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Original Research Article

Assessment of variability of essential oil components in different accessions of Damask rose (*Rosa damascena Mill.*) by multivariate analysis

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ABSTRACT

In this work, 22 Damask rose accessions were cultivated in a randomized complete block design with 3 replications. Then, chemical components of essential oils (EOs) were identified and subsequently characterized using gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS) techniques. Accordingly, sixteen chemical compounds were identified, which accounted for about 85.3-99.8% of the EOs. Accessions were classified in 4 groups by cluster analysis. Discriminant function analysis (DFA) was found to be in line with the results of cluster analysis approach. In addition, using principal component analysis (PCA), the first 5 components had Eigen values higher than 1. *n*-Eicosane, *n*-heneicosane, *n*-tricosane, *n*-nondecane and *n*-tetradecanal had maximum portions in first component, while citronellyl acetate, geranial and *n*-undecanol had the maximum portions in the second component. Accessions of Isfahan5, Kermanshah5 and Kermanshah6 had the maximum percent of geraniol and citronellol, the two key chemical components, responsible for EO quality.

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1. Introduction

In many parts of the world, such as Turkey, India, Bulgaria, Italy, Iran and Spain, different varieties of aromatic roses are grown and their aromatic constituents are widely used. Damask rose (*Rosa damascena* Mill.) is among the most precious essential oil (EO) bearing plants in the market (Babu et al., 2002; Nunes and Miguel, 2017). It has been well-documented that Damask rose EO is widely used in various pharmaceutical, food, perfume and cosmetics industries. Rosa EOs have some remarkable therapeutic properties, *e.g.*, respiratory antiseptic having notable anti-inflammatory, mucolytic, expectorant, decongestant and antioxidant characteristics and are also able to act as potential symptomatic prophylactics and drugs (Mileva et al., 2021). Furthermore, remarkable antimicrobial, anti-inflammatory, antioxidant, anticancer, protective neuronal, cardiac, gastrointestinal and hepatic effects of this herbal species have been the subjects of some of the previously reported papers in the literature (Nayebi et al., 2017; Nunes and Miguel, 2017). The EO of rose consists of some valuable natural compounds involving geraniol and citronellol, constituting most of the relevant chemical profiles (Lawrence, 1991). Using the group replacement chromatography technique combined with gas chromatography with polar, semi-polar and non-polar columns, Kovats was able to isolate and identify a total of 127 compounds in rose EO. He calculated and reported the retention time and retention index of each constituent component (fraction) in rose EO (Kovats, 1987). In another report, five different rose species were analyzed by different gas chromatographic-based techniques. The partial least squares regression (PLSR) outputs accounted for the presence of the relationship between key aroma compounds and characteristic aromas of rose EOs, as well (Xiao et al., 2018). The main compounds of rose EO were *n*-nonadecane

