International Journal of Research Studies in Biosciences (IJRSB)
Volume 3, Issue1, January 2015, PP 205-210
ISSN 2349-0357 (Print) & ISSN 2349-0365 (Online)
www.arcjournals.org

Biological Activities of the Methanol Extract of Cultivated Jordanian Fresh and Dried Mint Species (*Mentha Longifolia*)

Nawal H.Al. Bahtiti*

Basic sciences, Engineering Faculty, ASU (private)
Applied Science Private University
Amman-Jordan
nawal_ha@yahoo.com

Abstract: *The essential oil of mint species are widely used in food, pharmaceutical and cosmetic industries, growing throughout the world. Therefore, it is very important to know the chemical characteristics of the fresh and dried oil for economic use and enhanced performance of the end products. This study was carried out to determine mineral content, essential oil chemical composition, antimicrobial and antioxidant activities of essential oil of Jordanian fresh and dried cultivated mint species (*Mentha longifolia*) It is found that the mint oil is a greenish yellow with acid value of 0.83, relative density of 0.912, and a refractive index of 1.451. The effect of the microwave drying method on the content of volatile compounds was estimated. HPLC analysis of the essential oil identified 20 and 16 compounds of the oil from* fresh and dried, *respectively. It was determined that essential oil of* Mentha longifolia *contains pulegone and isomenthone as major components, in both fresh and dried mint leaves. Treatment of the oil exhibited strong antimicrobial activity against Escherichia coli, Bacillus cereus, Candida albicans and Staphylococcus aurous. Antioxidant activities of the oils were the same for both* dry and fresh M. longifolia *against DPPH (IC_{50} = 9 and 10 μg/ml, respectively) and 2-fold and 4-fold higher than shoot extracts (IC_{50} = 20 and 48 μg/ml, respectively). Moreover. In the same way, the capacity to inhibit superoxide anion was very significant for the two oils (0.1 μg/ml for* M. longifolia*).The antioxidant activity of essential oil of mint species lowered DPPH activity compared to ascorbic acid.*

Keywords: Mentha longifolia**,** *essential oil; pulegone, isomenthone; biological activity, DPPH activity*

1. INTRODUCTION

Mint species have been exploited by man for more than two thousand years. *Mentha* species are widely used in conventional medicine, for their antispasmodic, antiseptic and emmenagogue effects [1] moreover, their essential oils are used in chewing gums, alcoholic beverages, cosmetics, perfumes, toothpastes and mouthwashes [2] The plant is mainly used as salad, spice and for tea besides mint herbage used for wool dyeing [3].

Mentha longifolia or wild mint is a fast-growing, perennial herb that has creeps along an underground rootstock. [4] The color of the leaves varies from light and dark green to grey the small flowers of *Mentha longifolia* are crowded into spikes at the tip of the stems. Varying in color from white to mauve, this wild mint flowers throughout the summer months (November to April).. *Mentha longifolia* is known as horse mint because the leaves are usually unpleasantly scented [5] this exotic mint is a very popular herb and is also cultivated commercially for its essential oils that are used medicinally and in confectionary. Cultivated in Jordan since ancient times, its origin has been lost but it has managed to become naturalized throughout the world in a range of different forms [5] Found in most parts of the country and easy to harvest, wild mint is a popular traditional medicine. It is mainly used for respiratory ailments but many other uses have also been recorded. It is mostly the leaves that are used, usually to make a tea that is drunk for coughs, colds, stomach cramps, asthma, flatulence, indigestion and headaches. Externally, wild mint has been used to treat wounds and swollen glands. In her book Traditional healing herbs, [6] .Margaret Roberts mentions the different uses of *Mentha longifolia* and *M. aquatica*, which are delicious in salads and vegetable dishes. She also mentions that *M. longifolia* subsp. *capensis*, with its strong smell rubbed onto the body and bedding, is used to keep mosquitoes away. The present study is aimed at assessing fresh and dry herb quality with respect to its mineral contents

and essential oil components and antimicrobial and antioxidant activities of essential oils of *Mentha longifolia.*

2. MATERIALS AND METHODS

Plant Materials: Plant some cutting purchased mint in containers filled with potting mix enriched with compost. And kept the plants in a sunny spot. Drying leaves were performed in microwave.

