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Composition of Volatile Oil and Methanolic Extract of Jordanian *Melissa Officinalis L.* and actions against Human Cancer Cell Lines

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ABSTRACT

The essential oil of Jordanian *Melissa officinalis* L. were obtained by hydro-distillation and analyzed by Gas Chromatography – Mass Spectrometry. Components representing 96.40% of the total oil were identified. The methanolic extract and the volatile oil of *Melissa officinalis* L, were tested and showed anti-proliferation activities against *3 cancer* cell lines.

Keywords: Melissa officinalis L, Jordan Flora, LC-Ms/Ms analysis, Cancer Cell line Methanolic Extract.

INTRODUCTION

Most people living in less developed countries rely almost exclusively on traditional medicines for their healthcare needs. In Jordan also many people use herbal medicines as alternative, additional or complementary medicine[1-2]. However, most of the plants used in traditional medicine in Jordan lack detailed phytochemical study and biological evaluation[3].

One of the most interesting medicinal plant species in Jordan is *Melissa,* a genus of the madder family Labiatae[4]. It is widely cultivated in Europe and the United States[5].There has been considerable interest in the biological effects of essential oils from a variety of plants[6] and in their antimicrobial[7] and antioxidant properties[7-9]. The content and composition of the oil *Melissa officinalis* vary with its origin within a given country[10], from country to country[11-13], and under the influence of nitrogenous fertilizers[14] and growth regulators[15]. This

variability increases the importance of the study of a wide range of *Melissa* samples. In addition to the *melissa* essential oil composition, the aromatic and polyphenolic composition of herbal tea made from lemon balm (*Melissa)* has been reported[16].

Malissa has traditionally been used to treat a wide range of conditions such as fever, flatulence, headache, influenza and toothache[2, 5]. Numerous specific biochemical activities have been reported[17] such as acetylcholinesterase inhibition and antioxidant activity[18] and its use for the treatment of cancer[19, 20] and of diabetes[21] have been evaluated.

Milessa occurs rarely in Jordan but is found in restricted regions at Wadi Rajeb. It is a neglected, underutilized plant and threatened by wild herbs. Preliminary work was carried out by Syouf with collections from 3, not previously, studied sites in Jordan[22] the locations are shown in Table 1-1.

The essential oil composition of *Melissa* from Jordan and its anti-cancer proliferative activity have not been studied previously. Herein the analysis of the essential oil from these *Melissa* species is described for the first time.

MATERIAL AND METHOD

Plant materials

A large sample of wild *Melissa officinalis* SP were collected in Jordan in 2011. The plants were identified by Dr. Maha Al- Syuof. (Biodiversity department, NCARE[22]) .The aerial parts of the plants were dried at room temperature and then coarsely powdered.

Extraction, isolation and identification of the essential oil

Dried leaves of *M. officinalis* were subjected to hydrodistillation for 3 hours using a Clevenger

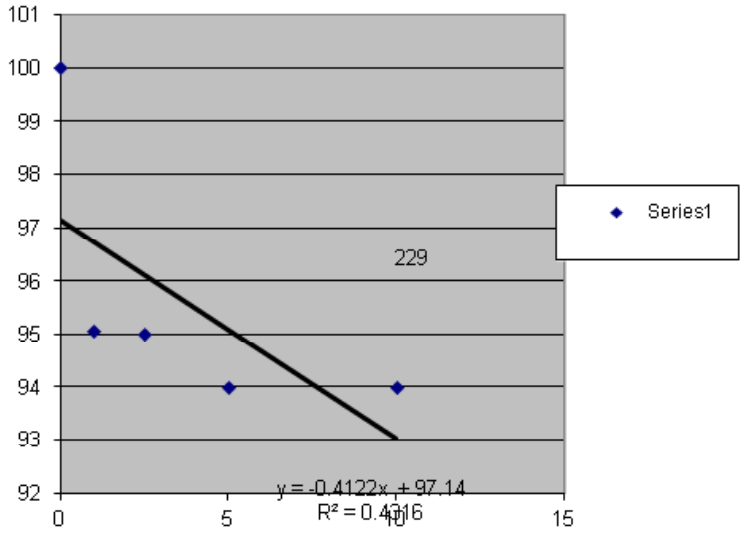

Fig. 1-1 : IC50 effect of the plant volatile oils

Table 1-1: Collection of wild *Melissa officinalis* from 3 sites in Jordan, with GIS data

