Kurdistan Journal of Applied Research (KJAR) | Print-ISSN: 2411-7684 – Electronic-ISSN: 2411-7706 | kjar.spu.edu.iq
Volume 2 | Issue 1 | June 2017 | DOI: 10.24017/science.2017.1.11

Chemical composition and antifungal activity of *Mentha spicata* L. plant from Sulaimaniyah in Iraq

Lana MA. Mohammed
Sulaimani Polytechnic University
Technical college of Health,
Medical Laboratory Department,
lana.amin@spu.edu.iq

Tara Faeq M. Salah
Medical analysis Department,
Sulaimani Polytechnic University,
Sulaimani, Iraq
tara.faeq@spu.edu.iq

Karzan O. Qader
Biology Department,
Sulaimani Polytechnic University,
Sulaimani, Iraq
karzan.omer@univsul.edu,iq

Abstract: Aerial parts of Mentha plants were collected from Sulaimaniyah city, during 15th Mar to 1st July 2016. Clevenger apparatus was used for plant sample distillation and then obtained essential oils were analyzed by GC and GC/MS. The GC–MS analysis resulted in the detection of thirty eight compounds which made up 100% of total plant essential oil. The major components of the oil were (54.44%) of 2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (R)-, (27.72%) of D-Limonene, (3.29%) of 2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, trans-, (2.16%) of Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.83%) of Bicyclo[3.1.0] hexan-2-ol,2-methyl-5-(1-methylethyl)-(1.alpha.,2.beta.,5.alpha.) and (1.03%) of 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-. The significant inhibition percentages of Mentha spicata plants extract on the Aspergillus niger mycelial growth were 16.78%, 18.92%, 23.21%, 28.57% and 36.78%, in 32%, 34%, 36%, 38% and 40% dilution respectively, while other concentrations showed no significant effect.

Keywords : *Mentha spicata* L; essential oil; antimicrobial activity; *Aspergillus niger*.

1. INTRODUCTION

Aspergillus niger is a worldwide distributed filamentous ascomycete fungi that is considered as one of the most common spices of genus *Aspergillus*. *A. niger* lives as saprophyte in many various environments. It was isolated from numerous habitats such as soil, water, dead plant, stored grain, compost piles decayed vegetables and fruits. It causes many diseases on plant because of its ability to produce hydrolytic and oxidative enzymes which could involve in breakdown of plant lignocellulos [24]. It is known as a main causative agent of black mold and rot disease on different fruits and vegetables also commonly used in food industry because of production of extracellular organic acids materials [24, 23, 22, 27]. *A. niger* has been tagged with the generally recognized as safe status from the US Food and Drug Administration [25]. It is considered as important fungi that has role in the global carbon cycle [3]. Moreover, *A. niger* can be considered as an opportunistic infectious agent of different human diseases. It causes severe lung disorder i.e. aspergillosis if sufficient amount of its spore inhaled by human. It has ability to produce certain mycotoxin which can cause several problems of liver, kidney, skin, muscle, nervous system, digestive tract, respiratory organs, genital organs etc [28]. Moreover, *Aspergillus niger* can produce ochratoxin A and fumonisin B2 and aflatoxins [20, 1].

A plant that is known as a strong antifungal medical plant is *Mentha spicata* L. (Spearmint). It is known as a member of Lamiaceae family. Spearmint is a type of the plant that grows in the moist areas without production of seed and increased only by vegetative methods which is rhizomes [18]. It is herbaceous and perennial aromatic herbs which essential oil of this plant used in different industries such as cosmetics, fragrances, flavors, aromatherapy, phototherapy, nutrition and spices [11]. The medical activity of Mentha species L. (Spearmint) is related to the presence of the two main compound groups: phenolic and essential oil compounds. The main phenolics component of Spearmint includes derivatives of caffeic acid and glycosidic forms of the flavonoids luteolin, apigenin, eriodictyol and naringenin [15]. *Mentha spicata* is characterized by a high carvone and limonene, however the carvone content of *Mentha cardiaca* is the same but it has higher limonene content [12]. It has been shown that Mint oil (oil obtained from Mentha spicata) exhibits antifungal activity against different pathogenic plant fungi such as Alternaria alternata, Aspergillus niger and Fusarium sp. by using agar well diffusion method [2]. Spearmint was taken as a tea to treat general digestive and used in commercially manufactured product, cooking and medicine [16]. Moreover, two species of spearmint plants were diagnosed and it has been reported that their essential oil

have strong antimicrobial activity [8]. Also studies of [26] on various antifungal properties of essential oil of *Mentha spicata* showed that mentha essential oil has fungi toxic effect.

