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Comparative Chemical Composition Analysis of Essential Oils in Different Populations of Damask Rose from Iran

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ABSTRACT

Damask rose (*Rosa damascena* Mill.), belonging to the Rosaceae family, is a unique species. One of the major and popular growing regions of Damask rose is Kashan and its rose essential oil has unique scent and global reputation. The aim of this study was to compare the variation that naturally exists in quantity and quality of essential oils in different populations and selection of the best population. Unfortunately, there is inadequate information about flower oil yield of different populations of *R. damascena* from different regions of Kashan. Therefore, flowers of *R. damascena* Mill. were collected from fifteen important rose oil production regions of Kashan, Iran. The chemical composition of essential oil was analyzed by gas chromatography coupled with mass spectrometry. As a result, based on the dendrogram obtained from cluster analysis of chemical component data, fifteen *R. damascena* populations were grouped into three clusters. A total of fifty-five compounds were identified and quantified by GC–MS analysis in the rose oil. The essential oil contents (w/w) were ranged from 0.0020% to 0.0190% after isolation in Clevenger apparatus. The major components of the oil contained limonene (0.4–12.8%), 2-phenylethyl alcohol (1.0–1.3%), citronellol (16.2–57.8%), geraniol (0.9–14.1%), methyleugenol (0.5–2.5%), heptadecane (0.8–3.0%), 1-nonadecene (2.1–7.5%), nonadec-9-ene (14.9–30.2%), eicosane (1.0–3.3%), heneicosane (5.8–18.6%), tricosane (0.9–5.2%), and pentacosane (0.3–2.1%). The essential oil of Josheghan was considered to have a high quality in terms of richness in citronellol, geraniol, and 2-phenylethyl alcohol monoterpenes and has good potentials as antioxidant and strong fragrance in cosmetic and pharmaceutical industry.

Keywords: Cluster analysis, Citronellol, Geraniol, Gas chromatography, *Rosa damascena* Mill.

INTRODUCTION

Rosa L. as a major genus belongs to the family Rosaceae and comprises 200 species and up to 18000 cultivars (Gudin, 2000). They are mostly deciduous shrubs (Carins, 2003) distributed in the temperate zones of the northern hemisphere with showy and colorful flowers (Horn, 1992). One of the most important *Rosa* species is *R. damascena* Mill. of which some cultivars are used for oil production and others are cultivated throughout the world as garden roses (Guenther, 1952). It is called as Damask rose because, in the beginning, this species was introduced from Damascus to the Europe (Gault and Synge, 1987). *Rosa damascena* primarily grew in their natural habitat and still this plant is wild in some countries like Caucasus, Syria, Morocco and Andalusia (Chevallier, 2001). The origin of Damask rose is Iran and the Middle East region (Zargari, 1992; Krussman, 1981). This type of rose is known as national flower of Iran.

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Rosa damascena essential oil is used in the pharmaceutical industries as antibacterial (Basim and Basim, 2003), antioxidant (Ozkan *et al*., 2004), anticonvulsant (Kheirabadi *et al*., 2008), anti–infective, anti–inflammatory properties (Basim and Basim, 2003) and rose-flower extract is used in the anti–HIV (Mahmood *et al*., 1996) and anti–aging (Jafari *et al*., 2008). Rose oil is used in traditional medicine for the treatment of stress related conditions, in aromatherapy for treatment of cardiac diseases and it is widely used in perfumery and cosmetic industry as a base component of many modern perfumes and cosmetics (Bekhradi and Khayat Kashani, 2006).

Essential oil from *R. damascena* is a complex mixture of more than 300 different components in various concentrations, whose amount and composition vary widely between specimens and cultivars. Identifying the percentage of major components is one of the important parameters, which determines the quality of rose oil (Boelens and Boelens, 1997). *Rosa damascena* essential oil is of worldwide economic importance and the demand and its price is growing continuously in the national and international markets. Thus, essential oils represent a significant upstream business opportunity for the world agricultural sector (Kumar, 2013). High quality rose oil should have a specific gravity of 0.861 g, a reflective index of 1.46, an optical rotation between –3 to –4, and a congealing point between 18°–22 °C (Weiss, 1997).

The present industrial facilities require large amounts of rose flowers for single distillation because 3500 to 4000 kilograms of rose flowers are necessary to produce 1 kg of rose oil. Therefore, the best genotypes should be identified and used based on the percentage and components of essential oil (Farooq, 2011).

Previously, some reports have been published on the quality and quantity of chemical and volatile compounds of *R. damascena* essential oils from different parts of the world including Iran, Turkey, Bulgaria, China, Saudi Arabia, Pakistan, India and etc. According to their results, citronellol, geraniol, 2-phenylethyl Alcohol, 1-nonadecene, nonadec-9-ene, eicosane, nerol, heneicosane, tricosane, α–guaiene, eugenol, and geranyl acetate have been considered as the main components of essential oils (Jirovetz *et al*., 2005; Khan and Rehman, 2005; Loghmani–Khouzani *et al*., 2007; Gochev *et al*., 2008; Yassa *et al*., 2009; Zeinali *et al*., 2009; Batooli and Safai–Ghomi, 2010; Moeina *et al*., 2010; Yousefi *et al*., 2011; Kurkcuoglu *et al*., 2013; Halavani, 2014).

One of the major and popular growing regions of Damask rose is Kashan, whose rose essential oil has unique scent. Therefore, the aim of the present work was to evaluate and compare the variation naturally existing in quantity and quality of essential oils isolated from different *R. damascena* populations from various areas of Kashan, as one of the most important rose oil production of Iran. From a commercial point of view, such study may eventually lead to the identification and selection of the best populations with the highest oil contents and the most favorite component profiling. The results could also provide practical information for future collection of Damask rose germplasm and breeding program.