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(25.5%), citronellol (17.7%), geraniol (13.3%), *n*-nonadecane (14.2%), citronellol (27.0%) and geraniol (18.7%) in A6 genotype (Osko, Iran) (Rezaee et al., 2004). Moreover, screening of the chemical profiles of Iranian *Rosa damascena* landraces EOs in different semi-arid and cool conditions led to the identification of *n*-nonadecane (34.2%), *n*-heneicosane (21.2%), citronellol (8.5%), *n*-hexadecanol (5.6%) and *n*-tricosane (6.4%) as the major chemical constituents (Yousefi and Jaimand, 2018). The main constituents of the EOs obtained from the flowers of three *Rosa damascena* Mill. genotypes (Kashan region, Iran) were citronellol (34.7%), nonadecane (14.5%) and heneicosane (10.3%) (Batooli and Safaie-Ghomi, 2012). Also, citronellol was the main compound of rose EO in some accessions from Iran (Shamspour and Mostafavi, 2011). On the other hand, 35 compounds were identified when analyzing Damask rose EO and the main constituents were reported as nonadecane (39.7%), heneicosane (32.4%), docosane (7.3%) and citronellol (6.1%) (Moein et al., 2010). The main compounds of Bulgarian rose have been reported as citronellol (8.8-48.2%), geraniol (8.8-23.3%), nonadecane (8.8-18.8%) and nerol (4.19-12.9%) (Dobreva et al., 2013). The most prevalent compounds characterized in the EOs of two Damask rose genotypes from Iran (Kashan) were geraniol (21.8%), *n*-nonadecane (23.3%) and citronellol (12.0%) (Jaimand et al., 2005b). Using solid phase extraction technique combined with GC/MS instrumentation, 41 compounds have been characterized in Rosa hybrid EO with citral, *n*-nonane, *n*-butyl acetate and *n*-decane as the major compounds (Kim et al., 2000). Finally, three novel eugenol disaccharides have been isolated from a methanolic extract of rose flowers using multilayer coil countercurrent chromatography and the relevant structures were characterized using two-dimensional NMR-based techniques (Straubinger et al., 1999). As being reported by Yousefi et al. (2016), no significant difference was observed between different *Rosa damascena* accessions for some natural compounds, viz. citronellol, geraniol, α-cadinene, heptadecane, eicosane, hexadecanol, tetradcanol, tetradcanal and citronellol acetate. However, there were significant differences in the levels of pentadecane, nonadecane, heneicosane, docosane, dihydrolinallol, geranial and methyl tetradecanoate at the level of 1% as well as tricosane and undecanol quantities at the level of 5%. Based on the characterized EO chemical profiles, 15 populations of *Rosa damascena* were clustered into 3 groups in which the main components of the EOs were found to be limonene (0.4-0.8%), 2-phenyl ethyl alcohol (1.0-1.3%), citronellol (8.2-57.2%), geraniol (1.9-14.1%), methyl eugenol (0.5-2.5%), heptadcane (0.8-3.0%), 1-nonadecane (1.2-5.7%), 9-nonadecane (2.3-9.1%), eicosane (1.3-3.0%), heneicosane (5.8-6.2%), tricosane (0.2-0.5%) and pentacosane (0.3-1.2%) (Toluei et al., 2019). Tabaei Aghdaei et al. (2007 and 2018) have recently reported a significant mean square among landraces of *Rosa damascena* EO. Additionally, Babaei et al. (2007) used molecular markers to assess the variation of 40 Iranian *R. damascena* genotypes from different regions and observed genotypic polymorphism. Some other studies on genetic diversity of *R. damascena* species did not reveal any polymorphism among their genotypes collected from various regions of Turkey and Bulgaria (Baydar et al., 2004; Rusanov et al., 2005). Yousefi and Tabaei-Aghdaei (2018) reported that there is no considerable genetic diversity among Iranian *R. damascena* landraces. Regarding the microsatellite profiles, Rusanov et al. (2005) have suggested a common origin for twenty-six oil-bearing *Rosa damascena* accessions and 13 garden Damask roses. Zeinali et al. (2009) have studied the cluster analysis, based on morphologic and yield traits of some Iranian *Rosa damascena* genotypes and revealed that Khuzestan and Shiraz genotypes were the most related ones, while the most independent genotypes were from Western and Eastern Azerbaijan. Pezeshkpour and Afkar (2019) used multivariate analyses for agronomic traits of Lentil (*Lens culinaris* Medik.) genotypes. More recently, using hierarchical clustering, Deepika et al. (2021) have classified 107 rice germplasms into 18 divergent clusters. Furthermore, sixty-four breed wheat genotypes have been studied using multivariate and cluster analyses representing that 61 genotypes and 3 checks were grouped into eight clusters (Mecha et al., 2017). Factor analysis also showed three independent factors that explained 71% of the total variability in 28 winter rapeseed cultivars (Sharafi et al., 2015). In the present study, we used multivariate analysis to assess the relationship between chemical compounds of the separated EOs and variation between Damask rose accessions based on EO chemical components to introduce high accessions of Damask rose for EO quality and geographical relationships between accessions.

2. Experimental

2.1. Plant material

In the present report, 23 accessions of Damask rose (*Rosa damascena* Mill.) were collected from some regions of Isfahan and Kermanshah Provinces, Iran and the relevant specifications of accessions are given in Table 1.