This study evaluated and determined the essential oil composition of *Mentha spicata* plant. Also antifungal activity of the *Mentha spicata* plant has been studied against growth of *Aspergillus niger* fungi.

2. MATERIALS AND METHODS

2.1. Isolation and identification of the fungi

Black conidia spores of *Aspergillus niger* on infected onion bulbs were collected and transferred to potato dextrose agar plates in the medical laboratory in Sulaimaniyah city. Later, PDA plates were incubated with incubation period 5-6 days, at 25˚C ± 2˚C for complete fungal growth. For purification of *Aspergillus niger* method of single spore and morphological characteristics were used. Identification was done based on standard keys [4, 19].

2.2. Preparation of Plant Materials

Young leaves and branches of Mentha plants were collected from cultivated plant in Sulaimaniyah city 15th Mar to 1st July 2016. Dehydration and milling have been done for all collected parts of plants and then the plant samples were kept at appropriate position considering temperature and light until the next step. Later, essential oil was taken from 100 g of the milled sample in hydro distillation method with the help of Clevenger set in 500 mL of distal water, kept at 4 °C until use [18].

2.3. Effect of plant extract on radial growth of *Aspergillus niger*:

Agar dilution technic was performed for detecting the inhibitory effect of different concentration of Mentha plant extract on radial growth of *Aspergillus niger*. Twenty different concentrations of Mentha extract were added to PDA medium, that include (2%, 4%, 6% 8%, 10%, 12%, 14%, 16%, 18%, 20%, 22%, 24%, 26%, 28%, 30%, 32%, 34%, 36%, 38%, 40%) each treatment was replicated 3 times. On the center of each Petri plate 1 centimeter diameter of fungal block from 4-day-old colony of *Aspergillus niger* was inoculated and incubated at 25˚C ± 2˚C. The measuring of fungal growth diameter of fungal growth colony was performed after 4 days of inoculation. Three replicates were used within each treatment. The effect of Mentha plant extract on fungal growth was calculated by using the following formula [21].

[Growth inhibition% = [(growth in control – growth in sample)/growth in control] × 100].

2.4. Gas chromatography

GCMS was done at GC-MS lab, college of agriculture university of Basra. One µl of the essential oils was injected into the GC-MS instrument using a micro syringe and the scanning was done for 20 min. GC Program was (GCMS-QP2010 Ultra, Ion Source Temp: 200.00 °C , Interface Temp. : 280.00 °C, Solvent Cut Time :3.00 min, Detector Gain Mode :Relative, Detector Gain :0.70 kV +0.10 kV, Start Time :3.00min, End Time :19.00min, ACQ Mode :Scan, Event Time :0.50sec, Scan Speed :1666 , Start m/z :50.00, End m/z :800.00). After separation of oil compounds, they extracted from the column and followed by the process of detection by a detector with consequence of electronic signal creation after any detection. The size of the signal was depended on the concentration of the samples.

2.5. Statistical Analysis

The effect of Mentha plant extract with different concentration on radial growth of *Aspergillus niger* assessed by a one way analysis of variance (ANOVA). Mean difference between Mentha plant extract was separated by Fisher's [7] test significant difference (LSD) at 1% significant probability level.

3. RESULTS

The GC–MS analysis of the essential oil of *M. spicata* L. resulted in the detection of thirty eight compounds which made up of 100% of total plant essential oil. The major compounds were; (54.44%) of 2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (R)-, (27.72%) of D-Limonene, (3.29%) of 2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, trans-, (2.16%) of Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.83%) of Bicyclo[3.1.0] hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.) and (1.03%) of 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-. See the retention times and chemical composition of essential oils of Mentha spicata in (Table 1).

This research also investigated the antifungal activity of *Menth spicata* extract against *Aspergillus niger* growth activity. It was found that the Mentha plant extrac dilutiont has significant inhibitory effect on *Aspergillus niger* mycelial growth when compared with control in 32%, 34%, 36%, 38% and 40% dilution with average inhibitory growth percentage 16.78%, 18.92%, 23.21%, 28.57% and 36.78% respectively (Figure 1).

