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Original article

Analysis of essential oils of *Origanum vulgare* from six production areas of China and Pakistan

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ABSTRACT

Origanum vulgare L., Lamiaceae, from six different production areas of China and Pakistan were analyzed via gas chromatography equipped with a flame ionization detector (GC-FID) and examined for their volatile constituents by gas chromatography-mass spectroscopy (GC-MS). This procedure allowed the identification of 11 to 46 components among six production areas, representing 98.5% to 99.9% of the total oil extracted. The yields of the essential oil of the six production areas of *O. vulgare* ranged from 0.1 to 0.7%. The class of oxygenated monoterpenes was predominant in all the essential oils. However, samples S5 and S6 have high content of sesquiterpene hydrocarbons (33.7 and 43.7%); while sample S6 is high on oxygenated sesquiterpene (32.9%). The principal component analysis of *O. vulgare* was employed to provide a comprehensive evaluation of essential oil components. The cluster analysis of *O. vulgare* was classified into three subsets, characterized according to the major essential oil components. The current study investigated the composition differences of essential oil among six production areas offering foundation for quality control, resource optimization, and clinical treatments.

Introduction

Origanum species from the Lamiaceae family, which is an important culinary herb in world trade, are widely distributed in the fields of China and some central Asian countries (Hudaberdi, 2004) and have been reported to be widely used as a traditional remedy to treat various ailments, such as whooping and convulsive coughs, digestive disorders, and menstrual problems (Ozbek et al., 2008). The essential oil of this plant has proven antimicrobial, fungicidal, and antioxidant properties (Lagouri et al., 1993; Busatta et al., 2007; Bouhdid et al., 2008;). The essential oil composition of *Origanum* spp. has been extensively investigated (Lawrence, 1984; Kokkini et al., 1997; Reverchon, 1997; Russo, 1998; Chalchat and Pasquier, 1998; D'Antuono et al., 2000), which had reported differences between the many species.

In the last decade, *Origanum vulgare* L., a member of the Lamiaceae family, has been a valuable source of natural products to maintain human health for a long period of time, and subjected to intensive analysis for natural therapies (Force et al., 2000). The dried herb and the essential oil of *O. vulgare* are used in medicine (Hummer et al., 1999). The aroma, flavor, and pharmaceutical properties of *O. vulgare* are products of

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its essential oil which consists mostly of monoterpenes and sesquiterpenes. *O. vulgare* can be used to prevent colds, treat acute gastroenteritis, abdominal pain, irregular menstruation, pruritus, and other diseases (Hudaberdi, 2004).

The differences in the essential oil composition among the different production areas may be due to the developmental stages or variations in cultivation conditions of the plant, or as a result of structural or physiological modification of the plant caused by specific environmental factors (phenotypic plasticity). Thus, composition differentiation requires a detailed analysis using gas chromatography-mass spectroscopy (GC-MS) and the application of principal component analysis. Composition analysis via GC-flame ionization detection (FID) and GC-MS of the eessential oil (Zhang et al., 2010; Ma et al., 2010; Zhou et al., 2011) has been previously reported.

There have been previous investigations on essential oil content and chemical composition of *O. vulgare* from the Kumaon Himalaya (Verma et al., 2010), Campania (Southern Italy) (De Martino et al., 2009), Bosnia (Stoilova et al., 2008), Corsica (Lukas et al, 2008), Bulgaria (Kula et al., 2007), and Lithuania (Mockute et al., 2004); the antioxidant activity of *O. vulgare* from Tunisia (Mechergui et al., 2010); as well as the antibacterial activities of *O. vulgare* (Ozkalp et al., 2010; Sarac & Ugur, 2008).

However, there is no report on the essential oil composition of *O. vulgare* from production areas in China and Pakistan. The aim of the current investigation was to analyze the chemical composition of the essential oil of six different production areas of *O. vulgare* in China and Pakistan. Meanwhile, the differences in chemical composition of essential oil among the six production areas were compared.

Materials and methods

Plant material

Fresh samples (whole plant) of *Origanum vulgare* L., Lamiaceae, were collected during flowering phase in August 2011, from six different production areas in China and Pakistan. More than ten specimens per collection site were collected. Voucher specimens from each site were deposited in the Traditional Chinese Medicine Ethnic Herbs Museum (TCMEHM) of Xinjiang Medical University under the name *O. vulgare*. The samples were identified by Yonghe Li, chief apothecary of the Chinese medicine hospital of Xinjiang. Table 1 summarizes the information about the collection sites, voucher numbers, and essential oil yields (%, referred to dry plant material), obtained by hydro-distillation in a Clevenger-type apparatus for 6 h.