Number	Collection location	JO. NO.	Elevation	Latitude N	Longitude E
1	Kufrangeh	3098	506	32 18 23.5	35 41 50.0
2	Wadi Rajib	3099	300	32 14 31.5	35 41 54.1
3	Rajib (Al Tal)	3100	418	32 13 59.5	35 41.33.3

Table 1-2: The chemical composition for the volatile oil of *Melissa officinalis* SP

No	Rt	Content	AI	KI	Compounds
					MEL98R22 LEV
1	10.523	0.513	1044	1050	<(E)-B>OCIMENE
2	13.956	0.109	1122	1126	<alpha->Campholenal
3	15.567	0.331	1160	1164	Pinocarvone
4	16.320	1.659	1174	1177	Terpinen-4-ol
5	19.149	0.331	1238	1241	Cumin aldehyde
6	21.323	0.010	1289	1290	Thymol
7	22.899	0.401	1325	1327	<p->Mentha-1,4-diene-7-ol
8	24.653	2.516	1374	1376	<alpha->copaene
9	24.999	3.141	1387	1388	<beta->Bourbonene
10	25.225	2.724	1381	1382	Panasinsene
11	26.572	3.417	1417	1419	<(E)->Caryophellene
12	28.053	1.931	1449	1415	Amorpha-4,11-diene
13	29.089	28.847	1478	1479	<Gamma->Muurolene
14	31.850	3.530	1548	1549	Elemol
15	33.166	43.556	1582	1583	Caryophellene Oxide
16	34.128	2.006	1608	1608	Humulene epoxide II
17	36.435	1.431	1680	1680	Elemol acetate
18	42.000	2.471			disappear
					MEL98R22 ST
1	10.557	0.1	1044	1050	<(E)-B>OCIMENE
2	12.183	0.1	1086	1088	<alpha->Terpinolene
3	14.551	0.2	1135	1139	<trans->pinocarveol
4	15.367	trace	1160	1164	<cis->Chrysanthenol
5	16.321	0.3	1174	1177	Terpinen-4-ol
6	19.126	trace	1238	1241	Cumin aldehyde
7	20.428	trace	1264	1267	E-citral
8	21.309	trace	1298	1299	Carvacrol
9	22.496	0.2	1315	1316	<(2E,4E)->Decadienal
10	24.648	2.1	1374	1376	<alpha->copaene
11	24.998	2.0	1387	1388	<beta->Bourbonene
12	25.221	20.9	1381	1382	Panasinsene
13	26.508	15.8	1417	1419	<(E)->Caryophellene
14	28.047	4.5	1449	1415	Amorpha-4,11-diene
15	29.023	22.2	1478	1479	<Gamma->Muurolene
16	31.847	trace	1548	1549	Elemol
17	33.060	30.5	1582	1583	Caryophellene Oxide
18	41.041	0.2	1800	1800	isotorquatone
19	42.409	0.4			Unkown
Normal Monoterpenes					0.2%
Oxygenated Monoterpenes					2.6%
Normal sesquiterpenes					39.1%
Oxygenated sesquiterpenes					53.9%

type apparatus[23]. The oil was collected, dried over anhydrous sodium sulphate and stored in the dark in a refrigerator until analyzed.

Gas Chromatographic – Mass Spectral (GC-MS) Analysis

About 1 µl aliquot of each oil sample, diluted in n-hexane, was subjected to GC-MS analysis. GC-MS analysis was performed using a Varian Chrompack CP-3800 GC/MS/MS-200 (Saturn, Netherlands) equipped with split-splitless injector and DB-5 (5% diphenyl, 95% dimethylpolysiloxane) capillary GC column (30m x 0.25mm ID, 0.25 µl film thickness). The carrier (ultra-pure helium) flow rate was 1ml/min. The column temperature was kept at 100°C for 3 min and then programmed at rate of 10°C/min up to 250°C, and then held at 250°C for 60 min. The total run time was 56.98 min the mass detector was set to scan ion between 35-500 *m/z*. A mixture of n-alkanes (C_8-C_{20}) was analyzed separately under the same conditions using the same DB-5 column. The compounds in the volatile oil s were identified, using built in libraries (NIST Co and Wiley Co, USa)

Identification of the compounds

The compounds were identified by comparing the retention time, retention index and mass spectrum of the chromatographic peaks with that of the standards available .