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	4.767	386449	0.41	.alpha.-Pinene
2	5.274	468651	0.50	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-
3	5.333	666776	0.71	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-
4	5.485	615211	0.65	.beta.-Myrcene
5	5.583	421585	0.45	Hydroxysecobarbital glucuronide 1,3-di-methyl deriv. methyl este, tris(O-trimethylsily
6	5.820	152396	0.16	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-
7	5.985	26041889	27.72	D-Limonene
8	6.167	90973	0.10	2-Oxatrispiro[4.0.5.2.5.0]nonadec-3-ene-1,12-dione, 13-(Z)-(4-propionyloxybenzoyln
9	6.311	317664	0.34	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-
10	6.457	1717860	1.83	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-
11	6.627	101655	0.11	Cyclohexene, 1-methyl-3-(1-methylethenyl)-, (+/-)-
12	6.813	324690	0.35	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-, [1R-(1.alpha.,2.beta.,5.alpha.)]-
13	7.042	165776	0.18	trans-p-Mentha-2,8-dienol
14	7.199	134125	0.14	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, trans-
15	7.566	115389	0.12	2,2'-Bis[4-(4-nitrobenzylideneamino)phenyl]-5,6'-bibenzimidazole
16	7.670	966659	1.03	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-
17	7.858	2028893	2.16	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-
18	8.101	3086245	3.29	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, trans-
19	8.251	699717	0.74	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-
20	8.365	51142529	54.44	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (R)-
21	8.434	464046	0.49	Cobalt, (2-bromo- eta.-5-indenyl)-bis(trisopropylphosphite-P-)
22	8.631	109257	0.12	11-Tridecyn-1-ol
23	9.054	81439	0.09	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, acetate, (1.alpha.,2.beta.,5.alpha.)-
24	9.127	85275	0.09	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, acetate, cis-
25	9.236	106129	0.11	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene)-
26	9.370	194759	0.21	trans-Carveyl acetate
27	9.645	848248	0.90	Cyclobuta[1,2:3,4]dicyclopentene, decahydro-3a-methyl-6-methylene-1-(1-methylethy
28	9.972	732087	0.78	Caryophyllene
29	10.052	87660	0.09	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-m
30	10.179	127158	0.14	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (
31	10.498	501640	0.53	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-m
32	10.758	94090	0.10	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (
33	10.819	86829	0.09	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-
34	11.041	114173	0.12	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R
35	11.599	112561	0.12	Cubenol
36	11.801	297522	0.32	.tau.-Cadinol
37	11.908	104814	0.11	.alpha.-Cadinol
38	11.956	145455	0.15	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.a
		93938274	100.00	

Table 1. Chemical composition of essential oils of Mentha spicata from Sulaimaniyah city.

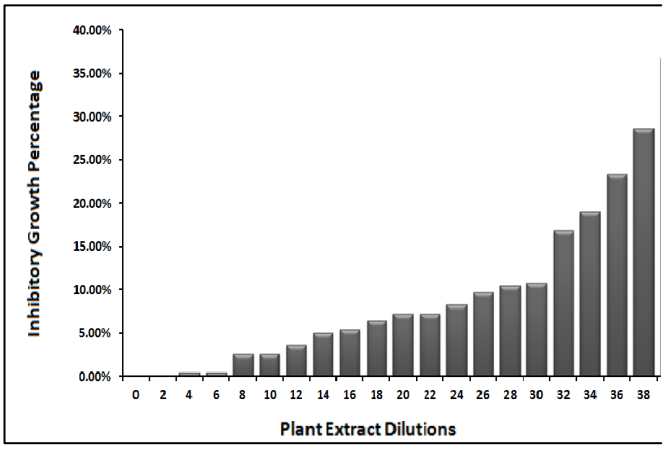

Figure 1; Antifungal activity of *Mentha spicata* extract with different dilutions on *Aspergillus niger* growth.