MATERIALS AND METHODS

Plant Material

The whole rose flowers (petals and sepals) were harvested at the time of full bloom in May and June 2015 from fifteen important rose oil production regions of Kashan (Table 1, Figure 1). The samples were harvested in the morning to get the maximum oil content. Flowers were randomly sampled from more than fifty plants for each population. All collected specimens were cultivated populations. The environmental conditions and substrates are almost similar for most populations.

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Table 1. *Rosa damascena* populations collected from different regions of Kashan.

Number	Abbreviation	Collection Site	Voucher Number	Altitude(m)
1	Gha	Ghazaan	UKH[a] 209	2316
2	Qam	Qamsar	UKH 210	1900
3	Zey	Zeynabad	UKH 211	2370
4	Mar	Margheh	UKH 212	2378
5	Via	Viduja	UKH 205	1988
6	Vid	Viduj	UKH 206	1976
7	Bar	Barzok	UKH 213	1600
8	Mas	Mashhad Ardahal	UKH 207	1770
9	Kas	Kashan	UKH 214	982
10	Jos	Josheghan	UKH 208	2345
11	Aza1	Azaran (Valley)	UKH 202	1931
12	Aza2	Azaran (Plain)	UKH 201	2700
13	Ezn	Eznaveh	UKH 200	2690
14	Gho	Ghohrud	UKH 203	2400
15	Kam	Kamoo	UKH 204	2345

[a] University of Kashan Herbarium

Figure 1. Distribution map of *R. damascena* populations collected from different regions of Kashan.

Hydro–Distillation

To isolate the essential oil, three hundred grams of fresh flowers were mixed with distilled water (700 mL) and placed in a flask (2 L) connected to the condenser of a Clevenger apparatus (Clevenger, 1928). The isolation continued for 3.5 hours and the essential oil and water mixture were finally separated by decantation. The essential oils were dried over anhydrous sodium sulfate (Na_2SO_4), stored in a dark glass bottle, and kept at low temperature (–20 °C) until analysis. The oil contents were calculated as proportion (%) of flower weight.

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GC–MS Analysis

The essential oil was analyzed with gas chromatography–mass spectrometry (GC/MS) for identifying the essential oil components. The chromatograph apparatus was Agilent technology 6890A system coupled with mass spectrometer model 5973N, construction Agilent Company (Palo Alto, CA, USA) equipped with a Quadrupole detector. The analysis was performed under the following conditions:

Capillary column of HP–5MS (30 m length, 0.25 or 0.32 mm id, film thickness 0.25µm); injector and detector temperature 250 °C. Stove heat program started at 60 °C and increasing to 246 °C rising at 3 °C.min^{-1}.; split ratio 1:50, detector 70 eV, carrier gas: helium (1.5 Ml.min^{-1}.); sample injected 1 µL. MS interface temp: 250 °C; MS mod: EI; mass range: 40-500 u; scan speed: 769 u/s. Data handling was made through Chemstaion G1701CA version C.00.00.

Identification and Data Analysis

Components of essential oil were identified by comparison of mass spectra of each peak (Figures. S1-S15, supplementary material) with those in a mass spectra library (The Wiley Registry of Mass Spectral Data, 6th Ed.) and confirmed by comparison of retention indices relative to n–alkanes obtained by GC with those of authentic samples (Adams, 1989).

A data matrix based on component of essential oil percentage was assembled and tree was reconstructed with the unweighted pair–group method with arithmetic averages (UPGMA) (Sneath and Sokal, 1963) and Average Distance coefficients using MVSP software Vers. 3.2 (Kovach, 1985-2002). Essential oil variations among populations were analyzed using Principal Coordinates Analysis (PCO) performed in MVSP software (Vers. 3.2). Charts were created in the Microsoft Excel, 2010. The percentage composition of samples was calculated from the GC peak areas.

RESULTS AND DISCUSSION

Essential Oil Components

The aim of the present study was to analyze the content (w/w) and the chemical composition of the essential oils from *R. damascena* cultivated in different areas of Kashan, an important region of Iran in rose oil industry. The essential oil yields of the samples ranged from 0.0020 to 0.0190 (w/w %). The yield of oil from *R. damascena* in different regions of Kashan decreased in the order of Azaran (valley) (0.0190%)> Kashan (0.0170)> Zeynabad (0.0150)> Azaran (plain) (0.0148)> Josheghan (0.0138)> Ghazaan (0.0132)> Ardahal (0.0130)> Margheh (0.0120)> Barzok (0.0110)> Viduj (0.009)> Ghohrud (0.0044)> Qamsar (0.0042)> Kamoo (0.0037)> Viduja (0.0034)> Eznaveh (0.0020). The results regarding oil yield of petals and sepals from different *R. damascena* populations are presented graphically in Figure 2. According to the results, *R. damascena* population from Azaran1 contained the highest amount of oil, while the lowest value was seen

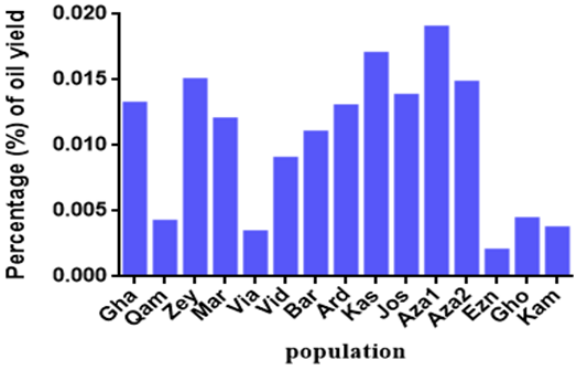

Figure 2. Percentage of oil from petals and sepals (W/W) in several *R. damascena* populations collected from different regions of Kashan.

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Table 2. The essential oil composition of *R. damascena* populations from different regions of Kashan.