Essential oil isolation

Dried undamaged *O. vulgare* whole plants (flowers, stems, and leaves) from the six different production areas (100 g each, $n = 5$) were submitted to hydro-distillation (Chu et al., 2011) in a Clevenger-type apparatus for 6 h. At the end of distillation, the oils were collected, dried with anhydrous sodium sulfate (Na_2SO_4) prior to analyses, measured, and transferred to glass flasks and stored at 4°C.

Gas Chromatography (GC) analysis

GC was carried out using an Agilent 6890 N GC-FID system (Liu & Du, 2011), equipped with a flame ionization detector (FID) on an Agilent capillary column, HP-5 (30 m × 0.32 mm; film thickness 0.25 µm) (Agilent Technologies, America). The column temperature was programmed from 40°C to 250°C at a rate of 5°C/min. The column temperatures of injector and detector were set at 250°C. Helium was used as the carrier, in a flow rate of 1.0 ml/min.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS analysis was carried out using an Agilent 6890 N GC-FID system, equipped with a flame ionization detector (FID) on an Agilent capillary column, HP-5 (30 m × 0.32 mm; film thickness 0.25 µm) (Agilent Technologies, America). The column temperature was programmed from 40°C to 250°C at a rate of 5°C/min. The column temperatures of injector and detector were set at 250°C. Helium was used as the carrier, in a flow rate 1.0 ml/min. Split ratio was 1:100. GC-MS analyses were done in the EI mode at 70 eV, inlet temperature was 200°C and transfer line temperature was 250°C. The temperature program was the same with that of the GC analysis. The injected volume was 0.2 µl.

Identification of components

The identification of the components and peak identification were done by comparing their retention time with respect to the *n*-alkane series (C_6-C_{22}) internal standards under identical experimental conditions (Adams, 2001). The mass spectra

Table 1
Specimen vouchers numbers, collection sites, and average essential oil yields of the *Origanum vulgare* L.

Sample n°/ Voucher n° (TCMEHM)	S1/10011	S2/10012	S3/10013	S4/10014	S5/10015	S6/10016
Collection site	Kunlun Mountain of Hetian	Shangqiu of Henan	Pakistan	Hetian	Anhui	Yili
GPS coordinates	Longitude 79.3°, latitude 41.2°	Longitude 115.51°, latitude 33.43°	Longitude 72.13°, latitude 30.12°	Longitude 79.94°, latitude 37.16°	Longitude 116.23°, latitude 32.37°	Longitude 86.03°, latitude 43.63°
Essential oil yield (%, $n = 5$)	0.7	0.3	0.3	0.3	0.3	0.1

and relative Retention Index (RI) were compared with those of the commercial NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) mass spectral library (NIST 05 and NIST 05 s). The relative amounts of the individual components were calculated based on the GC integrator peak areas without using correction factors.

Similarity analysis

Calculating the similarity degree via vector included angle cosine method, to obtain the diversity among the six different productions.

Principal component analysis

Principal component analysis (PCA) was carried out using the statistical software SPSS (Version 17.0, StatSoft, USA). PCA was employed to provide an overview of the capacity to distinguish essential oil components based on GC-MS data (select variables > 0.2% of 42 essential oil components from six production areas).

Cluster analysis

Cluster analysis is used to assign a set of objects into groups called clusters, so that the objects in the same cluster are more similar in one way or another to each other than those in other clusters. The cluster analysis was based on the data (select variables > 0.2 % of 42 essential oil components from six production areas).

Results and discussion

Essential oil composition analysis via GC/MS

The essential oil is extracted by hydro-distillation of the whole plant and analyzed via GC-FID and GC/MS, which allowed the identification of 11 to 46 components among the six different production areas studied, representing 98.5% to 99.9% of the total oil. The essential oil from *Origanum vulgare* L., Lamiaceae, collected from the six production areas of China: Kunlun Mountain of Hetian, Shangqiu of Henan, Hetian, Anhui, Yili, and Pakistan were obtained in yields ranging from 0.1% to 0.7 % (v/w).

