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EFFECT OF DRYING ON CHEMICAL COMPONENTS AND ANTIOXIDANT OF ESSENTIAL OIL FROM *MENTHA SPICATA* LEAVES

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ABSTRACT
Mint (*Mentha spicata L.*) is an aromatic member of Lamiaceae family and considered one of the most important herbs traditionally produced in Sudan. Mint was chosen for this study because of its common use in traditional medicine. The aim of the present study was to extract, identify the chemical constituents and antioxidant of *Mentha spicata* essential oil. The fresh leaves of mint were collected from Omdurman vegetable market and divided into three groups. Group A fresh sample, group B dried under shade and group C dried under sun. The fresh leaves (group A) after the collection immediately brought to the lab. The essential oils from the leaves of mint were obtained by hydrodistillation and analyzed by GC/MS. 24, 23 and 20 components were identified for sample A, B and C respectively. The antioxidant activity was evaluated by 2, 2 Di (4 -tert -octyl phenyl) - 1 - picryl – hydrazyl (DPPH) free radical. The essential oils exhibited a DPPH scavenging activity of 70%, 60%, 60% and 84% for sample A, B, C and the standard respectively.

KEYWORDS: Fresh leaves, shade-dried, sun-dried, mint.

INTRODUCTION
Medicinal and aromatic plants (MAPs) produced and offered a wide variety of products, from crude materials to processed and packaged products like pharmaceuticals, herbal remedies, dietary supplements, varnishes and insecticides (Ohrmann 1991, Gorecki 2002, Lange 1996). An estimated number of 70, 000 plant species are used in folk medicine world wide (Farnsworth and Soejarto 1991). As a consequence, there is an enormous demand in botanicals for domestic use and for commercial trade resulting in a huge trade on local, regional, national and international level (Bhattarai 1997; Lange 1998; Lange 2002; Kupe *et al*., 2000; Kathe *et al*., 2003).

Traditional medicines are used widely through out the world. As the name implies, these treatments are a part of the traditions of each country that have been handed down from generation to generation. Acceptance of traditional medicines by a population is largely conditioned by cultural factors. Acknowledging the potential value of traditional medicine for the expansion of health service, the World Health Assembly (WHA) passed a number of resolutions in 1976 to draw attention to the potential reserve constituted by traditional medicine. In 1977, WHA urged countries to utilize their traditional system of medicine.

In 1979, WHA called for a comprehensive approach to the subject of medicinal plants. Medicinal plants are the backbone of traditional medicine. In his struggle against disease, man has used plants as medicinal agents. The discovery and application of antibiotics infectious disease led to the development of the pharmaceutical industry in the second half of the last century that done much to combat disease in man.

Most herbal medicines of current interest originated from the ancient civilization of Africa, Asian subcontinent, north, central and south American (Phillipson, 2001). Approximately 3,000 species are used by an estimated 200,000 indigenous traditional healers.

Sudan is the largest country in Africa with a diverse flora. Most of the Sudanese people in rural areas rely on traditional medicine for the treatment of many infectious diseases. Sudanese traditional medicine is characterized by a unique combination of knowledge and practices of Arabic, Islamic and African culture (Elhamidi 1970, El kamali and Elkhalifa 1997).

Infectious diseases are the world's leading cause of premature deaths (Emori and Gaynes 1993), there fore, there is continuous and urgent need to discover new antimicrobial compounds with diverse chemical

structures and novel mechanisms of action. On the other hand, viral infectious are very common and responsible for a variety of infectious disease ranging from the common cold to uniform fatal rabies and AIDS. In contrast to the enormous amount of antimicrobial drugs, very few effective antiviral drugs are available. Mint was first described by Linnaeus (1753) from specimens that had been collected in England; he treated it as spices, but it is now universally agreed to be a hybrid (Harley, 1975). Traditionally, mint is used for both culinary as well as medicinal purposes. Mint leaves, fresh or dried, are used in teas, beverages, Jellies, syrups, candies and ice creams. Mint essential oil and menthol are extensively used as flavorings in breath fresheners, drinks, antiseptic mouth rinses, tooth paste, chewing gum, desserts and candies, such as mint (candy) and mint chocolate.