NO	Components	RI [a]	RI [b]	Formula	Percent (%) Populations													
					Gha	Qam	Zey	Mar	Via	Vid	Bar	Mas	Kas	Jos	Aza1	Aza2	Ezn	Gho	Kam
1	Methylcyclopentane	627	620	C_6H_{12}	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Pyridine	753	740	C_5H_5N	0	0	0	0	0	0	0	0	0	0	0	0	0.12	0	0
3	Octane	800	772	C_8H_{18}	0	0	0.18	0	0	0	0	0	0	0	0	0	0	0	0
4	Heptanal	882	889	$C_7H_{14}O$	0	0	0	0	0	0	0	0.12	0	0	0	0	0	0	0
5	Alpha-Pinene	926	918	$C_{10}H_{16}$	0	0.09	0.32	0	0	0	0.13	0.07	0	0	0	0	0	0	0
6	Myrcene	991	975	$C_{10}H_{16}$	0	0.14	0.25	0	0	0	0	0	0	0	0	0	0	0	0
7	Decane	1000	983	$C_{10}H_{22}$	0	0.22	0.46	0	0.25	0	0.25	0.20	0.23	0.12	0.21	0	0.12	0	0.28
8	Beta-Phellandrene	1031	993	$C_{10}H_{16}$	0	0.11	0.23	0	0	0	0	0	0	0	0	0	0.07	0	0
9	(6Z)-2,6-Dimethylocta-2,6-diene	1004	1008	$C_{10}H_{18}$	0.68	0.43	0.48	1.01	0.65	0.48	0	0.58	0.64	0.38	0.36	0.34	0.51	0.36	0
10	Limonene	1013	1018	$C_{10}H_{16}$	1.87	5	12.80	3.59	6.87	4.59	6.94	3	0	1.58	2.19	4.55	3.45	0.40	8.78
11	Linalool	1097	1091	$C_{10}H_{18}O$	0.47	0.74	0.53	0.35	0.43	0.44	0.39	0.83	1.28	0.44	0.27	0.54	0.79	0	0.25
12	Rose oxide	1127	1102	$C_{10}H_{18}O$	0	0.40	0.56	0.38	1.32	0	0.5	0.50	0.24	0.46	1.07	0	0.26	0	0
13	2-phenylethyl Alcohol	1110	1113	$C_8H_{10}O$	0	0.95	0	0	1.44	0	0	1.27	0	1.02	1.09	0	0.96	0	0
14	Benzene, 1,2,4,5-tetramethyl-	1124	1116	$C_{10}H_{14}$	0	0	0	0	0	0	0	0	0	0	0	0	0.10	0	0
15	Terpinen-4-ol	1177	1173	$C_{10}H_{18}O$	0	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0
16	Dodecane	1200	1186	$C_{12}H_{26}$	0	0.21	0	0	0	0	0	0	0	0	0	0	0.30	0	0
17	Citronellol	1229	1237	$C_{10}H_{20}O$	43.67	33.04	51.43	41.39	40.68	36.21	29.52	40.03	42.04	57.77	47.14	48.92	43.02	26.80	16.18
18	2,6-Octadienal, 3,7-dimethyl-, (E)-	1250	1270	$C_{10}H_{16}O$	0	0.13	0	0	0	0	0	0.23	0	0	0	0	0.16	0	0
19	Geraniol	1253	1257	$C_{10}H_{18}O$	3.38	3.03	1.91	2.54	1.54	0	0.92	5.30	14.9	5.02	2	0	4.53	1.39	0
20	Citronellyl-acetate	1340	1354	$C_{12}H_{22}O_2$	0	0.11	0	0	0	0	0	0.27	0	0	0	0	0.13	0	0
21	Geranyl Acetate	1363	1377	$C_{12}H_{20}O_2$	0.22	0.45	0.15	0.49	0	0	0	0.94	1.26	0.29	0	0	0.65	0	0
22	Tetradecene	1385	1382	$C_{14}H_{28}$	0	0.20	0	0	0	0	0	0.18	0	0	0	0	0.31	0	0
23	Methyleugenol	1390	1402	$C_{11}H_{14}O_2$	1.55	1.56	1.25	1.70	2.47	1.68	0.66	0.81	2.07	0.50	1.48	1.17	0.68	0.74	0
24	Beta-Elemene	1391	1386	$C_{15}H_{24}$	0	0.53	0	0	0	0	0	0	0	0	0	0	0	0	0
25	Tetradecane	1400	1390	$C_{14}H_{30}$	0	0.12	0	0	0	0	0	0	0	0	0	0	0.12	0	0
26	Beta-Caryophyllene	1418	1410	$C_{15}H_{24}$	0.51	0.65	0.29	0.50	0.27	0.52	0.32	0.18	0.64	0	0	0	0.46	0.72	0.51
27	Alpha-Guaiene	1439	1430	$C_{15}H_{24}$	0.37	0.63	0.29	0.54	0.45	0.71	0.29	0	0.36	0.14	0	0	0.23	0.87	0.76
28	Alpha-Humulene	1446	1444	$C_{15}H_{24}$	0.39	0.51	0.31	0.51	0.34	0.43	0.22	0	0.44	0.14	0	0	0.24	0.61	0.53
29	Germacrene-D	1480	1474	$C_{15}H_{24}$	0.88	0.96	0.45	0.96	0.47	0.60	0.39	0.11	0.82	0.21	0.28	0	0.40	1.50	1.13
30	Eugenol Acetate	1482	1483	$C_{12}H_{14}O_3$	0.68	0.99	0.57	0	0.53	0	0.34	0.48	1.99	0	0.58	0	0.68	0	0
31	Pentadecane	1500	1486	$C_{15}H_{32}$	0.49	0.31	0	0.23	0.46	0.29	0.19	0.25	0.29	0.14	0.44	0.41	0.14	0.28	0.23
32	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	1503	1495	$C_{15}H_{24}$	0	0.18	0	0	0	0	0	0	0	0	0	0	0	0	0
33	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4dimethyl-7-(1-methylethenyl)-,[1s-(1a,7a,8Ab)]-	1508	1498	$C_{15}H_{24}$	0.32	0.62	0.33	0.54	0.56	0.77	0.34	0	0	0	0	0	0.24	0.77	0.68

[a] Retention Indices published value (NIST Chemistry WebBook), [b] Relative Retention Indices as determined on a HP-5MS column using the homologous of n-alkanes

Continued…

Continued of Table 2.

