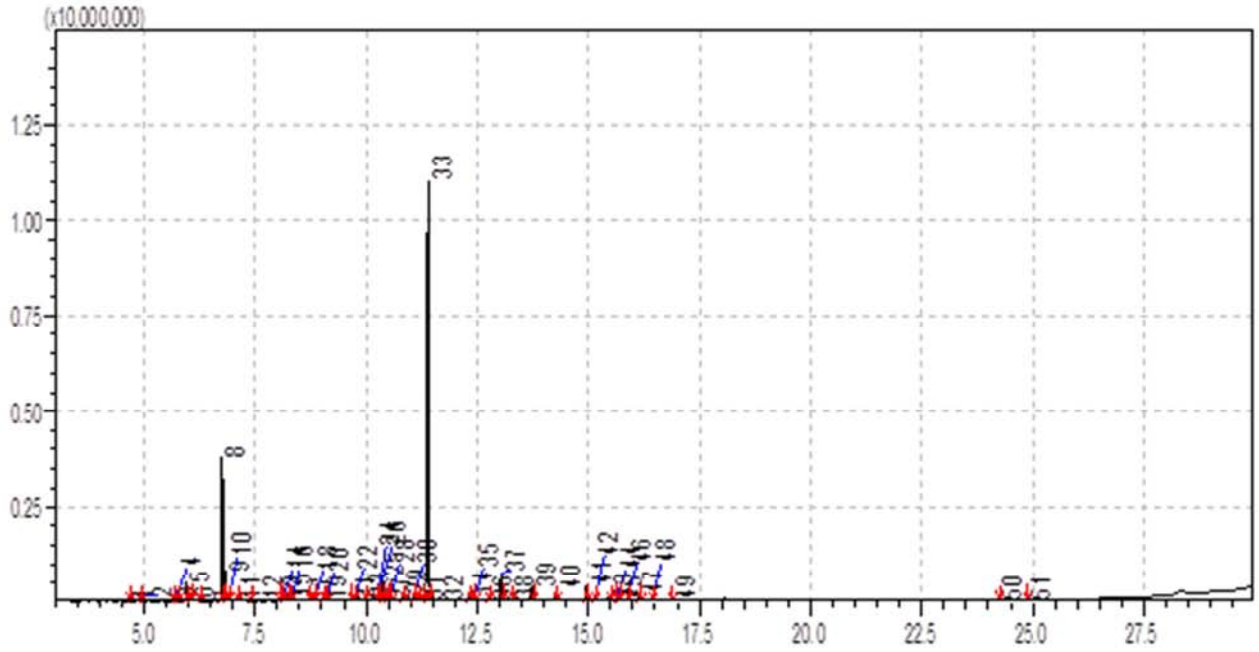

Figure 1. *GC Chromatogram of essential oil of Mentha viridis.*

4. Conclusion

The essential oil of *M. viridis* L. obtained by hydrodistillation and their antimicrobial activity was tested by disc diffusion method. The medicinally important constituents are the essential oils, which contain about 1.75% of the leaves. The major components of essential oils are Carvone and Limonene. Therefore, antibacterial activity may likely to be associated with a high concentration of methyl acetate. Finally, it can be concluded that the active chemical compounds present in *M. viridis* L. should certainly find a place in the treatment of various bacterial infections. The results showed that present study are very stimulating and indicate that this herb should be studied to explore its potential activity in the treatment of infectious diseases as well. It was established that the herbs containing the high concentrations of oil inhibited the growth of microorganisms and results were compared with antibiotic Gentamycin commonly used therapeutically and they showed less strong inhibition for Gram negative bacteria and pronounced inhibition for Gram positive bacteria.

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