MATERIALS AND METHODS

Plant material

Long leaves of mint were collected from Omdurman city "vegetable market". The leaves samples were divided into three groups: fresh (A), dried under shade (B) and dried under sun (C). Sample (A) was sent directly without delay to the National center for Research in Khartoum for analysis.

Extraction of mint oil

The method described by Sukhdev *et al*., (2008) was adopted for extraction: 600, 200 and 200g from the samples, A,B,C respectively were placed separately in 2000 ml round bottom capacity flasks, and 1000ml of distilled water was added to each flask using a Clevenger type apparatus. The system was heated to 100°C for about four hours until the volume of the oil above the water layer in the receiver became constant. The oil was pipetted, dried over anhydrous sodium sulphate and stored in a dark container at 4°C in a refrigerator till used.

Evaluation of antioxidant activity of the extracted oils

The effect of extracted oils on 2.2-Di (4 - tert-octylphenyl) - 1- picryl - hydrazyl (DPPH) were assayed using the method of Shimada *et al*., (1992), with some modification. The test samples were dissolved in Dimethyl sulfoxide (DMSO) while DPPH, was prepared in ethanol. In 96- well plate, the test samples were allowed to react with DPPH free radical for half an hour at 37°C.

After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated as control group. All test and analysis were run in triplicate.

Gas Chromatography / Mass Spectrometry (GC/MS) analysis

GC/MS analysis was carried out with a Fisons Instrument GC2010, equipped with mass selective detector and quad rupole analyzer (FTD). The injection mode is split, and column oven temperature 35.0 ° C. The flow control mode by pressure 61.8 kpa, column flow 1.20 ml/min. Helium was used as a carrier gas at a linear velocity 39.4 cm/sec. the injection temperature was 250°C. Data acquisition was performed with Mass Lab Software for the mass range 30-600 u with a scan speed of 833scan/sec. The identification of compounds was performed by comparing their mass spectra with data mass spectra library spectra. The identification of compounds was also based on the kovats retention indices.

RESULTS AND DISCUSSION

Percentage yield of the essential oils

The results of the hydrodistillation of the leaves of the three samples A, B and C are summarized in table (1). The low yield of sample A is due to high content of water in fresh sample but the low yield of sample C is due to evaporation of some compounds by sun compared to sample B which have been dried under shade.

Table 1: Percentage of essential oils yield.

Sample	Weight of the samples(g)	Volume of the extracted oils (ml)	%Yield(v/w)
A	600	3.6	0.6
B	200	5.9	2.95
C	200	4.6	2.3

Where A: fresh leaves, B: dried under shade and C: dried under sun.

DPPH scavenging activity

The percentage of DPPH radical scavenging activity of essential oils were shown in table (2). The essential oil of sample A exhibited DPPH scavenging activity of 70%, the essential oil of samples B and C exhibited DPPH scavenging activity of 60% where as for Propyl Gallate (standard) was found to be 84%. The essential oil of sample A was found to be more effective than sample B and C essential oils.

Table 2: Effect of essential oils on DPPH assay.

Sample	% of activity ± SD
A	70 ± 0.01
B	60 ± 0.06
C	60 ± 0.21
PG	84 ± 0.02

SD: Standard deviation.
DPPH: 2,2 Di (4 -tert -octyl phenyl) - 1 - picryl - hydrazyl.
PG: Propyl Gallate.

GC-MS analysis for sample A

Table (3) shows the compounds identified in the fresh leaves essential oil of Mint in order of elution. 24 different components were identified, representing 99.27% of the compounds in the oil.

Table (3): Compounds identified for sample A determined by GC-MS.

