

Comparative Analysis of the Essential Oils of *Teucrium polium* L. Grown in Different Arid & Semi Arid Habitats in Jordan

Fatma U. Afifi^{1✉}, Barakat. E. Abu –Irmaileh² and Rinad. A. Al-Noubani³

¹ Faculty of Pharmacy, University of Jordan, Amman, Jordan.

² Faculty of Agriculture, University of Jordan, Amman, Jordan.

³ Faculty of Graduate Studies, University of Jordan, Amman, Jordan.

ABSTRACT

Teucrium polium is a widely-used medicinal herb in Jordan. This herb is an endogenous plant species that is distributed over Irano-Turanian as well as Mediterranean regions, receiving total annual precipitation of 50 to 350 mm. The species is one of the most variable species; variability is partly phenotypic as well as seasonal within the country. Data on its taxonomic status to subdivisions is lacking. This research is intended to record the possible variations in the constituents of extracts of plants collected from different regions. Hexane extracts were prepared from *T. polium* specimens, collected at pre-flowering stage from five different locations and from plants grown in the greenhouse and analyzed by gas chromatography (GC). The results indicated the presence of several volatile compounds such as menthone, germacrene, linalool, ρ -cymene, carvone, and β -caryophyllene. Samples from different locations showed qualitative and quantitative variations in the oil composition. GC analysis showed also that the highest number of volatile compounds was detected in samples collected from the greenhouse and Jdaideh, while the lowest number of volatile compounds was from Gawa'a samples (both Mediterranean type-regions).

Keywords: *Teucrium polium*, Labiatae, essential oil composition, Dead Sea, Jordan.

INTRODUCTION

Volatile oils are odorous principles found in various plant parts. Environmental factors such as duration of sunshine, temperature, soil, ground water, availability of nutrients are known to affect the presence and/or the concentrations of the active principles, including the volatile oils of various medicinal herbs. Geographical location of the growing area and the time of collecting the plants are additional factors influencing the phytochemical composition⁽¹⁻⁶⁾. The oils, flavonoids,

terpenes, and other active constituents of several medicinal plants, such as *Thymus piperella* L., *T. vulgaris* L., *Satureja montana* L. and *Origanum syriacum* L. showed different patterns in different environments^(1, 3, 7, 8).

Teucrium polium (germander), which is known locally as "Ja'ada", is one of the medicinal plants in Jordan and used in the folk medicine very frequently for the treatment of gastrointestinal/abdominal ailments and for its hypoglycemic properties^(9, 10). Germander is a perennial plant that belongs to the family Labiatae and grows naturally in different bio-geographical zones with diverse habitats and landscape elements. The occurrence of volatile oils in mature germander has been investigated by several researchers from different Mediterranean countries⁽¹¹⁻¹⁶⁾. A comparison of these studies showed

Received on 8/7/2008 and Accepted for Publication on 24/8/2008.

✉ E-mail: fatueafi@ju.edu.jo

qualitative and quantitative variations in the oil composition, related to the different environments, plant habitats, and climatic conditions where the plant was grown. Variability in germander constituents in arid regions in Jordan has not been investigated. This research is intended to shed a light on the possible variations in oil composition of germander specimens collected at the pre-flowering stage in different environments, since the survey carried out by Al-Noubani⁽¹⁷⁾ showed that the inhabitants of the arid and semi-arid regions collect germander at pre-flowering stage for medicinal use.

EXPERIMENTAL SECTION

Study area

Germander is widely spread in Mediterranean, Sudanian and Irano-Turanean regions in Jordan. The selected study site was found in and around the natural reserve, the Mujib Nature Reserve, recognized as such by the Jordanian Royal Society for the Conservation of Nature (RSCN)⁽¹⁸⁾ (Figure 1). Wadi Mujib Area (220 km²) is located 120 km south of Amman, between the districts of Karak and Madaba. This part of Jordan is characterized by diverse bio-geographical zones (Figure 1). The average annual rainfall, the mean annual temperature and the altitudes of the locations from which the plant specimens were collected are given in tables (1) and (2) based on RSCN data⁽¹⁸⁾. In addition to the germander specimens collected from these different study areas, twenty plants of *T. polium* collected from natural habitat in Batheyeh were planted in 30 cm diameter pots for establishment in the green house of the Faculty of Agriculture, University of Jordan. The first cut was taken after flowering on July 2004.