NO	Components	RI [a]	RI [b]	Formula	Percent (%)														
					Populations														
					Gha	Qam	Zey	Mar	Via	Vid	Bar	Mas	Kas	Jos	Aza1	Aza2	Ezn	Gho	Kam
34	Delta-Cadinene	1524	1520	$C_{15}H_{24}$	0	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0
35	Hexadecane	1600	1593	$C_{16}H_{34}$	0	0.18	0	0	0	0	0	0	0	0	0	0	0.18	0	0
36	Heptadec-8-ene	1664	1676	$C_{17}H_{34}$	0.33	0.16	0	0	0	0	0	0.12	0	0	0.25	0	0	0	0
37	Heptadecane	1700	1695	$C_{17}H_{36}$	3.04	1.70	1.16	2.34	2.34	1.86	2	1.59	2.94	0.77	2.32	1.83	1.15	2.12	2.53
38	Farnesol	1706	1754	$C_{15}H_{26}O$	0.75	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	Trans-Farnesol	1707	1704	$C_{15}H_{26}O$	0	0	0	0	0	0	0	0	0	0	0	0	0.07	0	0
40	Octadecane	1800	1796	$C_{18}H_{38}$	0.28	0.31	0	0	0.28	0	0.25	0.23	0.29	0.15	0.27	0	0.27	0.34	0.38
41	(E)-5-Octadecene	1811	1793	$C_{18}H_{36}$	0	0	0	0	0	0	0	0	0	0	0	0	0.16	0	0
42	1-Octadecene	1845	2074	$C_{18}H_{36}$	0	0	0	0	0.24	0	0	0	0	0	0.33	0	0	0	0
43	1-Hexadecanol	1860	1830	$C_{16}H_{34}O$	0	0.11	0	0	0	0	0	0	0	0	0	0	0.22	0	0
44	Nonadec-9-ene	1875	1910	$C_{19}H_{38}$	22.34	24.45	17.72	28.16	7.61	27.32	33.29	22.35	21.16	19	25.49	25.98	18.27	29.72	37.35
45	Palmitic acid	1984	1970	$C_{16}H_{32}O_2$	0	0	0	0	0	0	0	0	0	0	0	0	0.59	0	0
46	Nonadecane	1900	2065	$C_{19}H_{40}$	0	0	0	0.32	0	0	0	0	0	0	0	0	0	0.38	0.42
47	Eicosane	2000	2002	$C_{20}H_{24}$	1.28	2.46	1.04	1.83	1.86	2.97	2.25	2.21	1.25	1.38	2.16	1.33	1.84	3.33	3.63
48	10-Heneicosene (c, t)	2060	2078	$C_{21}H_{42}$	0	0.28	0	0	0	0	0	0.19	0	0	0	0	0.17	0	0
49	1-Octadecanol	2074	2091	$C_{18}H_{38}O$	0	0.28	0	0	0	0	0	0.23	0	0	0.24	0	0.26	0	0.25
50	Heneicosane	2100	2108	$C_{21}H_{44}$	5.75	12.82	6.06	9.73	8.85	15.34	16.76	12.43	5.86	8.22	9.40	10.82	12.55	20.86	18.55
51	Docosane	2200	2198	$C_{22}H_{46}$	0	0.29	0	0	0	0.42	0.28	0.31	0	0	0	0	0.28	0.63	0.53
52	Tricosane	2300	2303	$C_{23}H_{48}$	0.97	3.08	0.93	2.29	1.71	4.02	2.92	3.2	1.22	1.21	1.89	2.38	2.94	5.75	4.94
53	Pentacosane	2500	2498	$C_{25}H_{52}$	0	1.01	0.32	0.59	0.41	1.36	0.84	1.17	0.46	0.42	0.55	0.71	0.97	2.06	1.76
54	Tetracosane	2567	2400	$C_{24}H_{50}$	0	0.18	0	0	0	0	0	0.17	0	0	0	0	0.16	0.36	0.33
55	Hexatriacontane	3600	3550	$C_{36}H_{74}$	0	0	0	0	0	0	0	0	0	0.48	0	0	0	0	0
	Total Monoterpen hydrocarbons				2.55	5.77	1.67	4.6	7.52	5.07	7.07	3.65	0.64	1.96	2.55	4.89	4.03	0.76	8.78
	Total Monoterpen oxygenated				47.74	38.02	54.58	45.15	49.97	36.65	31.33	47.65	59.72	64.03	50.48	49.46	49.54	28.19	16.43
	Total Sesquiterpen hydrocarbons				2.47	4.15	1.67	3.05	2.09	3.03	1.56	0.29	2.26	0.49	0.28	0	1.57	4.47	3.61
	Total Sesquiterpen oxygenated				0.75	0	0	0	0	0	0	0	0	0	0	0	0.07	0	0
	Total Phenylpropanoids				2.23	2.55	1.82	1.70	3	1.68	1	1.29	4.06	0.50	2.06	1.17	1.36	0.74	0
	Total Nonterpenoids				34.61	48.37	27.87	45.49	24.01	53.58	58.4	44.95	33.7	31.89	43.55	43.46	40.78	65.83	71.18
	Unknown				0	0.22	0	0	0	0	0	0	0	0.16	0	0	1.25	0	0
	Total identified				87.13	99.81	87.61	99.99	88.03	94.94	99.36	99.1	100	100	100	98.98	98.36	99.99	99.55

[a] Retention Indices published value (NIST Chemistry WebBook), [b] Relative Retention Indices as determined on a HP-5MS column using the homologous of n-alkanes

in Eznaveh population. This showed a significant variation in the oil contents among different Iranian populations of *R. damascena*. Lower variations reported in the essential oil from Bulgarian or Turkish roses ranged from 0.032–0.049% (from flower)(Kovatcheva *et al*., 2011) or 0.032–0.040% (from petals)(Baydar and Baydar, 2005) (w/w), respectively. The reason for these relatively low variations might be related to the fact that *R. damascena* from these countries mainly originated from cultivated single genotypes which have been vegetatively propagated for years (Agaoglu *et al*., 2000).