Essential oil Extraction: Essential oils of fresh and dried mint herbs were isolated by hydro-distillation for 2.5 the isolated oils were stored in tightly closed vials at +4 ºC until analysis

Mineral content of samples: The plant extracts were digested with a digestion mixture of HNO_3 and H_2O in the ratio of 3: 1. The resulting solution after microwave digestion was filtered through Whatmann filter paper and diluted to 50 ml with Millipore water. A sample blank containing only acid mixture was served as a control. The control and the digested samples were subjected to mineral content analysis by Perkin Elmer 2380 atomic absorption spectrophotometer. Electrode-less discharge lamp (EDL) for selenium and hollow cathode lamps for magnesium, chromium, manganese, iron, cobalt and copper are used as light sources to provide specific wavelength of the elements to be determined. Acetylene gas was used to provide constant thermal energy for atomization process .Argon gas was used as the carrier gas for purging purpose and graphite furnace was used for the analysis of Selenium

2.1. HPLC Analysis

The analytical HPLC system employed consisted of a JASCO high performance liquid chromatography coupled with a diode array detector (MD910 JASCO, Tokyo, Japan). The analytical Data were evaluated using a JASCO data processing system (DP-L910/V). The separation was achieved on a Waters Spherisorb ®5µm ODS2 4.6 ×250mm column (Milford, MA, USA) at ambient temperature. The mobile phase consisted of water with 1%glacial acetic acid (solvent A), water with 6% glacial acetic acid (solvent B), and water-acetonitrile (65:30 v/v) with 5% glacial acetic acid (solvent C). The gradient used was similar to that used for the determination of phenolics in wine [7] with some modifications: 100% A 0–10 min;100% B,10–30min;90% B/10% C,30–50 min;80% B/20% ,50–60 min;70%B/30% C,60–70 min;100% C,70–105 min; 100% A,105–110 min; post time 10 min before next injection. The flow rate was 0.5 mL/min and the injection volume was 20 µL. The monitoring wavelength was 233. The identification of each compound was based on a combination of retention time.

2.1.1. Antimicrobial Activity

Minimum Inhibitory Concentration (MIC) tests: The samples were tested for their antimicrobial testing in vitro by the agar dilution technique. All samples were dissolved in Dimethyl Sulphoxide Solvent (DMSO) for the antimicrobial test and the solutions were sterilized by membrane filtration. Aliquots of samples were diluted with melted typtic Soy agar, tryptone, soytone, sodium chloride and agar to give concentrations of 2000, 1500, 1000, 500, 250, 125, 62.5 and 31.3: g/mL.

The essential oil was tested against microorganisms including *E. coli*, *S. aureus*, and *C. albicans*. Bacterial strains were cultured overnight in Nutrient Broth (NB) at 37°C, with the exception of *C.albicans* (30°C).

2.1.2. Antioxidant activity

DPPH assay

Hydrogen atoms or electrons donation ability of the corresponding oils was measured from the bleaching of purple colored methanol solution of DPPH. This spectrophotometric assay uses stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH) as a reagent [8][9]. Fifty microliter of the oil in methanol was added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. The same procedure was repeated with the ascorbic acid as positive controls. Inhibition free radical DPPH in percent (I%) was calculated in following way: *I%* = (*A*blank - *A*sample/*A*blank) X 100where *A*blank is the absorbance of the control reaction (containing all reagents except the test compound), and *A*sample is the absorbance of the test compound.

3. RESULTS AND DISCUSSION

3.1. Mineral Contents of Samples

Minerals are of critical importance in the diet, even though they comprise only 4–6% of the human body [6]. Their excess or deficiency in organs and tissues leads to diseases**.** It is very important to know the possible influence of metals on pharmacological properties of herbal infusions [10] Mineral analysis of mint species showed that Ca content ranged from 1448 to 1850 mg kg-1, Fe from 87.47 to 210 mg kg-1, Na from 344 to 194 mg kg-1, P from 276 to1340 mg kg-1, K from 1924 to 7300 mg kg-1, Mg from 602 to 305 mg kg-1, Cu from 1.542 to 2.25 mg kg-1, Mn from 11.482 to 350 mg kg-1 and Zn from 2.41 to 1.61 mg kg-1 (Table 2).