The main components of these essential oil, from sample S1 were β-citronellol and citronellol acetate (85.3% and 5.2%); thymol and citronellol acetate (42.9% and 12.2%). From sample S2; β-citronellol, thymol, and citronellol acetate (72.7%, 7.2% and 5.9%). From sample S3, β-citronellol and *trans*-geraniol (75% and 7.7%). From sample S4, eucalyptol, caryophyllene, eugenol methyl ether, and citronellol acetate (20.8%, 10.2%, 9.8% and 8.8%, respectively). From sample S5, caryophyllene oxide, caryophyllene, citronellol acetate; and germacrene D (32.9, 17.8, 10.2 and 9.8%, respectively) from sample S6, respectively (Table 2). According to Table 2, the principal components of the essential oil from the six different localities were β-citronellol (72.7% to 85.3%), citronellol acetate (8.8% to 12.2%), thymol (1.5% to 42.9%), caryophyllene (0.4% to 17.7%). The samples from the six different localities contain in all the compositions, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes. The class of the oxygenated monoterpenes compound was predominant in all the essential oils, whereas sample S6 location had less than the other production areas. Samples S5 and S6 have a high sesquiterpene hydrocarbons (33.7% and 43.7%) content; while sample S6 has high oxygenated sesquiterpene (32.9) content. Meanwhile, the composition of monoterpene hydrocarbons in samples S3, S4, and S6 production areas (Table 3) cannot be determined. The differences of essential oil composition among the different production areas may be due to the developmental stages of the plants or the variations in cultivation conditions, or as a result of the structural or physiological modifications of the plant caused by specific environmental factors (phenotypic plasticity).

Compared with other previous similar works reporting that β-sitosterol (I), dancosterol (II), and fifteen compounds were isolated from the chloroform fraction of *O. vulgare*,planted in Hubei (Sun et al., 2007); The other experiments showed some differences within the area variation, nevertheless all samples contained the same eight compounds: 3-octanone, myrcene, *p*-cymene, γ-terpinene, thymol, carvacrol, α-caryophyllene, caryophyllene oxide. Furthermore, the relative amounts of these eight compounds in all samples were above 70% (Zan et al., 2013).

Table 2
The main components of essential oil from S1 to S6.

Sample n°	S1	S2	S3	S4	S5	S6
Main components	A 85.3%	B 42.9%	A 72.7%	A 75%	eucalyptol (20.8%)	caryophyllene oxide (32.9%)
	C 5.2%	C 12.2%	B 7.2%	D 7.7%	caryophyllene (10.2%)	caryophyllene (17.8%)
			C 5.9%		eugenol methyl ether (9.8%)	C 10.2 %

A, β-citronellol; B, thymol; C, citronellol acetate; D, *trans*-geraniol.

Table 3
Essential oil composition identified by GC-MS of *Origanum vulgare* L. collected from six different production areas of China and Pakistan.

n°	Compounds	Rt	RI	Peak Area (%)					
				S1	S2	S3	S4	S5	S6
1	α-pinene	8.094	933	0	0	0	0	0.2	0
2	sabinene	9.283	973	0	0	0	0	0.5	0
3	β-pinene	9.446	979	0	0	0	0	1.1	0
4	1-octen-3-ol	9.493	980	0	0.7	0	0	0	0
5	3-octanone	9.632	985	0	0.6	0	0	0	0
6	β-myrcene	9.763	989	0	0	0	0	0.4	0
7	3-octanol	10.013	998	0	0.4	0	0	0	0
8	α-terpinene	10.655	1018	0	0.4	0	0	0	0
9	*m*-cymene	10.903	1026	0	7.4	0.3	0	0.9	0
10	limonene	11.045	1030	0	0	0	0	1.8	0
11	1,8-cineole	11.151	1034	0	0	0	0	20.8	0
12	γ-terpinene	11.959	1059	0	1.9	0	0	0.4	0
13	β-linalool	13.274	1101	0.4	0.4	0.4	0.5	5.5	3.2
14	α-thujone	13.518	1109	0	0	0	0	1.7	0
15	*cis*-rose oxide	13.592	1111	1.8	0	3.8	4	0.3	0
16	β-thujone	13.868	1120	0	0	0	0	0.4	0
17	rose oxide	14.106	1128	0.9	0	1.9	2.1	0	0
18	sabinol	14.571	1142	0	0	0	0	0.3	0
19	β-citronellal	14.898	1153	1.2	0	0.6	0.4	0	0
20	l-borneol	15.622	1176	0	1.2	0	0	0	0
21	terpinen-4-ol	15.864	1183	0	0.9	0	0	0.4	2.7
22	α-terpineol	16.315	1198	0	0	0	0	0	3.9
23	citronellol	17.184	1227	0	12.2	0	0	8.8	10.2
24	β-citronellol	17.223	1229	85.3	0	72.7	75	0	0
25	thymol methyl ether	17.284	1230	0	4.2	0	0	0.5	0
26	benzene,1-methoxy-4-methyl-2-(1-methylethyl)	17.573	1240	0	0	0.6	0.3	0	0
27	carvone	17.779	1247	0	0	0	0	0.8	0
28	geraniol	17.904	1251	0	0.2	1.1	7.7	0.5	0
29	citronellyl formate	18.56	1273	0	0	0.2	0.2	0	0
30	thymol	19.177	1294	0	42.9	7.2	0	1.5	0
31	*p*-cymen-2-ol	19.425	1302	0	7.5	0	0	2.4	0
32	thymol acetate	20.677	1346	0	0	0	0	0.2	0
33	citronellol acetate	20.747	1349	5.2	0.5	5.9	3.4	0.4	0
34	carvacrol acetate	21.224	1366	0	0	0	0	0.5	0
35	acetic acid, geraniol ester	21.564	1377	0.3	0	0.3	0.3	0	0
36	α-copaene	21.637	1380	0	0	0	0	1.7	0
37	eugenol methyl ether	22.21	1400	0	0	0	0	9.8	3.4
38	β-caryophyllene	22.893	1426	0.4	7.8	1	0.5	10.2	17.7