Additionally, a high variability in the chemical composition of rose oil from Kashanian populations has been detected by GC–MS analysis. More than fifty-five components were identified in the essential oil of *R. damascena* populations. The identified components along with their percentage are shown in Table 2. A total of 23, 43, 25, 21, 24, 18, 23, 31, 21, 22, 22, 12, 41, 21 and 21 compounds were identified in the essential oil of *R. damascena* from Ghazaan, Qamsar, Zeynabad, Margheh, Viduja, Viduj, Barzok, Mashhad Ardahal, Kashan, Josheghan, Azaran1, Azaran2, Eznaveh, Ghohrud and Kamoo, respectively. Among the components, thirteen included limonene, 2-phenylethyl Alcohol, citronellol, geraniol, methyleugenol, heptadecane, 1-nonadecene, nonadec-9-ene, eicosane, heneicosane, tricosane, and pentacosane were identified in all populations, representing more than 88% of the total oil (Table 2).

Alcoholic monoterpene citronellol, which is an important constituent in *R. damascena* (Kovatcheva *et al*., 2011) had the highest value in *R. damascena* from Josheghan (57.8%) and the lowest value in *R. damascena* from Kamoo (16.2%). Citronellol was the dominant compound in oils of all Kashanian populations. The percentage of this component in the oil of *R. damascena* from other populations was in the following order: 51.4% (Zeynabad), 48.9% (Azaran2), 47.1%, (Azaran1), 43.7% (Ghazaan), 43.0% (Eznaveh), 42.0% (Kashan), 41.4% (Margheh), 40.7% (Viduja), 40.0% (Ardahal), 36.2% (Viduj), 33.0% (Qamsar), 29.5% (Barzok) and 26.8% (Ghohrud) (Table 2). This compound, as one of the most important ingredient responsible for scent of rose oil, has not been observed in oils isolated from *R. damascena* cultivated in Guilan (north of Iran) and India (Yassa *et al*., 2009; Perumal *et al*., 2012). In these studies, rose essential oils were isolated from rose petals. *Rosa damascena* from Pakistan and south part of Iran have also been reported to contain a low value of citronellol (3.7% and 6.1%, respectively) (Moeina *et al*., 2010; Khan and Rehman, 2005). Also, Khan and Rehman used petals to isolate essential oils. The composition of the major oil components in the rose populations from Iran and other countries is compared in Table 3.

Geraniol, as another major component, was found to be highest (14.1%) in the oil isolated from the *R. damascena* population from Kashan. The values were 5.3%, 5.0%, 4.5%, 3.4%, 3.0%, 2.5%, 2.0%, 1.9%, 1.5% and 1.4% which were related to *R. damascena* populations from Mashhad Ardahal, Josheghan, Eznaveh, Ghazaan, Qamsar, Margheh, Azaran1, Zeynabad, Viduja and Ghohrud, respectively. This component was not detected in the essential oil from Viduj and Azaran2. Although, according to the findings of Loghmani–Khouzani *et al*. (2007), geraniol was among the main components of *R. damascena* from Viduj regions (18.1%), it was not observed in Viduj population. This might be related to the different flower harvesting time or sample-to-sample variations.

2-Phenylethyl alcohol is another important constituent of rose oil for fragrance, but low values of this compound were detected or even absent in some populations tested in the present study. Among the populations, Viduja, Mashhal Ardahal, Azaran1, Josheghan, Eznaveh and Qamsar contained 1.4%, 1.3%, 1.1%, 1.0%, 1.0% and 1.0% 2-phenylethyl alcohol, respectively. This

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Table 3. The comparison of major essential oil composition percentage of *R. damascena* in other populations from Iran and different countries.

Components	Percent (%)													
	Populations from other studies													
	Qamsar	Dorrin	Viduj	Khonab	Vidor	Kahriz No	Guilan	South of Iran	Chin	Saudi Arabia	Bulgaria	Pakistan	India	Turkey
2-Phenylethyl Alcohol	0.2	–	0.4	0.4	0.3	3.1	0.9	–	–		–	70.9	27.2	–
Linalool	–	–	0.3	–	–	–	3.8	–	–	8.0	–	1	–	–
Rhodinol	–	–	–	–	–	–	–	–	–	–	–	2.7	–	–
Citronellol	26.1	23.6	32.5	14.9	47.4	34.7	–	6.1	30.7	23.0–28.0	23.0	3.7	–	38.7
Nerol	–	–	–	–	1.2	5.0	3.1	–	5.8	6.0–11.0	7.1	–	–	8.3
Geraniol	–	5.7	18.1	–	–	–	15.1	–	16.1	14.0–20.0	19.0	1.5	–	17.2
Eugenol	–	–	–	–	–	–	–	–	–	–	–	1.7	–	–
Methyleugenol	2.6	0.9	0.6	1.8	–	–	–	–	–	–	–	–	–	–
Beta–Caryophyllene	–	–	–	–	–	7.8	–	–	–	–	–	–	–	–
Germacrene–D	–	–	–	–	–	–	–	1.8	–	–	–	–	–	–
Pentadecane	2.7	1.3	0.1	1.0	0.2	–	–	–	–	–	–	–	–	–
Hexadecane	–	–	–	–	–	–	–	–	–	–	–	–	7.8	–
Cis–farnesol	–	–	1.6	–	–	–	–	–	–	–	–	–	–	–
Heptadecane	–	1.6	1.4	1.6	1.1	–	2.9	2.4	–	–	–	–	–	–
Octadecane	–	–	–	–	6.1	–	2.8	–	–	–	–	–	10.5	–
Nonadec-9-ene	5.0	4.3	4.9	3.3	2.6	0.6	18.6	5.7	–	–	–	–	–	–
Nonadecane	40.7	39.7	24.0	10.4	–	14.7		39.7	17.0	11.0–16.0	11.1	–	3.2	7.2
9–Eicosene	–	–	–	–	–	–	0.3	–	4.7	–	–	–	–	–
Eicosane	1.7	1.9	1.3	20.5	0.7	–	–	4.5	–	–	–	–	0.2	–
Heneicosane	13.9	–	9.6	7.3	17.5	10.3	–	32.4	7.0	7.0	–	–	10.5	–
Docosane	0.4	19.5	–	7.0	–	–	–	7.3	–	–	–	–	–	–
Tricosane	–	–	–	–	–	4.3	–	–		–	–	–	1.2	–
Citronellyl acetate	–	–	0.1	–	–	–	–	–	–	–	–	2.5	–	–
n—Tricosane	–	–	–	–	–	–	16.7	–	–	–	–	–	–	–
Pentacosane	–	–	–	2.4	–	–	3.4	–	–	–	–	–	–	–
Trisilocendecanol	–	–	–	–	–	–	–	–	–	–	–	–	3.5	–
Hexatriacontane	–	–	–	–	–	–	24.1	–	–	–	–	–	–	–