Table1. *Chemical composition of (Mentha Longifolia fresh and dry leaves from RI*

No.	Compound	IR	% fresh	% dry
1	α-pipene	0946	1.696	1.650
2	β-Pinene	0980	3.568	3.232
3	α-Terpinolene	1088	0.323	-
4	Menthofuran	1164	0.543	0.422
5	Cyclohexanone, 3-vinyl-3-methyl	1237	2.406	2.213
6	Piperitone oxid	1252	6.777	6.433
7	Borneol, acetate	1282	0.503	0.500
8	Pulegone	1290	19.585	16.601
9	2,3-Dimethylhydro quinone	1398	1.107	1.00
10	Pulespenone	1354	6.462	5.532
11	Piperitenone oxide	1363	5.085	3.427
12	Sesquiterpene hydrocarbons	1380	0.555	0.453
13	β – elemene	1382	0.244	-
14	1, 8- Cineole	1385	4.636	6.971
15	β-Caryophyllene	1390	0.470	0.436
16	β-Cubebene	1454	0.281	-
17	α-Humulene	1468	.563	0.835
18	β-Farnesene	1468	.315	0.230
19	isomenthone	1480	15.058	9.960
20	γ-Cadinene	1524	0.200	-

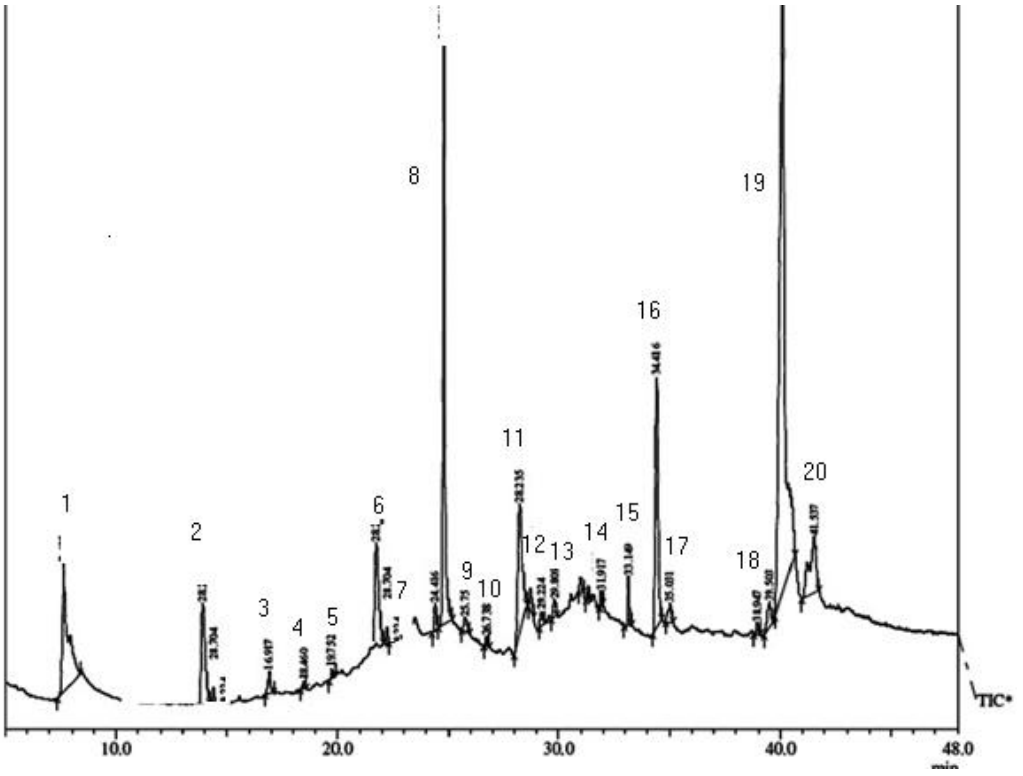

Fig1. *Chromatogram of extracted oil from fresh leaves of Mentha Longifolia*

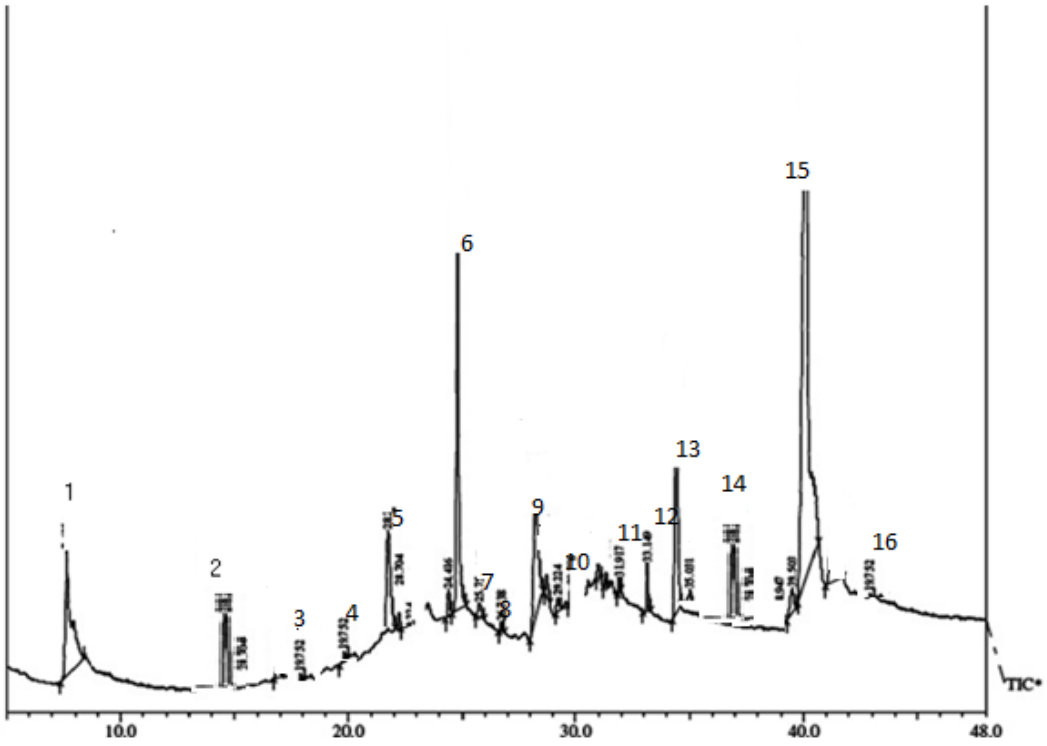

Fig2. *Chromatogram of extracted oil from dried leaves of Mentha Longifolia*

Table2. *Mineral Content of dry and fresh mint*

Serving Size: 100g or 3.5oz			
Element	Amount (mg)		RDI%
	Dry	Fresh	
Calcium	1488	1850	149%
Iron	87.47	210	486%
Magnesium	602	305	150%
Phosphorus	276	1340	28%
Potassium	1924	7300	41%
Sodium	344	194	15%
Zinc	2.41	1.61	16%
Copper	1.542	2.25	77%
Manganese	11.482	350	574%
Selenium	~	-	50%
Fluoride	~	-	1.1%

*Recommended daily intake (RDI)**

The World Health Organization cites maximum permissible levels in raw plant materials for copper as 20 mg kg-1 [10][11]. This study showed that Cu content of mint species was lower than that recommended by World Health Organization [11] Mint tea is widely used as herbal tea; therefore, mineral content of its herbs can meet daily elemental mineral demand of human body when consumed as herbal tea.