Plant materials

Samples of *T. polium* were collected from the study area and green house at pre-flowering stage and taxonomically identified by one of the authors (B. Abu-Irmaileh). The aerial parts of *T. polium* specimens were dried at room temperature in the shade and ground. Voucher specimens were deposited in the herbarium of the Faculty of Agriculture and Faculty of Pharmacy.

Extract preparation

The plant materials were extracted separately with five different solvents (petroleum ether, ethylacetate, chloroform, ethanol (96%), and water) for thin layer chromatographic (TLC) screening. For each extract preparation, 1 g plant material and each 10 mL solvent was used. Extraction was carried out by gentle heating in water bath and keeping the decoction overnight before filtration. After filtration, the different extracts were used for TLC analysis. For GC and GC/MS analysis, one gram of ground material of *T. polium* samples were extracted in three replicates with each 10 ml n-hexane by gentle heating in a water bath, then they were filtered and used for GC and GC/MS analysis⁽¹⁹⁾.

Analysis

GC analysis was performed on a Hewlett Packard apparatus model 5890 series II with an attached flame ionization detector and Hewlett Packard 3395 integrator. GC/MS (Shimadzu QP 2010) analysis was performed at the Department of Pathology, Microbiology and Forensic Medicine, Faculty of Medicine, University of Jordan. Samples of 1 µL each were injected into the GC injection port preheated at 90° C. The samples were eluted in a Treb-Merck- α Si column (HP-5; 30 m x 0.25 mm) employing dry nitrogen gas as elution carrier at a flow rate of 1 mL/minute. The temperature of the GC column was adjusted according to the following program: 45° C over 3 minutes then heated at 4° C per minute up to 250° C. Eluted samples were detected by the attached MS apparatus configured to measure *m/z* up to 500. The chemical identities of the detected components were determined in comparison with the data bank spectra (NIST Libraries, USA) provided by the instrument software.

RESULTS

The ethnobotanical survey carried out in the study area showed that the majority of inhabitants of Mujib area use *T. polium* at pre-flowering stage⁽¹⁷⁾. No volatile substances were detected by TLC from the different extracts prepared from the plant samples collected at pre-

flowering stage using ready coated analytical TLC plates (Silica gel 60F₂₅₄, Merck, Darmstadt, Germany) and development and visualization systems as described by Wagner and Bladt⁽²⁰⁾. GC and GC/MS analysis was performed on n-Hexane extracts of *T. polium* samples collected at the pre-flowering stage. The identified volatile substances by GC/MS analysis are listed in Table (3). This table indicates that limonene and spathulenol were the only volatile secondary metabolites detected in all samples. Several other volatile compounds such as bisabolol, germacrene D, linalool, p-cymene, (+)-carvone and others were detected in varying concentrations in oils from different locations. Samples from different locations showed qualitative and quantitative variations in the oil composition. GC analysis showed that samples collected from the greenhouse and Jdaideh (a Mediterranean type-region) contained 18-20 different volatile compounds, while samples collected from Gawa'a (another Mediterranean type-region) contained only 12 volatile compounds. On the other hand, in samples collected from the Irano-Turranian Region (Tlail and Batheyeh), 14 volatile compounds were detected. Fenchole and α -cadinole were only found in samples collected from Tlail location, and γ -Elemene and oleamide were only found in samples collected from plants grown in the greenhouse. Analysis of germander plants, brought from Batheyeh and grown in the greenhouse, indicated the presence of ten additional volatile compounds compared to the plants collected from the natural site. In addition, the concentrations of the identified volatile oils were different. Samples from the greenhouse contained by far larger quantities of germacrene D and spathulenol while an obvious decrease in the concentration of the other volatile constituents was detected.