Table 3 *cont.*

nº	Compounds	Rt	RI	Peak Area (%)					
				S1	S2	S3	S4	S5	S6
39	α-*trans*-bergamotene	23.17	1436	0	0	0	0	1.6	0
40	(Z)-β-farnesene	23.634	1453	0	0	0	0	0.8	0
41	α-humulene	23.871	1462	0.6	0.4	0.6	0.5	4.9	5.6
42	γ-muurolene	24.34	1483	0	0.4	0	0	0	0
43	germacrene D	24.54	1487	0	0.3	0	0.2	0.5	9.8
44	isoeugenol methyl ether	24.794	1497	0	0	0	0	0.5	0
45	germacrene B	24.922	1501	0	0	0.3	0.4	0	0
46	(*E*,*E*)-α-farnesene	25.003	1504	0	0	0	0	0	3.8
47	β-bisabolene	25.149	1510	0	0.7	0	0	2.2	6.8
48	γ-cadinene	25.364	1519	0	0.3	0	0	0	0
49	δ-cadinene	25.454	1523	0	0.6	0	0	0.8	0
50	β-sesquiphellandrene	25.585	1527	0	0	0	0	0.7	0
51	elemicin	26.084	1547	0	0	0	0	0.5	0
52	ledol	26.101	1548	1.2	0	1	0.7	0	0
53	myristicin	26.74	1573	0	0	0	0	0.4	0
54	viridiflorol	27.018	1584	0.2	0	0	0	0	0
55	spathulenol	27.043	1585	0	0.3	0.7	1.3	0.5	0
56	caryophyllene oxide	27.199	1590	0	2.2	0.4	0.5	2.5	32.9
57	*cis*-asarone	27.705	1611	0	0	0	0	3.21	0
58	1,5,5,8-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	27.895	1619	0	0	0	0.5	0	0
59	myristicin	27.937	1621	0	0	0	0	2.5	0
60	cubenol	27.999	1623	0	0	0	0.23	0	0
61	asarone	28.569	1647	0	0	0	0	1.3	0
62	1,2-dimethoxy-4-(2-methoxy-1-propenyl) benzene	29.207	1673	0	0	0	0	3.62	0
63	α-bulnesene	29.33	1679	0	0	0	0	0.2	0
64	apiol	30.2	1716	0	0	0	0	0.3	0
65	dill apiol	31.594	1777	0	0	0	0	0.5	0
	Class composition								
	Monoterpene hydrocarbons			0.12	2.3	0	0	4.5	0
	Oxygenated monoterpenes			91.3	65.4	87.7	89.6	43.4	20
	Sesquiterpene hydrocarbons			1.2	10.4	1.6	1.5	33.7	43.7
	Oxygenated sesquiterpene			1.5	2.5	2.1	3.2	3	32.9
	Total			95.2	80.6	91.4	94.3	84.6	96.6
	Total identified			99.3	99.4	98.6	98.5	99.8	99.9
	Total								
	Total identified								

RI, retention indices relative to C_6-C_{22}. N-alkanes on the HP-5 column.
Values of peak area (%) less than 0.2 % are deleted.

Table 4
Proximity Matrix for Similarity analysis.

	Cosine of Vectors of Values					
	1:1	2:2	3:3	4:4	5:5	6:6
1:1	1.000	0.002	0.994	0.994	0.005	0.003
2:2	0.002	1.000	0.094	0.003	0.216	0.179
3:3	0.994	0.094	1.000	0.991	0.015	0.012
4:4	0.994	0.003	0.991	1.000	0.009	0.010
5:5	0.005	0.216	0.015	0.009	1.000	0.376
6:6	0.003	0.179	0.012	0.010	0.376	1.000

Similarity analysis

According to the calculations of the similarity degree via vector included-angle cosine method, *O. vulgare* sample S1 is similar to samples S3 and S4, which indicated that the three samples (S1, S3, and S4) have little differences in composition (Table 4).