4. DISCUSSION

Several studies have been reported on *Mentha spicata* essential oil composition around the world. While by far there are no reports about *Mentha spicata* essential oil composition in Iraq specifically in Sulaimaniyah city. The GC–MS analysis of the essential oil of *Mentha spicata* L. resulted in the detection of thirty eight compounds which made up of 100% of total plant essential oil (Table 1). The major components of the oil were (54.44%) of 2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (R)-, (27.72%) of D-Limonene, (3.29%) of 2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, trans-, (2.16%) of Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.83%) of Bicyclo[3.1.0] hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.) and (1.03%) of 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-. The dominant chemo types of *Mentha spicata* that found in Greece were linalool, carvone/dihydrocarvone, piperitone oxide/piperitetone oxide, and menthone/isomenthone/ pulegone, respectively [14]. While, the major components of *Mentha spicata* that found in Bejaia location (Algeria) were carvone, limonene, and 1,8-cineole [5]. The chemical composition showed that the plant leaves and branches in Sulaimaniyah had composition different to those of other *Mentha spicata* essential oils analyzed from Bejaia location (Algeria) and Greece.

The inhibition percentages of *Mentha spicata* plants extract on the *A. niger* mycelial growth was shown in Figure 1. The percentage of mycelium growth inhibition were 16.78%, 18.92%, 23.21%, 28.57% and 36.78% in 32%, 34%, 36%, 38% and 40% dilution respectively. It is known that the antifungal effect of plant extract depends on plant species and its chemical components. It has been considered that there is a bond between the chemicals in plant essential oil and the antimicrobial activity [6]. Several published researches have been reported the antimicrobial activity of the *Mentha spicata's* essential oils against Gram positive and Gram negative bacterial, yeast, and fungal strains. *Menthe spicata's* essential oil was used as an antimicrobial agent against *Aspergillus flavus* NRRL 391 and *Candida albicans* ATCC 1024. The result showed inhibition in mycilial growth with zone diameters > 40 mm [17]. According to another study on antifungal effect of menthe sp. essential oil against several plant pathogrnic fungi, *Aspergillus niger* showed more sensitivity than other tested fungi. Furthermore, the diameter of growth inhibition obtained for *Mentha spicata* essential oil against *Aspergillus ochraceus* NRRL 3174 was 43 mm [9]. It has been revealed that *Mentha spicata* showed positive antimicrobial effect on bacterial and fungal strains [9, 13]. Moreover, *Mentha spicata* essential oil showed high antimicrobial activity against *Candida albicans* strain.

5. CONCLUSION

The study of growth inhibitory effect of Mentha spicata extract with different dilutions has given encouraging results, showed their potential use in the management of various plant and human diseases that caused by *Aspergillus niger*. In addition, this study showed the major chemical composition of young leaves and branches of essential oils from *Mentha spicata* plant in Sulaimaniyah city. The essential oil that obtained from *Mentha spicata* plant was characterized by GC-MS and 38 volatile compounds were identified which made up 100% of the total essential oil.

6. REFERENCES

[1]. AHA. Al-Abdalall, "Production of aflatoxins by Aspergillus flavus and Aspergillus niger strains isolated from seeds of pulses," Journal of Food Agriculture and Environment, 7 33-39, 2009.

[2]. F. Aqil, AZ. Beng, I. Ahmad, "In vitro toxicity of plant essential oils against soil fungi," J. Med. Arom. Pl. Sci. 23:177-181, 2000.

[3]. S. Baker, "Aspergillus niger genomics: Past, present and into the future," Medical Mycology 44 17-21, 2006.

[4]. C. Booth, "The Genus Fusarium. Common wealth Mycological Institute," Kew, Surrey, 237, 1971.

[5]. F. Brahmi, A. Adjaoud, B. Marongiu, D. Falconieri, , D. Yalaoui-Guellal, , K. Madani, and M. Chibane, "Chemical and biological profiles of essential oils fromMentha spicataL. leaf from Bejaia in Algeria," Journal of Essential Oil Research, 28, 3 2016, 211-220, 2016.

[6]. K. Deans, Svoboda, "The antimicrobial properties of marjoram (Oroganum majorana L.) volatile oil," Flavour Fragr.J., 1990.5:187-190, 1990.

[7]. A. Fisher, F.R Immer, and O. Tedin, "The Genetical Interpretation of Statistic of the Third Degree of Study of Quantitative Inheritance" Genetics, 17, 107-114, 1932.

[8]. H. Hafedh, T. Najla, N. Emira, S. Mejdi, F. Hanen, K. Riadh, B. Amina, "Biological activities of the essential oils and methanol extract of tow cultivated mint species (*Mentha longifolia* and *Mentha pulegium*) used in the Tunisian folkloric medicine," World Journal of Microbiology and Biotechnology, vol 25, 12, pp 2227–2238, 2009.