The identification of other components was by computer matching with the Wiley, NIST and ADAMS libraries[24] based on their retention indices[25] determined by reference to a homologous series of *n*-alkanes, (C8-C20) and by comparison of their mass spectral fragmentation patterns with those reported in the literature[24] and stored on the MS library data system. Using the van den Dool equation[26], to get RI values helps by predicting the closet component from the top ten component summary which are given from the data system.

$$RI = 100 \times n + 100 \times \left(\frac{tx - tn}{t(n+1) - tn}\right)$$

tx = retention time of unknown component.
tn = retention time of preceding n-alkane.
*t(n+*1*)* = retention time of following n-alkane.
n = carbon number of preceding n-alkane.
RI: arithmetic index as reported in literature.
KI: Kováts index as reported in literature.

RESULTS

In the Table (1-2).which follow the major oil components (component %>3) are indicated in bold. The total amount content was 0.1 ml.

The Chemical composition of *Melissa officinalis* from Wadi Rujb /Ajlun is shown in Table 1-4. The amount of oil collected was 0.2 ml.

Antiproliferative Activity Against Human Cancer Cell Lines

The methanolic extract of *Melissa officinalis* SP was tested on three cancer cell lines namely : two types of colorectal (SW480), (HCT116), and prostate (PC3).As were the volatile oils of the plants.

These cancer cell lines were treated with different concentration of plant extract (10µg/ml,25 µg/ml,50 µg/ml,100 µg/ml), and for the volatile oil (1 µg/ml,1.5 µg/ml,5 µg/ml, 10 µg/ml) , for 72 hr.

The cells growth were evaluated using MTT assay as illustrated.

Table 1-3: Major component in *Melissa officinalis* sp

98R22 Leaf	Major Compound	%Content >3
1	<(E)->Caryophellene	3.4
2	<beta->Bourbonene	3.1
3	<Gamma->Muurolene	28.8
4	Elemol	3.530
5	Caryophellene Oxide	43.556
98R22 Stem	Major Compound	Content >3%
1	Panasinsene	20.9
2	<(E)->Caryophellene	15.8
3	Amorpha-4,11-diene	4.5
4	<Gamma->Muurolene	22.2
5	Caryophellene Oxide	30.5

Table 1-4: Chemical composition of *Melissa officinalis* SP Wadi Rujb /Ajlun

No	Rt	Content	AI	KI	MEL99R31LEV
1	10.112	0.1	1032	1037	<(Z)-B>Ocimene
2	14.568	0.1	1137	1142	<trans->Sabinol trans for OH vs IPP
3	15.408	0.1	1160	1164	Pinocarvone
4	16.337	0.1	1174	1177	Terpinen-4-ol
5	18.821	1.6	1235	1238	Neral
6	20.119	2.6	1160	1164	<(Z)->Isocitral
7	24.642	3.1	1374	1376	<alpha->copaene
8	24.986	3.2	1387	1388	<beta->Bourbonene
9	25.148	1.1	1389	1390	<Gamma->Elemene
10	26.510	16.4	1417	1419	<(E)->Caryophellene
11	28.037	4.3	1449	1451	Amorpha-4,11-diene
12	29.030	19.9	1478	1479	<Gamma->Muurolene
13	31.841	2.4	1548	1549	Elemol
14	33.092	39.8	1582	1583	Caryophyllene Oxide
15	34.112	1.7	1608	1608	Humulene epoxide II
16	36.425	2.3	1668	1669	<(Z)->Caryophyllene<14-hydroxy-9-EPI
17	42.261	0.1			Unkown
					MEL99R31ST
1	10.559	0.0	1044	1050	<(E)-Beta->Ocimene
2	12.744	0.1			3-methyl-2-(2-methyl-2-butenyl)-furan
3	14.660	0.1	1137	1142	<trans->Sabinol trans for OH vs IPP
4	15.445	0.1	1160	1164	Pinocarvone
5	16.280	0.3	1174	1177	Terpinen-4-ol
6	19.564	0.1	1238	1241	Cumin aldehyde
7	20.477	6.7	1264	1267	E-citral
8	21.486	4.2	1299	1299	<cis-á ->Necrodol acetate
9	24.983	3.8	1387	1388	<beta->Bourbonene
10	25.523	2.2	1389	1390	<Gamma->Elemene
11	26.493	15.6	1417	1419	<(E)->Caryophellene
12	28.003	4.3	1449	1451	Amorpha-4,11-diene
13	29.003	17.4	1478	1479	<Gamma->Muurolene
14	33.040	42.6	1582	1583	Caryophyllene Oxide
15	36.523	1.9	1668	1669	<(Z)->Caryophyllene<14-hydroxy-9-EPI
16	41.035	0.1	1800	1800	Isotorqutone
17	42.238	0.5			Unkown
Normal Monoterpenes					3.2%
Oxygenated Monoterpenes					4.3%
Normal Sesquiterpenes					48%
Oxygenated Sesquiterpenes					46.3%

