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-Cardveyl 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
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. Antimicrobial Activity of Reference Drugs Against Standard Microorganisms

### 2.6.1. Antimicrobial 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%), (-)-(Z)-8-p-Menthene-2-yl, acetate, trans (2.59%), Cyclohexanol, 2-methyl - 5- (1 methylethyl) (2.36%), Eucalyptol (2.28%), 3-Hexadecyne (1.82%), Caryophyllene (1.72%), Beta-myrcene (1.43%), Trans-Carveyl acetate (1.37%), (-). Beta-Bourbonone (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 compiled 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|
### 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-Menth- 2-yl, acetate, trans | 13.034 | 1416968  | 2.59    | 196|
| 4   | Cyclohexanol, 2-methyl-5-(1-methyl ethenyl) | 10.305 | 1292867  | 0.07    | 222|
| 5   | Eucalyptol                      | 6.831    | 1247941  | 2.28    | 154|
| 6   | 3-Hexadecyne                    | 10.369   | 996739   | 1.82    | 222|
| 7   | Caryophyllene                   | 14.969   | 944275   | 1.72    | 204|
| 8   | Beta – myrcene                  | 15.136   | 91508    | 0.17    | 204|
| 9   | Alpha – farnesene               | 15.487   | 127863   | 0.23    | 204|
| 10  | Humulene                        | 15.621   | 46076    | 0.08    | 204|
| 11  | Tricycletetra (4.4.0.0(2,8))decane-4-ol | 15.681 | 65147    | 0.12    | 152|
| 12  | 1,6-Cyclodecadiene, 1-methyl-5-methylene 8-(1-methylethenyl), (S-(E), E) | 15.871 | 56572    | 0.10    | 204|
| 13  | Gamma, muurolene                | 16.131   | 482831   | 0.88    | 204|
| 14  | 1,5 – cyclodecadiene, 1,5 – dimethyl – 8-(1 methylthylidene), (E, E) | 16.426 | 100538   | 0.18    | 204|
| 15  | Copeane                         | 16.859   | 48564    | 0.09    | 204|
| 16  | Alpha – farnesene               | 24.244   | 22037    | 0.04    | 204|
| 17  | Phytole                         | 24.852   | 62925    | 0.11    | 296|

| 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|
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| 10  | Humulene                        | 15.621   | 46076    | 0.08    | 204|
| 11  | Tricycletetra (4.4.0.0(2,8))decane-4-ol | 15.681 | 65147    | 0.12    | 152|
| 12  | 1,6-Cyclodecadiene, 1-methyl-5-methylene 8-(1-methylethenyl), (S-(E), E) | 15.871 | 56572    | 0.10    | 204|
| 13  | Gamma, muurolene                | 16.131   | 482831   | 0.88    | 204|
| 14  | 1,5 – cyclodecadiene, 1,5 – dimethyl – 8-(1 methylthylidene), (E, E) | 16.426 | 100538   | 0.18    | 204|
| 15  | Copeane                         | 16.859   | 48564    | 0.09    | 204|
| 16  | Alpha – farnesene               | 24.244   | 22037    | 0.04    | 204|
| 17  | Phytole                         | 24.852   | 62925    | 0.11    | 296|

Table 2. Major Chemical composition of essential oil of M. viridis.
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    | 24252| 0.04  | 136|
| 5   | Gamma – Terpinene                                                       | 7.375    | 20560| 0.04  | 136|
| 6   | Cyclohexene, 1-methyl –4-(1-methyl ethylidene)                          | 8.015    | 9667 | 0.02  | 144|
| 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 – (E)-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    | 31975| 0.06  | 136|
| 12  | Bicycle (4.1.0)hept – 2-ene                                             | 9.662    | 62468| 0.11  | 94 |
| 13  | L. alpha – Terpinol                                                     | 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 ethylene), cis                 | 10.446   | 36957| 0.07  | 152|
| 16  | Butanoic acid, 3-methyl, hexyl ester                                    | 11.198   | 36957| 0.07  | 152|
| 17  | Cyclohexane, 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  | Neodihydroocarvone                                                      | 12.756   | 28278| 0.05  | 154|
| 20  | Cyclohexane, 1-ethyl- 1-methyl-2-(1-methylene)l-4-(1-methyllethylene)    | 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 aureas    | 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 aureas    | 16                    | 15                                          | 13              | 12   |
| Pseudomonas aeruginosa  | 16                    | 14                                          | 12              | 11   |
| Tested fungi used       | 16                    | 14                                          | 12              | 11   |
| 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.
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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