Peak	R. Time	Area %	Name of compound
1	10.862	0.70	Alpha-Pinene, Bicyclo [3.1.1] hept-2-ene, 2.6.6- trimethyl.
2	12.234	0.49	Beta-phellandrene, Cyclohexene, 3-methylene -6- (1-methylethyl)
3	12.323	0.89	Bicycle [3.1.1] heptane, 6, 6-dimethyl -2-methlene
4	12.846	2.21	Beta –myrcene, 1,6- Octadiene, 7-methyl -3-methlene.
5	13.098	0.28	3-Octanol, n-Octan-3-01, Ethylamycarbinol.
6	14.102	18.15	1- Limonene, Cyclohexene, 1-methyl -4- (1-methyl ethenyl), p-Mentha- 1,8 diene.
7	14.168	1.91	Eucalyptol
8	17.094	0.28	3- Octanol, acetate
9	19.423	2.83	Dihydrocarvone, Cyclohexanone, 2-methyl -5- (1-methyl ethenyl)
10	20.201	0.84	2- Cyclohexen 1-01,2 – methyl -5-(1- methyl ethenyl)
11	20.881	63.26	2- Cyclohexen -1-one, 2-methyl -5-(1-methyl ethenyl), 2- methyl -5-ispropenyl -2-Cyclohexenone.
12	21.067	0.26	Pentaborane, Boronhydride
13	21.787	0.10	Carvone oxide, Cis-4- Ispropenyl -1- methyl-7-oxabicyclo [4.1.0] heptan-2-one.
14	22.043	0.24	Dihydroedulan II, 2H-1-Benzopyran,3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl.
15	23.078	1.60	Dihydrocarvyl acetate, cyclohexanol, 2-methyl-5-(1-methyl ethenyl), acetate
16	23.325	0.06	2-Cyclohexen-1-01,2-methyl-5-(1-methyl ethenyl), acetate, Cis-p-Mentha-6,8-dien-2-01, acetate
17	24.019	2.47	2-Cyclohexen-1.01,2-methyl-5-(1- methyl ethenyl), acetate,(IR-trans)
18	24.674	0.85	Beta – Bourbonene
19	25.599	0.98	Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-,ethylene.
20	26.326	0.41	Cyclohexanol,2-methyl-3-(1- methyl ethenyl), acetate.
21	27.167	0.48	1,6-Cyclodecadiene, 1-methyl-5-methylene-8- (1- methyl ethenyl).
22	40.350	0.29	2-Hexadecen-1-01,3,7,11,15-tetra methyl, phytol.
23	40.519	0.22	13(16), 14-Labdien-8-01
24	50.556	0.19	Tetratetracontane

GC-MS analysis for sample B

Table (4) shows the compounds identified in the leaves (dried under shade) essential oil of Mint in order of elution. 25 different components were identified, representing 100.00% of the compounds in the oil.

Table (4): Compounds identified for sample B determined by GC-MS.

Peak	R. Time	Area %	Name of compound
1	10.355	0.29	Beta- pinene, Bicyclo [3.1.1] heptane, 6,6-dimethyl-2-methylene
2	10.869	1.42	Alpha-pinene, Bicyclo [3.1.1] hept-2-ene,2,6,6-trimethyl
3	12.251	0.63	Beta-phellandrene, Cyclohexene, 3-methylene-6-(1- methyl ethenyl)
4	12.336	1.41	Bicyclo [3.1.1] heptane, 6,6-dimethyl-2-methylene.
5	12.864	3.64	Beta-Myrcene, 1,6-Octadiene,7-methyl-3-methylene.
6	13.113	0.41	3-Octanol, n-Octan-3-01, Ethylamyl carbinol
7	14.128	25.83	dI-limonene- Cyclohexen, 1-methyl-4-(1- methyl ethenyl), 1-P-metha-1,8-diene.
8	14.191	2.59	Eucalyptol
9	17.098	0.43	3-Octanol, acetate
10	19.430	3.58	Cyclohexanone, 2-methyl-5-(1- methyl ethenyl), p-menth-8-en-2-one.
11	20.246	0.82	2-Cyclohexen-1-01,2-methyl-5-(1- methyl ethenyl)
12	20.653	1.24	Cis-Carveol
13	20.881	47.25	2-Cyclohexen-1-one,2-methyl-5-(1-methyl), 2-methyl-5-isopropenyl-2-Cyclohexenone, Carvol.
14	21.068	0.21	Hexaborane-12, pentaborane, borohydride
15	22.049	0.43	Dihydroedulan II, 2H-Benzopy rane, 3,4,4a,5,6,8a- hexahydro- 2,5,5,8a-tetramethyl.
16	23.081	2.12	Dihydrocarvyl acetate, Cyclohexanol, 2-methyl-5-(1- methyl ethenyl), acetate.
17	23.326	0.08	2-Cyclohexen-1-01,2-methyl-5(1-methyl), acetate, (IR-Cis)
18	24.022	2.90	2-Cyclohexen-1-01,2-methyl-5-(1- methyl ethenyl),acetate, menthe-6,8-dien-2-01
19	24.676	1.37	Beta-Bourbonene
20	25.599	1.51	Bicyclo [7.2.0] undec-4-ene,4,11,11-trimethyl-8-methylene
21	26.214	0.13	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1- methyl ethenyl).
22	26.326	0.70	7-Oxabicyclo [4.1.0] heptane, 1-methyl-4-(2-methyl Oxiranyl).
23	27.166	0.53	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1- methyl ethenyl).