Principal component analysis (PCA)

To explore the relationship between the samples from various regions and their relation to specific volatile compounds, the GC-MS data was subjected to PCA. This analysis was employed to provide an overview of the capacity to distinguish essential oil components based on GC-MS data (select variables > 0.2% of 42 essential oil components from six production areas). As a result, the rate of accumulation of the previous three major compounds reached 99.6% (> 85%), which were 80.3%, 13.3%, and 6.0%, respectively. The first main compound found had high yields of eucalyptol and eugenol methyl ether; the second major compound was rich in germacrene D and caryophyllene oxide; and the third major element was rich in thymol.

The scree plot graph (Fig. 1), a plot of eigenvalue as a foundation of the eigenvalue number, was used to decide the number of principal components needed to be retained. The scree graph for the data (select variables > 0.2% of 42 essential oil components from six production areas) exhibited an ideal pattern, which has a relatively large disparity among the eigenvalue of factors 1, 2, and 3, hence, the three factors provided the most information. According to the principal component factor and weighted comprehensive scores, samples S2, S5, and S6 have better quality (Shanqiu of Henan, Abhui and Yili).

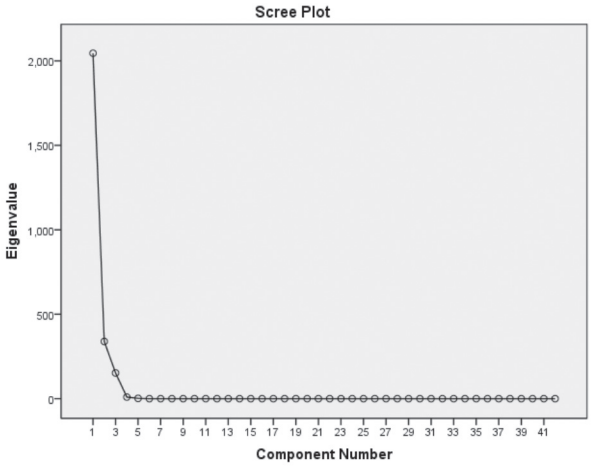

Figure 1 - Scree graph of essential oil from six different production areas.

Cluster analysis

Cluster analysis, can classify the number of samples studied into a number of groups, according to the chemical composition of essential oil by 'magnifying' their similarities. Results obtained from the cluster analysis showed the existence of a high inter-production variability within the essential oil of *O. vulgare* From the six production-samples submitted to multivariate analysis, two well-defined groups of essential oil were differentiated by cluster analysis (Fig. 2). Based on the data, (select variables > 0.2% of 42 essential oil components from six production areas) two subclusters can be observed: the first subset contains three production sites of samples S1, S3, and S4 (Kunlun Mountain of Hetian, Pakistan, Hetian), the second subset includes samples S2 (Shangqiu of Henan), S5 (Anhui) and S6 (Yili).

This current study was the first to determine the essential oil composition to conduct similarity analysis, PCA, and cluster analysis of *O. vulgare* from the six different production areas in China and Pakistan. The main aim of the present study was to offer research basis.

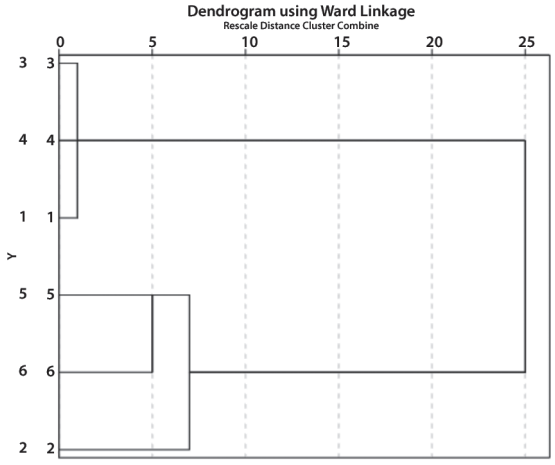

Figure 2 - Cluster analysis graph (select composition > 0.2 %) of the essential oil from six different production areas.

Authors' contributions

HG (PhD student) contributed in collecting plant sample and identification, running the laboratory work, drafted the paper. WL counted and analysied the data. GL contributed in collecting plant sample and identification. XZ contributed to the laboratory work and critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts interest.

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