2.2. Experimental conditions

In April, seedlings were planted in Mehregan Research Station of Kermanshah located at 20 km of Kermanshah to Sanandaj road with 34°.9'' latitude and 47°.9'' longitude, 1270 m altitude, average annual rainfall 470.7, absolute minimum temperature -13 °C, absolute maximum temperature + 40.5 °C, average annual temperature 13.8 °C. The sampling area has a semi-arid steppe climate class with heavy to very heavy soil texture, pH between 7.4 to 8.4 and an average pH of 7.7. The percentage of organic matter was 0.38 to 1.3% and the lime quantity within the range 15-30%. Seedlings planted in 3×3 m distances in a randomized complete block design. Plants were irrigated once a week by drip irrigation. No chemical or toxic fertilizers were used during the project and mechanical methods were used to control weeds.

Table 1
Specifications of accessions of *Rosa damascena* Mill.

Accession name	Abbrev.	Accession source	no	Accession name	Abbrev.	Accession source	no
Isfahan1	Isfah	Chamoo	33	Kermanshah2	Kermansh2	Kermanshah	41
Isfahan2	Isfah2	Chamoo	34	Kermanshah3	Kermansh3	Mehregan	42
Isfahan3	Isfah3	Ardehal	35	Kermanshah4	Kermansh4	Harsin	43
Isfahan4	Isfah4	Chamoo	36	Kermanshah5	Kermansh5	Kandolah	44
Isfahan5	Isfah5	Chamoo	37	Kermanshah6	Kermansh6	Malavi	45
Isfahan6	Isfah6	Qamsar	38	Kermanshah7	Kermansh7	Mian-darband	46
Isfahan7	Isfah7	Ardehal	39	Kermanshah8	Kermansh8	Javanrood	47
Isfahan8	Isfah8	Chamoo	40	Kermanshah9	Kermansh9	Mahidasht	48
Isfahan9	Isfah9	Chamoo	4	Kermanshah10	Kermansh10	Kangarshah	49
Isfahan10	Isfah0	Isfahan	5	Kermansh11	Kermansh11	Sardehlagh	50
Kermanshah1	Kermansh1	Mehregan	21	Kermanshah12		-	-

2.3. Extraction and EO isolation

The rose flowers were harvested early in the morning and immediately after transferred to the laboratory. 500 g of fresh petals were used for EO isolation. EOs were extracted by water distillation for 3 hours, using the Jaimand-Rezaie status based on the Clevenger and British pharmacopoeia (1993) under the same conditions (Jaimand et al., 2005a). The EO samples were dehydrated with sodium sulfate anhydrous and kept in a refrigerator (4 °C) until injection into a chromatographic apparatus.

2.4. GC and GC/MS analyses

After diluting 1 µL of EO in 2 µL of dichloromethane, diluted oil samples were analyzed by a Thermo-UFM (Ultra-Fast Model) gas chromatograph equipped with Chrom-Card A/D data processor, cap-column Ph-5 (non-polar) (Thermo Fisher company) with a length of 10 m, an inner diameter of 0.1 and a thickness of 0.4 µM. The inner surface of the device was coated with a stationary phase consisting of dimethyl siloxane phenyl (5%) (Thermo Fisher, Italy). Column temperature program was as follows. The initial temperature was adjusted at 60 °C and programmed to reach a final temperature of 285 °C with a general ramp of 80 °C/min. The column temperature was set at 285 °C for 3 minutes. The detector used was of flame ionization detector (FID) type operating at 290 °C. The temperature of the injection chamber was 280 °C, carrier gas was helium and inlet pressure to the column was set at 0.5 kg/cm^2. GC/MS device (Varian 3400) connected to mass spectrometer (Saturn II) consisted of an ion telephoto system operating at an ionization energy of 70 eV. The GC/MS system was equipped with a DB-5 semi-polar column with the general characteristics as follows. Length: 30 m, inner diameter: 0.25 mm and thickness of static phase layer equal to 0.25 microns. Column head gas pressure was set at 35 psi. The column was programmed from an initial temperature of 40 °C to the final temperature of 250 °C with an increasing speed of 4 °C/min. The injection chamber and line transfer temperatures were respectively adjusted at 260 °C and 270 °C. The retention indices were calculated by injection of normal hydrocarbons (C_7-C_{25}) under the same conditions with EO samples, by a computer program. The predominant constituent components of the EOs were identified by comparison of their mass spectral patterns with different sources and the reports of the corresponding libraries (Adams, 2007; Davies, 1990; Kovats, 1987; Shibamoto, 1987).