GC-MS analysis for sample C

Table (5) shows the compounds identified in the leaves (dried under sun) essential oil of Mint in order of elution. 20 different components were identified, representing 99.82% of the compounds in the oil.

Table (5): Compounds identified for C sample determined by GC-MS.

Peak	R. Time	Area %	Name of compound
1	10.861	0.70	Alpha-pinene, Bicyclo [3.1.1] hept-2-ene, 2,6,6-trimethyl.
2	12.233	0.35	Beta-phellandrene, Cyclohexene, 3-methylene-6-(1- methyl ethenyl)
3	12.322	0.79	Bicyclo [3.1.1] heptane, 6,6-dimethyl-2-methylene.
4	12.844	1.79	Beta-myrcene, 1,6-Octadiene, 7-methyl-3-methylene.
5	13.096	0.18	3-Octanol, n-octan-3-01, Ethylamyl carbinol
6	14.096	15.28	1-limonene, Cyclohexene, 1-methyl-4-(1- methyl ethenyl), p-mentha-1,8-diene.
7	14.163	1.33	Eucalyptol
8	19.419	3.71	Carveol, dihydro, Cis-Cyclohexan 01,2-methyl-5-(1- methyl ethenyl)
9	19.635	0.29	Dihydro carvone, Cyclohexanone, 2-methyl-5-(1- methyl ethenyl)
10	20.184	1.11	2-Cyclohexen-1-01, 2-methyl-5-(1-methyl) Cis.
11	20.566	0.59	2-Cyclohexen-1-01,2-methyl-5- (1- methyl ethenyl), trans
12	20.881	66.09	2-Cyclohexen-1-one, 2-methyl-5-(1- methyl ethenyl)
13	21.075	0.08	Pentaborane (II), Boron hydride
14	22.039	0.33	Dihydroedulan II, 2H-1-Benzopyrane, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl
15	23.076	2.29	Dihydrocarvyl acetate
16	24.015	2.27	2-Cyclohexen-1-01,2-methyl-5-(1- methyl ethenyl), acetate (IR-Cis), p-mentha-6,8-dien-2-01,acetate.
17	24.670	0.86	Beta bourbonene
18	25.595	1.00	Trans-Caryphyllene, Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene.
19	26.321	0.49	7-Oxabicyclo [4.1.0] heptane, 1-methyl-4- (2- methyl oxiranyl).
20	27.162	0.29	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1- methyl ethenyl)

CONCLUSION

It is clear from the results shown in tables 1, 2, 3, 4 and 5 the drying condition has an effect on percentage yield, chemical constituents and the antioxidant of extracted essential oils. The GC/MS analysis showed three compounds in sample A and not appeared in the other two samples, these compounds are phytol (0.29%), 13(16), 14-labdien-8-ol (0.22%) and n-tetratetracontane (0.19%). Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyl ethenyl) (0.13**%)** appeared in sample B only**.**

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