The results also showed differences in the type of volatile oils from plants collected from Gawa'a and Jdaideh, although both are considered Mediterranean region. For example, samples from Gawa'a showed high amounts of caryophyllene oxide, α - Bisabolol oxide and δ -cadinene, which were absent in samples of Jdaideh. In addition, the concentration of spathulenol in Gawa'a samples was almost fifteen times that of Jdaideh.

DISCUSSION

Germander is a common medicinal herb in Jordan, very frequently used for the treatment of gastrointestinal/abdominal ailments, and for its hypoglycemic properties^(9, 10). This herb is an endogenous plant species that is distributed over Irano-Turranian as well as Mediterranean regions of the country. It can be found growing naturally at 300 m below sea level to above 750 m altitudes, in different bio-geographical zones with diverse habitats and landscapes receiving total annual precipitation of 50 to 350 mm. *T. polium* is one of the most variable species. Variability is **partly** phenotypic as well as seasonal within the country. Data on its taxonomic status to subdivisions is lacking.

Response of patients using this herb to help in curing gastrointestinal ailments varies according to the area from which the plant was collected. The results of analyzing samples, collected from different regions, indicated very clearly that differences in the type and in the concentration of volatile constituents exist (Table 3). Analysis of samples collected from different environments showed significant variations in type of oil as well as in the concentration of similar oil constituents. It was noticed that not all samples collected from different areas contained the same concentration of volatile oils even when collected at the same developmental stage. Limonene was consistently detected in all samples, with the highest concentration occurring in samples collected from Batheyeh, with the lowest average temperature (18.5°C) and lowest altitude (83 m). The amount of the volatile substances detected in samples grown in the greenhouse, originally taken from Batheyeh site, were in general less than those grown in their natural habitat, Batheyeh (Table 3). Samples taken from different locations of the same site (Jdaideh 1 and 2) also showed variations in type and concentration of their constituents.

Qualitative and quantitative variations in the oil composition, related to the different environments, plant habitats, and climatic conditions where the plant was grown have been reported by several authors⁽¹¹⁻¹⁶⁾. Environmental factors may be a major contributor to the variation in germander constituents. As the species is

described to be one of the most variable taxon that requires detailed taxonomic studies, variations in the constituents in samples collected from the same type biogeographical region could be due to the phenotypic subdivisions within the species⁽²¹⁾. Samples collected from Gawa'a and from Jdeideh showed significant variations in their constituents (Table 3), even though both sites are of Mediterranean type vegetation zone.

Infusions and decoctions are traditionally used dosage forms for treatment purposes. The volatile oils are present in these preparations only in minute amounts, practically in non-detectable concentrations. None of the volatile compounds could be identified from the aqueous extracts using TLC on silica gel. Two possible hypotheses could contribute to explaining the widely

accepted use of *T. polium* aqueous extracts by the inhabitants. Firstly, the presence of other water soluble substances might be responsible for the therapeutic activity of these commonly used preparations, or that the volatile substances are highly potent at these very low concentrations, capable curing the gastrointestinal/abdominal diseases, as reported by the inhabitants. Additionally, one should keep in mind that the gastrointestinal/abdominal diseases can be considered as "self-limiting diseases", which fade away with time, with or without treatment with *T. polium* extracts.

ACKNOWLEDGMENTS

The authors would like to thank Mr. Ismail Abaza for his technical assistance.