[9]. F. Anwar Hussain, , P.S. Nigam, M. Ashraf and A.H. Gilani, "Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four Mentha species," J Sci Food Agric., 90, 1827–1836, 2010.

[10]. J. Bouajila kaddem, M. Ennajar, A. Lebrihi, F. Mathieu and M. Romdhane, "Chemical composition and antimicrobial and antioxidant activities of Mentha (longifolia L. and viridis) essential oils," J. Food. Sci., 74, 358–363, 2009.

[11]. k. Kapp, "Polyphenolic and essential oil composition of Mentha and their antimicrobial effect," Helsinki, 2342---317X, 2015.

[12]. M. Kazemi, H. Rostami, S. Shafiei, "Antibacterial and Antifungal Activity of some Medicinal Plants from Iran," J. Plant Sci. 7(2):55-66, 2012.

[13]. S. Kizil, N. Hasimi, V. Tolan, E. Kilinc and U. Yuksel, "Mineral content, essential oil components and biological activity of two Mentha species (M. piperita L., M. spicata L.)," Turk. J. Field Crops, 15(2), 148–153,2010.

[14]. A. Kofidis, Bosabalidis and S. Kokkini, "Seasonal variation of essential oils in a linalool-rich chemotype of Mentha spicata grown wild in Greece," J. Essent Oil Res. Chowdhury, 16, 469–472 .19. J.U. 2004.

[15]. P. Kumar, , Mishra, S., Malik, A. and Satya, S., "Insecticidal properties of Mentha species: A review," Ind. Crop. Prod., 34(1): 802-817, 2011.

[16]. AY. Leung, "Encyclopedia of Common Natural Ingredients used in food, drugs and cosmetics," New York: John Wiley & Sons. p.231. Linnaeus C (1753). Species Plantarum. 2:576-577, 1980.

[17]. M. Mkaddem, J. Bouajila, M. Ennajar, A. Lebrihi, F. Mathieu and M. Romdhane, "Chemical composition and antimicrobial and antioxidant activities of Mentha (longifolia L. and viridis) essential oils," J. Food. Sci., 74, 358–363, 2009.

[18]. M. Moghtader, H. Salari, and A. Farahmand, "Evaluation of the antifungal effects of rosemary oil and comparison with synthetic borneol and fungicide on the growth of Aspergillus flavus," Journal of Ecology and the Natural Environment Vol. 3(6), pp. 210-214, 2011.

[19]. P.E. Nelson, T.A. Toussoun and W.F.O. Marasas, "Fusarium Species. In: An Illustrated Manual for Identification," Pennsylvania State University Press, University Park, 193, 1983.

[20]. P. Noonimabc, W. Mahakarnchanakulb, KF. Nielsend, JC. Frisvadd and RA. Samsona, "Fumonisin B2 production by Aspergillus niger in Thai coffee beans," Food Addit. Contam., 26 94-100, 2009.

[21]. D.K. Pandey, N.N. Tripathi, R.D. Tripathi and S.N. Dixit, "Fungitoxic and Phytotoxic properties of the essential oil of H. suaveolenszeitschrift fur pflanzenkraneaten and pflanzenschutz, 89: 344-349, 1982.

[22]. JR. Perfect, GM. Cox, JY. Lee, SW Chapman and CA. Kauffman, "The impact of culture isolation of Aspergillus species: A hospital-based survey of aspergillosis," Clinical Infectious Diseases 33 1824-1833, 2001.

[23]. G. Perrone, A. Susca, G. Cozzi K. Ehrlich and J.Vargas, "Biodiversity of Aspergillus species in some important agricultural products," Students Mycology 59 53-66, 2007.

[24]. JI. Pitt and AD. Hocking, Fungi and Food Spoilage. 2nd Edn., Springer, New York, 593, 1997.

[25]. KA. Powell, A. Renwick and JF. Peberdy, "The genus Aspergillus, from taxonomy and genetics to

industrial application," Plenum Press, New York, 1994.

[26]. k. Sandeep Ramesh, D. Anupam, "Antifungal properties of essential oil Mentha spicata l," var. MSS-5. Indian J.Crop Science. 1:197-200, 2006.

[27]. R. Sharma, "Pathogenicity of aspergillus niger in plants," Cibtech Journal of Microbiology 2319-3867, 2012.

[28]. MW. Truckesses and PM. Scott, "Mycotoxins in botanicals and dried fruit," A review. Food Addition Contamination 25 181-192, 2008.