2.5. Data analysis

Data were analyzed using some effective statistical approaches like multivariate analysis, cluster analysis using Ward's method with Euclidean distance, discriminant function analysis (DFA), principal component analysis (PCA) and Pearson's correlation coefficient by SPSS (ver.16) and MINITAB (ver.16) software. The abbreviated symbols used for chemical composition of the obtained and extended EO samples have been represented in Table 2.

3. Results and Discussion

3.1. Chemical profiles of the Damask rose (*Rosa damascena* Mill.) EOs

Using water distillation extraction, sixteen chemical compounds were identified in the EOs, which accounted for about 85.3-99.8% of the oil compositions (Table 2). These compounds include geraniol ($C_{10}H_{18}O$) and citronellol ($C_{10}H_{20}O$) two volatile terpenoid alcohol, geranial or ($C_{10}H_{16}O$) and *n*-tetradecanal ($C_{14}H_{28}O$)

Table 2
Specifications and quantities of EO Chemical compounds in different *Rosa damacena* accessions.

Chemical compounds	**Abbrev.**	**RI**	**Isfah1**	**Isfah2**	**Isfah3**	**Isfah4**	**Isfah5**	**Isfah6**	**Isfah7**	**Isfah8**	**Isfah9**	**Isfah10**
Dihydrolinalool	DHL	1134	0.7	0.2	6.8	0	0.8	0.8	0.5	0.3	0.6	0.5
Citronellol	CITOL	1228	25.8	16.5	15.9	8.8	47	23.3	15.8	21.8	18.2	19.7
Geraniol	GERO	1244	2.8	4.9	5.5	2.1	0.9	15.7	10.1	10.2	3.7	10.6
Geranial	GERAL	1271	32.6	12.2	8.6	3.2	5.1	25.8	20.9	16.3	19.3	19
Citronellyl acetate	CITACE	1349	0	0.3	0	0.2	2.6	0.2	0.2	0.5	1.1	0.3
n-Undecanol	UNDEC	1375	0.4	1	0	0.7	0.4	0.9	1	0.5	1.3	1.3
α-Cadinenee	CAD	1534	0	0	3.8	0	0	0	0	0	0	0
n-Tetradecanal	TDCANL	1611	0	2.8	0.4	0	0	0	0	0	2.9	0
n-Heptadecane	HDCAN	1700	0	0	0	5.8	0	2.5	0	0	0	0
n-Pentadecane	PDECAN	1500	0	0	0	0.6	0	0.3	0	0	0	0
n-Hexadecanol	HDCOL	1881	9.2	10.4	2.5	9.6	8	3.4	6.6	5	9.4	9.2
Methyl tetradecanoate	MTDCATE	1726	3	8.4	0	2.3	0.7	0.7	4.7	5.6	4.5	3
n-Nonadecane	NONADC	1900	12.3	25	12	41.1	16.6	15.5	17.8	14.3	19.8	12.2
n-Eicosane	EICO	2000	1.5	2.4	3.7	3.1	1.6	1.2	1.8	1.6	2	1.5
n-Heneicosane	HENICO	2100	8.6	11.2	21	16.5	7.8	6	9.1	7.8	0.7	8.6
n-Tricosane	TRICO	2300	2.9	4.5	12.9	4	2.3	1.3	3.1	2.8	3.3	2.9
Percentage of total compounds	-	-	**99.8**	**99.8**	**93.1**	**98.0**	**93.8**	**97.6**	**91.6**	**86.7**	**86.8**	**88.8**
Chemical compounds	**Abbrev.**	**RI**	**Kermansh3**	**Kermansh4**	**Kermansh5**	**Kermansh6**	**Kermansh7**	**Kermansh8**	**Kermansh9**	**Kermansh10**	**Kermansh11**	
Dihydrolinalool	DHL	1134	0.6	5.7	0	1	1	2.3	1.1	0	0.6	
Citronellol	CITOL	1228	23.9	3.7	5.5	32.8	7.8	19.3	54.8	29	23.9	
Geraniol	GERO	1244	5.6	0.5	1.4	2.8	3.8	4	6.0.	4.4	5.6	
Geranial	GERAL	1271	20.7	3	2.8	20.5	12.5	4.6	14.8	16.2	20.7	
Citronellyl acetate	CITACE	1349	0.7	0.5	0	0.5	0	1	1.1	0.3	0.7	
n-Undecanol	UNDEC	1375	2.2	0	0	0.8	1.5	1	1.4	0.3	2.2	
α-Cadinenee	CAD	1534	0.8	5.5	0	0.5	0	2.7	0	0	0.8	
n-Tetradecanal	TDCANL	1611	1.8	0.8	2.1	0	5.5	3	0.8	0	1.8	
n-Heptadecane	HDCAN	1700	0	0.4	0	1.5	0	0	0	0	0	