Table (1): Averages of annual precipitation and temperatures in the study area, for the year 2004

Site	Station	Average annual precipitation (mm)	Average annual Temperature (°C)
Gawa'a	Al- Rabbah	392.9	16.7
Jdaideh (1) and(2) ^a	Madaba	326.4	17.6
Tlail	Ghor Safi	144.6	25.6
Batheyeh ^b	Wadi Wala	267.1	18.5

^aJdaideh (1) is located within the Mujib Nature Reserve, Jdaideh (2) is located outside the Mujib Nature Reserve

^bfor the period (1962-2003)

Table (2): Description of the locations for the sample collection (longitude, latitude, altitude and the biogeographical zones)

Location	Longitude	Latitude	Altitude (m) asl ^a	Vegetation zone ^b
Gawa'a	31° 23' N	35° 39' E	873	M
Jdaideh 1(Inside Mujeb nature Reserve)	31° 30' N	35° 38' E	747	M
Jdaideh2 (outside Mujeb nature Reserve)	31° 30' N	35° 38' E	780	M
Tlail	31° 25' N	35° 35' E	240	I
Batheyeh	31° 25' N	35° 40' E	83	I

^aasl: above sea level;

^bM: Mediterranean , I: Irano-Turanean;

Table (3): Area percentages of volatile substances detected in *T. polium* collected at pre-flowering stage from different bio-geographical areas using GC-MS analysis.

Ret. time	Volatile compound	% of volatile substances in samples collected from the locations ^a :					
		<u>Tlail</u>	<u>Batheveh</u>	<u>Green House</u>	<u>Jdaideh 1</u>	<u>Jdaideh 2</u>	<u>Gawa'a</u>
5.567	Cyclohexane	3.35±0.96	1.53±0.77	-	-	-	2.32±0.09
5.858	6,6-Dimethyl-5,6-dihydropyran	-	-	-	3.38±0.12	2.88±0.76	-
6.175	Hexane, 3-methyl	-	1.12±0.04	-	-	-	0.79±0.03
6.450	Heptane 3,4,5-trimethyl	-	-	1.10±0.24	4.78±1.02	-	3.92±1.13
6.600	Isooctane	3.15±1.03	-	-	-	-	19.23±3.79
6.685	2, 4- Dimethyl-heptane	0.86±0.21	-	7.65±0.64	-	-	-
6.975	Heptane	-	3.28±0.23	1.12±0.10	-	-	4.36±0.44
7.475	2-Methyloctanal	-	7.32±0.09	0.97±0.09	-	-	-
7.758	Pentane 2.2.4-trimethyl	-	-	-	-	-	8.46±3.02
8.075	Octane, 4-methyl	0.13±0.04	-	0.38±0.03	-	-	-
10.342	4-Heptanone, 3-methyl	-	1.17±0.66	-	2.49±0.50	-	1.98±0.78
10.792	2-Methylpent-2-en-4-one	-	-	-	4.23±1.13	1.88±0.08	1.86±1.11
13.167	2-Hexenal	-	-	-	6.63±0.98	8.38±0.77	4.89±1.72
14.183	p-Cymene	-	-	-	0.55±0.09	0.42±0.23	-
14.233	D-Limonene	0.10±0.03	2.89±0.01	0.94±0.09	0.27±0.04	0.37±0.08	0.32±0.01
15.233	Nonane, 5-(2-methylpropyl)	0.26±0.08	-	1.01±0.27	-	-	-
15.517	Decane, 3, 7-dimethyl	0.92±0.11	0.35±0.11	3.84	-	0.57±0.23	-
15.733	2,6, Dimethyl-decane	0.10±0.03	-	8.37±0.87	1.75±0.10	1.54±0.23	0.15±0.05
15.925	cis-Sabinenhydrate	-	-	-	0.42±0.04	0.30±0.10	-
16.342	2,4-Dimethyl,1-decene	0.11±0.04	-	0.39±0.02	2.92±1.01	0.89±0.42	-