component was not present in essential oils other tested populations (Table 2).

The highest percentage of 2-phenylethyl alcohol was reported in a Pakistanian genotype (70.9%) (Khan and Rehman, 2005). In this research, high amount of 2-phenylethyl alcohol was due to the use of the distillation of recovered solvent method. It is hardly possible to hydro-distillate rose oil, because of very good solubility of this alcohol in water. This compound was also high and one of the main component of rose essential oil from India with a percentage of 27.2 (Perumal *et al*., 2012).

Methyleugenol is a naturally occurring carcinogenic phenylpropanoid compound (Rusanov *et al*., 2012) and is a controlled item (at least in Europe) in rose essential oil, due to alleged carcinogenic properties. Therefore, the variable concentration of methyl eugenol may be considered in priority when developing an agricultural selection program. This compound was observed in all populations, except Kamoo. This component was the highest and the lowest in populations from Viduja (2.5%) and Josheghan (0.5%), respectively. This component has a positive effect in rose scent erection (Baser, 1992) and anti bactericidal properties.

Among the components, Limonene was the major compound in all populations, except Kashan. Interestingly, Limonene, which was rarely reported in other studies (Jirovetz, 2002), ranged from 0.4% (Ghohrud) to 12.8% (Zeynabad) in the present study.

The highest value of heptadecane (3.0%) was found in rose oil isolated from Ghazaan population, while the population from Josheghan contained the lowest value of this component (0.8%). In this study, the highest (3.3%) and the lowest (1.0%) values of eicosane belonged to Kamoo and Zeynabad populations, respectively, while eicosane as high as 20.5% has been previously reported for Khonab (Loghmani–Khouzani *et al*., 2007). Nonadec-9-ene content had the highest values in Kamoo (7.5%) and the lowest in Josheghan (2.1%) populations. The amount of nonadecane was found at a high level (15.9–30.2%) in all tested populations. In this term, Barzok population had the highest content of nonadecane. Eicosane showed the highest value (3.3%) in Kamoo population, while Zeynabad population showed the lowest value of 1.0%.

The contents of nonadec-9-ene, nonadecane and eicosane (compounds that reduce the oil quality) were the highest in Kamoo population with the contents of 7.5%, 29.8%, and 3.3%, respectively. The lowest value of nonadec-9-ene was observed in *R. damascena* population from Josheghan (2.1%), whereas, higher values of nonadecane up to 40.7%, 39.7% and 39.7 % has also been previously observed in landrace from Qamsar, south of Iran, and Dorrin, respectively (Loghmani–Khouzani *et al*., 2007; Khan and Rehman, 2005). The maximum amount of heneicosane (20.9%) was observed in the oil isolated from Ghohrud population, while Ghazaan was the poorest population for this component (5.8%). The component heneicosane was observed in the Viduj population (15.3%) and was higher than that reported by Loghmani–Khouzani *et al*. (9.6%) (2007); however, there was no significant difference in heneicosane content of Qamsar population between the present study and Loghmani–Khouzani *et al*. results. The results also showed that tricosane was one of the major components in rose oil from all the populations tested in our study and ranged from 0.9% in Margheh to 5.2% in Ghohrud populations, however, it has not been reported in most other studies. Ghohrud also had the highest content of pentacosane (2.1%), while the minimum content of this compound was recorded in Zeynabad population (0.3%). The latter component was not present in the essential oil of Ghazaan population (Table 2). In this study, two major components of the oil were pentacosane and tricosane, which were also identified in Qamsar and Viduj populations, but, these compounds have not been detected in the oils isolated previously from

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these populations (Loghmani–Khouzani *et al*., 2007).

The chemical composition of Bulgarian rose essential oil was similar to that of rose essential oil from Turkey (Jirovetz *et al*., 2006; Gochev *et al*., 2013), which might emphasize again on the unique origin of these two European genotypes. Overall results showed huge variations not only in the quality but also in the quantity of the oils isolated from different populations of Iranian roses. This is seemingly related to the good potential of Iranian climates for trigging the specification of roses. Besides their genetic makeup, the variations in percentage of chemical composition in Iranian populations of roses could also be due to the differences in environmental culture condition, genotype, conditions for keeping the flowers after harvest until the time of extraction of the essential oil, distillation temperature, duration of extraction, extraction methods, developmental stage of plants, or harvesting time of flower.

From a commercial point of view, the amount of compounds such as citronellol, geraniol, and 2-phenylethyl alcohol is considered as one of the best criteria for assaying the quality of rose essential oil and has significant importance for the aroma quality (Chen *et al*., 1985; Sood *et al*., 1994). These compounds were reported to be the chief constituents in the rose oil (Tambe and Gotmare, 2016). Therefore, selection of the genotypes with the highest content of these valuable compounds would be a critical key to increase the oil productivity and to decrease the expense of oil production in future industry. The comparative percentage of these three compounds in *R. damascena* populations tested in our study is shown in Figure 3.