Table 1-5 : Major Components in the *Melissa officinalis* SP

99R31Leaf	Major compounds	% Content>3
1	< α->copaene	3.1
2	<β->Bourbonene	3.2
3	<(E)->Caryophellene	16.4
4	Amorpha-4,11-diene	4.3
5	<γ->Muurolene	19.9
6	Caryophyllene Oxide	39.8
99R31 Stem	Major compounds	% Content>3
1	E-citral	6.7
2	<cis-α->Necrodol acetate	4.2
3	<β->Bourbonene	3.8
4	<(E)->Caryophellene	15.6
5	Amorpha-4,11-diene	4.3
6	<γ->Muurolene	17.4
7	Caryophyllene Oxide	42.6

Table 1-6 : IC50 for Melissa officinals SP methanolic extract

	SW480	HCT116	PC3
0	100	100	100
10	100	100	100
25	100	100	100
50	85.51449	100	100
100	60.43956	69.11142	94.47674
IC50	130.2	180.68	H
	0.9459	0.7776	

Table 1-7: IC50 for *Melissa officinalis* SP volatile oil

	SW480	HCT116	PC3
0	100	100	100
1	86.95652	100	100
2.5	80.43478	100	100
5	67.3913	100	100
10	67	100	100
IC50	13.67	H	H
R2	0.745		

Table 1-8 : IC50 (µg/mL) of different plant fractions against different cancer cell line

	SW40	HCT116	PC3
Cultivated V. Oil	114	H	H
Wild V. Oil	13.67	H	H
Cultivated Crud Ext.	135	229	199.7
Wild Crud Ext.	130.2	180.68	H

CONCLUSIONS

Here in is reported the chemical composition of the volatile oils obtained by hydrodistillation from the two *Melissa* species, The plant also evaluated for its anti-proliferative activities using (SW480), (HCT116), and (PC3), cancer cell lines. Results revealed that the methanolic extract of *Melissa officinals* has an effect in cell viability. Further studies are needed for determination of the mode of action(s) of these plants antiproliferative activities.

The present study strengthen evidence that the search for new anti cancer agent should emphasize to the screening of natural flora of the different countries.

It can be noticed generally that the chemical composition of Jordanian *Melissa officinlis* sp ,and *Melissa officinalis* in other countries have not identical components .

The composition of the oil from *M. officinalis* harvested in Algeria was dominated by neral, geranial

and citronellal. This composition was qualitatively the same that the oils from Serbia (Dukic *et al*., 2004), Slovak[22], Egypt (Shalaby El-Gengaihi and Khattab, 1995), France (Carnat*et al*., 1998) and Iran (Sadraei *et al*., 2003);

However, limonene was the major component in the samples from Scotland (Damien *et al*., 2000) (57.5 %), neral was found with only (4.3 %) and geranial was completely absent. Basta *et al*. (2005) reported that caryophyllene oxide (12.6 %) and β-pinene (18.2 %) were also the most abundant constituents in the oil of *M. officinalis* from Greece but neral and geranial were not detected in the oil. Oils from Cuba[13]) and Brazil[15] were dominated by neral (29.9 % and39.3 %) and geranial (41.0 % and 47.3 %) respectively.

Which clearly leads to conclusion that the chemical composition of the plant and volatile oil composition may vary according to location.

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