Ret. time	Volatile compound	% of volatile substances in samples collected from the locations ^a :					
		<u>Tlail</u>	<u>Batheveh</u>	<u>Green House</u>	<u>Jdaideh 1</u>	<u>Jdaideh 2</u>	<u>Gawa'a</u>
16.683	Fenchone	0.33±0.08	-	-	-	-	-
17.142	Linalool	-	-	2.06±0.13	1.24±0.30	0.84±0.10	-
19.217	Menthone	0.56±0.20	-	-	1.74±0.09	2.65±0.35	-
21.892	(+) Citronellol	-	-	-	0.33±0.07	0.28±0.15	-
22.475	(+)-Carvone	0.88±0.06	-	-	0.96±0.08	1.35±0.35	-
22.917	trans-Linalooloxide	-	-	2.72±0.72	-	-	0.75±0.07
23.742	Dodecane, 4, 6-dimethyl	-	-	1.19±0.47	0.25±0.10	0.40±0.03	-
24.042	3,8-Dimethylundecane	-	-	1.66±0.08	3.39±0.97	3.06±1.01	0.89±0.08
24.508	2,6,11-Trimethyl-dodecane	-	-	2.75±0.11	-	-	0.98±0.00
24.517	Carvacrol	-	-	-	2.78±0.10	3.48±0.21	-
25.867	trans-Carveol	-	0.32±0.12	-	1.97±0.17	0.24±0.04	1.47±0.49
28.600	β-Caryophyllene	-	0.90±0.13	0.29±0.08	-	-	-
29.592	β-Farnesene	-	0.45±0.22	0.22±0.22	0.17±0.06	0.27±0.09	-
29.700	α-Humulene	-	0.58±0.05	-	-	-	-
30.208	Germacrene D	-	4.10±0.15	7.48±0.40	0.48±0.56	0.26±0.06	-
31.017	γ-Elementene	-	-	2.20±1.98	-	-	-
31.117	Thyme-camphor	-	-	10.79±0.34	4.75±0.30	8.42±0.13	0.76±0.06
33.650	Caryophyllene-oxide	1.25±0.21	3.25±0.37	0.92±0.33	-	-	2.80±0.37
33.792	Phenol, 2-methoxy-3-(2-propenyl)	-	-	12.79±3.73	12.01±0.44	13.61±0.69	-
34.867	Pentadecane	-	3.31±1.21	-	4.58±1.47	2.63±0.98	-
35.683	α-Cadinole	0.68±0.06	-	-	-	-	-
36.775	Octadecane	-	1.78±0.09	0.33±0.07	1.06±0.27	2.79±1.29	-
39.002	(+) Ledol	1.81±0.98	-	-	-	-	-
41.275	Diethylphthalate	24.11±1.02	7.30±1.11	1.56±1.02	-	-	1.18±0.93
41.408	Spathulenol	2.98±0.18	5.99±0.12	12.53±2.01	2.27±0.19	1.41±0.19	21.91±2.45
42.450	Humulene oxide	2.40±0.42	1.17±1.14	-	-	-	0.84±0.06
43.125	δ-Cadinene	-	-	-	-	-	3.61±0.95
43.542	α-Bisobalol-oxide	-	2.40±0.14	-	-	-	5.54±0.58

Ret. time	Volatile compound	% of volatile substances in samples collected from the locations ^a :					
		<u>Tlail</u>	<u>Batheveh</u>	<u>Green House</u>	<u>Jdaideh 1</u>	<u>Jdaideh 2</u>	<u>Gawa'a</u>
44.133	α -Bisobalol	-	1.87±0.19	-	3.04±0.26	3.12±0.30	2.72±0.25
44.642	Bisoflex DNP	36.48±2.04	-	-	6.41±2.28	12.80±1.79	-
49.233	Phthalic acid dinonylester	-	-	-	9.29±3.03		3.21±1.12
50.917	Bisoflex 91	-	6.83±2.01	-	7.66±2.27	6.41±1.82	-
51.500	Nonylphthalate	-	-	0.24±0.08	0.50±0.09	-	0.79±0.01
52.558	Oleoamide	-	-	0.98±0.31	-	-	-
53.275	Hexanedioic-acid	10.30±1.64	12.31±3.01	-	-	9.71±4.21	0.98±0.30
54.950	Nonacosane	5.60±0.72	10.68±0.47	-	-	-	3.29±0.09
55.750	n-Hexa-triocontane	3.64±0.19	19.10±0.85	6.52±0.53	7.70±0.10	9.04±0.06	-