Many other components that are present in low amount are important for the quality of the rose oil. The key flavor components that contribute to the distinctive scent of rose are beta-ionone beta-damascenone, beta-damascone, and rose oxide (Kovats, 1987; Ohloff, 1994). Beta-damascenone and beta-damascone compounds that are considered as the marker for the quality of rose oil and beta-ionone were not present in essential oils of all studied populations. The highest amount (0.8%) of rose oxide was in Viduja. This monoterpene was not observed in Ghazaan, Viduj, Kashan, Azaran2, Ghohrud, and Kamoo populations.

It was shown that the essential oil from Josheghan contained large amounts of monoterpenes (66%) that constitute more than half of the total oil. These compounds are mainly used in perfumes and scents; Kamoo population was characterized with a large content of sesquiterpenes and a less amount of monoterpenes (16.2%). In addition, the Kamoo's oil was rich in waxy and heavy compounds such as nonadecane, nonadec-9-ene, heneicosane, and tricosane comprising 60.2% of the oil and the long–chain compounds called Steroptens that decrease the quality of oil and cause perfume not to smell good. Besides, rose oxide, which is a fragrance chemical, was found in Josheghan (0.5%) rose oil, but was not present in Kamoo population.

Cluster Analysis and PCA

Cluster analysis was performed on the whole set of data with UPGMA method and percent similarity coefficients (Figure 4). Dendrogram showed 3 main clusters at 0/13 identity level.

Kashan population formed individual clusters I. Cluster II comprised of Ghohrud, Kamoo, Azaran2, Barzok and Viduj and Cluster III included nine populations: Viduja, Zeynabad, Josheghan, Azaran1, Margheh, Eznaveh, Mashhad Ardahal, Qamsar, and Ghazaan.

Based on the data from oil constituents, PCO analysis also classified the populations into three distinct groups (Figure 5), more supporting the results from cluster analysis by UPGMA. Eigenvalues and individual and cumulative percentages of variance among the data accounted for the first three axes of

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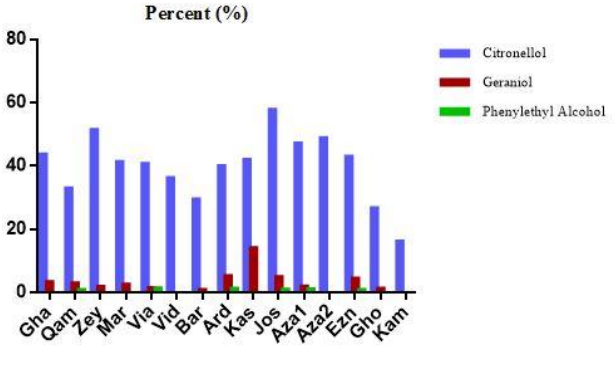

Figure 3. Comparative percentage of citronellol, geraniol and 2-phenylethyl alcohol among *R. damascena* populations in different regions of Kashan.

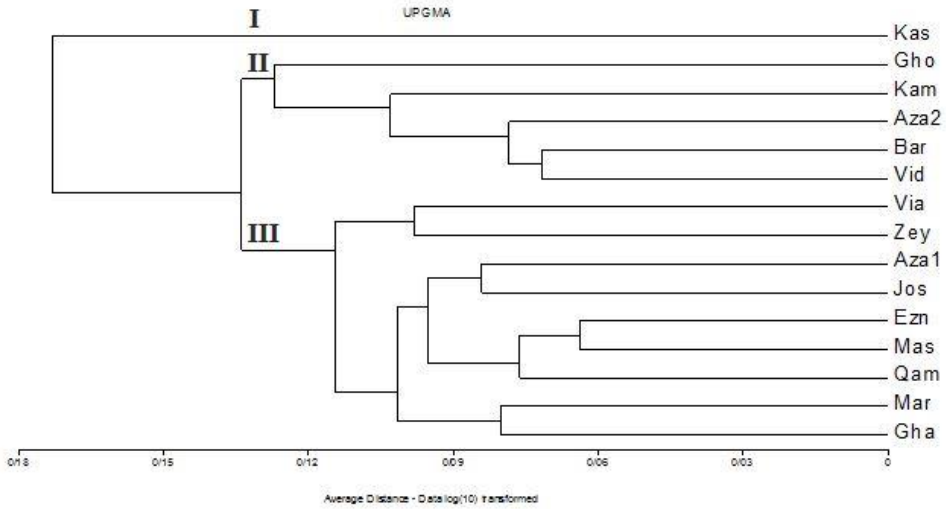

Figure 4. The UPGMA dendrogram of *R. damascena* populations in different regions of Kashan based on oil constituents.

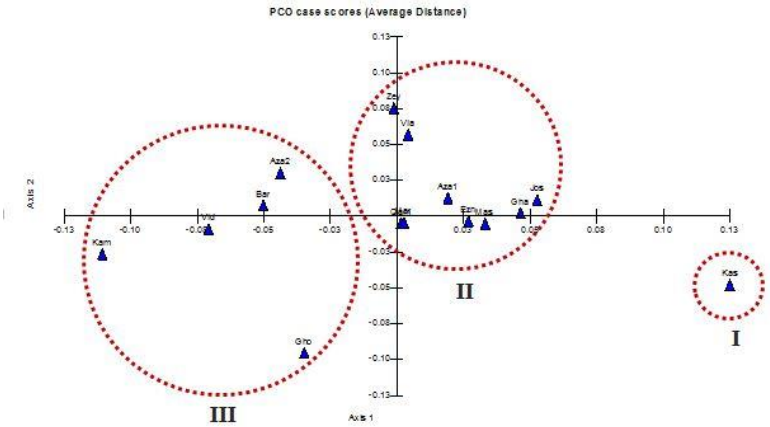

Figure 5. Plot of Principal Coordinates Analysis of *R. damascena* populations in different regions of Kashan for oil constituents.