3.2. Essential Oil Analysis

Essential oils extracted from leaves of Mentha longifolia L. fresh and dry leaves analysis revealed that M. longifolia was constituted by pulegone (19.60%) as a major component followed by isomenthone (15.058%), Piperitone oxid (6.77%), **Pulespenone** (6.45%), and piperitenone oxide (5.3%).

3.3. Antimicrobial Activity

The antimicrobial activities of *Mentha longifolia* essential oil against microorganisms were examined by the presence or absence of inhibition zones and zone diameter. As shown in Table 3

Table3. *Antimicrobial activity of the essential oil of fresh and dry Mentha*

Test bacteria	**Inhibition zone diameter (**ug/ml**)**	
	Mentha fresh	***Mentha dry***
Escherichia coli (E. coli)	62.5	31.5
Bacillus cereus (B.cereus)	125	62.5
Candida albicans	31.5	31.5
Staphylococcus aurous	62.5	31.5

3.4. Antioxidant Activity

The DPPH radical scavenging method was used to evaluate the antioxidant properties of Mentha longifolia in comparison with those of known natural and synthetic antioxidants, ascorbic acid and . Free radical scavenging capacities of the tested oil rose with increasing oil concentration and oil concentrations providing 50% inhibition (IC50) as shown in Table 4. According to the results obtained from the study, the highest radical scavenging activity was observed in the following order; fresh Mentha longifolia > ascorbic acid > dry Mentha longifolia. The free radical scavenging activity of the two mint species showed that the essential oils of *fresh and dry leaves* is more effective than those of *ascorbic acid* .