^amean value of three determinations ± standard deviation

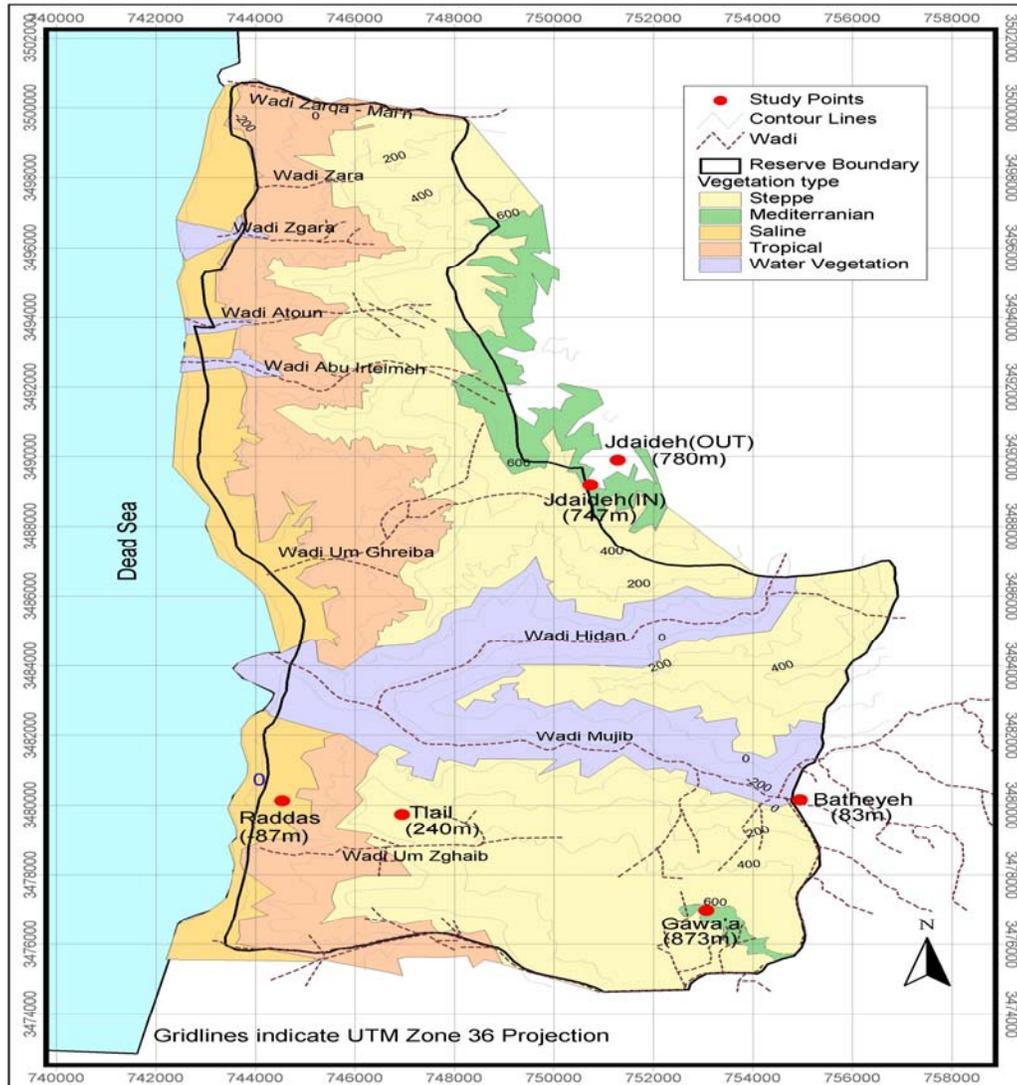


Figure (1): Mujib Nature Reserve showing the locations of *T. polium* collections

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