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the principle components analysis and are presented in Table 4.

These results revealed that the populations clustered in each group had the same quality but different quantitative composition.

Kashan population, which clustered individually, contained high amounts of linalool (1.3%), geraniol (14.9%), phenol, 2–methoxy–4–(2–propenyl)– acetate (2%), and geranyl acetate (1.26%) compared to other populations. Limonene was not detected in this population. In the group II (comprised Ghohrud, Kamoo, Azaran2, Barzok and Viduj), all populations had large amounts of sesquiterpenes which can reduce the quality of the rose oil. In group III (with populations of Viduja, Zeynabad, Joshaghan, Azaran1, Margheh, Eznaveh, Mashhad Ardahal, Qamsar, and Ghazaan), the plants have a large amount of citronellol, geraniol, and 2-phenylethyl alcohol. As a result, the petals of *R. damascena* from this group had gentle smell but possible powerful antioxidant potential. This group might have a negligible value to be used as a main constituent for the fragrance. Interestingly, all populations in group III had high levels of rose oxide. This compound also contributes to the flavor and scent of *R. damascena*.

Based on the results, there was no systematic trend between the geographical distance and the essential oil composition, so that the two populations of Azaran1 and Azaran2, despite their similar geographical location, were differentiated in terms of the compositions of essential oils and were divided in two groups. Because these two populations are topographically different i.e. one of them is located in the valley and other in the plain. Also, the Viduj and Viduja populations are geographically close but they were placed in two completely different groups. Qamsar and Ghazaan are near each other and environmentally similar, and were placed in one group. In this grouping, Kashan was placed in a separate group, it was different with the rest of the populations geographically and altitudinally. Margheh, Zeynabad and Ghohrud are geographically beside each other, but in terms of the chemical composition of essential oils, Ghohrud was separated from the others and placed in another group. Josheghan and Kamoo are similar in height, but they differ in chemical composition. Eznaveh and Mashhad Ardahal geographically are different from Viduja, Zeynabad, Josheghan, Azaran1, Margheh, and Qamsar, but in terms of chemical composition are in the same group. Therefore, these differences can be attributed to genetic differences and other ecological factors such as soil texture, gradient direction, microclimate, etc. It is suggested that the soil analysis further clarifies the interpretation of these groupings.

In conclusion, our study showed that there are significant differences in the quantity and chemical profile of the essential oils of *R. damascena* originating from different regions of Kashan. With respect to medicinal or pharmaceutical importance, population from Joshghan is suggested for the cultivation and exploitation of its oil in pharmaceuticals and cosmetics, especially due to its richness in citronellol and geraniol monoterpens. The observed variation in the oil quantity and chemical composition of the oils from different population can be attributed to physiological and ecological factors as well as genetic differences.

Table 4. Eigenvalues, individual and cumulative percentage variance of populations data accounted for the first three axes of the Principal Coordinates Analysis (PCO) for the *R. damascena* populations in Kashan.

	Axis 1	Axis 2	Axis 3
Eigenvalues	0.049	0.029	0.013
Percentage	41.808	20.503	10.717
Cumulative Percentage	41.808	62.311	73.028

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آنالیز مقایسه‌ای ترکیبات شیمیایی اسانس جمعیت‌های مختلف گل محمدی در ایران

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چکیده

گل محمدی (*Rosa damascena* Mill.) یک گونه منحصر به فرد از خانواده رزاسه است. یکی از عمده‌ترین و محبوب‌ترین مناطق کشت گل محمدی کاشان است و اسانس گل‌های آن دارای عطر و بوی منحصر به فرد و شهرت جهانی است. هدف از این مطالعه مقایسه تنوعی که به‌طور طبیعی در میزان و کیفیت اسانس جمعیت‌های مختلف وجود دارد و انتخاب بهترین جمعیت‌ها است زیرا اطلاعات جامع و کامل در مورد جمعیت‌های مختلف گل محمدی موجود در شهرستان کاشان وجود ندارد. ۱۵ جمعیت گل محمدی از مناطق اصلی و مهم تولید اسانس کاشان جمع‌آوری شد. ترکیبات شیمیایی اسانس با استفاده از دستگاه کروماتوگرافی گازی متصل به طیف‌سنج جرمی آنالیز و شناسایی شد. بر اساس دندروگرام حاصل از آنالیز خوشه‌ای ترکیب شیمیایی اسانس، ۱۵ جمعیت گل محمدی به ۳ گروه تقسیم‌بندی شدند. در کل بیش از ۵۵ ترکیب شیمیایی توسط دستگاه GC-MS شناخته شد. بازده اسانس گل‌ها پس از استخراج توسط دستگاه کلونجر۰/۰۰۲ – ۰/۰۱۹٪ (جرمی/جرمی) بود. عمده‌ترین ترکیبات اسانس شامل لیمونن (۰/۴–۱۲/۸٪)، ۲–فنیل‌اتیل الکل (۱–۱/۳٪)، سیترونلول (۱۶/۲–۵۷/۸٪)، ژرانیول (۰/۹–۱۴/۱٪)، متیل‌اوژنول (۰/۵–۲/۵٪)، هپتادکان (۰/۸–۳٪)، ۱– نونادکان (۲/۱–۷/۵٪)، ۹–نونادکان (۱۴/۹–۳۰/۲٪)، آیکوزان (۱–۳/۳٪)، هنیکوزان (۵/۸–۱۸/۶٪)، تریکوزان (۰/۹–۵/۲٪) و پنتاکوزان (۰/۳–۲/۱٪) هستند.اسانس جمعیت جوشقان با میزان بالای سیترونلول، ژرانیول و فنیل اتیل الکل که دارای پتانسیل بالقوه ضداکسیدانی و رایحه قوی در صنایع آرایشی و دارویی است به‌عنوان بهترین اسانس از لحاظ کیفی شناسایی شد.

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