Table4. *Antioxidant activity of **DPPH** antioxidants and extracted oil of fresh and dry mint*

Samples	**DPPH IC_{50} (μg/ml)**
Ascorbic Acid	45.43
Fresh mint	60.41
Dry mint	59.50

The most powerful scavenging compounds were reported to be 1, 8- cineole [12] The amounts of Pulegone, isomenthone and 1, 8-cineole in Mentha longifolia used in the present study were **19.585** %, **15.058** % and **4.636** %, respectively (Table 2). Essential oil percentage of menthone, isomenthone and 1,8 cineole of the present study are consistent with those of [12] The highest antioxidant properties of essential oils might be related to its phenolic contents like phenolic acids, as reported before[13]. Therefore, the reason of the poor activity of these essential oils, probably, is due to its lack or low amount of phenolic contents; synergistic or antagonistic effect of its components [14]. It has earlier been reported that plant phenols can behave as ROS (Reactive Oxygen Species) scavengers, metal chelators and enzyme modulators and prevent lipid peroxidation[15] The present study confirmed the antioxidant activity of such mint species

4. CONCLUSION

Mint species are used widely throughout the world as an important medicinal plant. Their oils are one of the most popular and widely used essential oils, mostly because of its main components such as menthol and carvone. The essential oils of mint specie leaves; either fresh or dried showed strong antimicrobial activity against *C. albicans. Microwave drying method was efficient in conserving properties of mint leaves* .The free radical scavenging activity of mint species showed that the essential oil *has* antioxidant activity.

ACKNOWLEDGEMENTS

The author acknowledges Applied Science Private University, Amman, Jordan, for the fully financial support granted of this research article. Sincere thanks to all my Colleagues at basic science department, for creating inspiring conditions for work. The author acknowledges Mr. Mazin Gannam at Pharma International Company for HPLC analysis.

REFERENCES

[1] Aflatuni, A. 2005. The yield and essential oil content of mint (*Mentha* ssp.) in Northern Ostrobothnia. (http://herkules.oulu.fi/ isbn9514277465/), Oulu University Press, Finland, 52 p..

[2] Baytop, T. 1984.Antioxidant properties and composition of aqueous extracts Therapy with medicinal plants in Turkey (Past and Present), No: 255. Istanbul, Publications of Istanbul University.

[3] Burits, M., F. Bucar, 2000. Antioxidant activity of *Nigella sativa* Chem. 51: 4563–4569.

[4] Codd, L.E.W. 1983. Southern African species of Mentha L. (Lamiaceae). *Bothalia* 14: 169-175.

[5] Codd, L.E.W. 1985. *Flora of southern Africa* 28,4 Botanical Research Institute, Pretoria

[6] .Edris, and etal. 2003. Evaluation of a chemotype of spearmint (*Mentha spicata* L.) grown in Siwa Oasis, Egypt. European Food Research and Technology, 218: 74–78

[7] Goldblatt, P. & Manning, J. 2000. *Wild flowers of the fairest Cape.* National Botanical Institute, Cape Town

[8] Mumcu, 2002. Antimicrobial activity and chemical composition of some essential oils. Archives of Pharmacal Research, 25: 860–864

[9] Dorman, H.J.D., M. Kosar, K. Kahlos, Y. Holm, R. Hiltunen, 2003.Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties, and cultivars. Food Chem. 51: 4563–4569.

[10] Hussain, A.I. 2009. Characterization and Biological Activities of Essential Oils of Some Species of Lamiaceae. PhD Thesis, 257p., Faisalabad, Pakistan

[11] Ozcan, M. 2004. Mineral contents of some plants used as condiments in Turkey. Food Chem. 84: 437–440.

[12] Queralt, L., and etal, 2005. Quantitative determination of essential and trace element content of medicinal plants and their infusions by XRF and ICP techniques. X-Ray Spectrom. 34: 213–217

[13] Rodrigo, R., C. Bosco, 2006. Oxidative stress and protective effects of polyphenols: Comparative studies in human and rodent kidney. A review. Comparative Biochem. Physiol. Part C, 142:317–327.

[14] WHO (World Health Organization), 2005. Quality control methods for medicinal plant materials

[15] Van Wyk, B. & Gericke, N. 2000. *People's plants*. Briza Publications, Pretoria.