Table 2 Continued

Chemical compounds	Abbrev.	RI	Kermansh3	Kermansh4	Kermansh5	Kermansh6	Kermansh7	Kermansh8	Kermansh9	Kermansh10	Kermansh11	
n-Pentadecane	PDECAN	1500	0.4	0.6	0	0.3	0	0.4	1.4	0	0.4	
n-Hexadecanol	HDCOL	1881	10	3.1	7.9	7.3	3	7.9	2.3	6.6	10	
Methyl tetradecanoate	MTDCATE	1726	4.3	0.7	2	3	12.2	4.9	1.7	3.4	4.3	
n-Nonadecane	NONADC	1900	13.4	15.6	29.4	12.2	28.3	24.4	5.5	20.3	13.4	
n-Eicosane	EICO	2000	1.4	13.2	4.4	1.4	3.6	2.2	2.1	1.7	1.4	
n-Heneicosane	HENICO	2100	7.2	28.1	29.8	7.4	12.3	11.9	2.8	8	7.2	
n-Tricosane	TRICO	2300	0.6	5.7	0	1	1	2.3	1.1	0	0.6	
Percentage of total compounds	-	-	**93.6**	**87.1**	**85.3**	**93.0**	**92.5**	**91.9**	**90.9**	**90.2**	**93.6**	

two terpenoid compound, *n*-eicosane ($C_{20}H_{24}$), *n*-heneicosane ($C_{21}H_{44}$) and *n*-tricosane ($C_{23}H_{48}$) from the group of straight line chain alkanes, dihydrolinalool acetate ($C_{12}H_{20}O_2$) and citronellyl acetate ($C_{10}H_{18}O$) from the group of monoterpene aldehydes, *n*-undecanol ($C_{11}H_{24}O$) and *n*-hexadecanol ($C_{16}H_{34}O$) tow long chain fatty alcohols, three alkanes, namely *n*-pentadecane ($C_{15}H_{32}$), *n*-heptadecane ($C_{17}H_{36}$) and *n*-nonadecane ($C_{19}H_{40}$), methyl tetradodecanoate ($C_{15}H_{30}O_2$) a fatty acid methyl ester, α-cadinenee ($C_{15}H_{24}$) a bicyclic sesquiterpene. It should be noted that recently some new techniques have been widely used for extraction of EOs from plant species (Mohammadhosseini, 2017; Mohammadhosseini et al., 2017; Hashemi-Moghaddam et al., 2018). The use of new EO extraction techniques in Damask rose may lead to the identification of more and different compounds. A simple perusal of the isolated oils represents that using these methods; the characterized profiles are different quantitatively and qualitatively in terms of distillation with water and steam. Microwave-assisted hydrodistillation (MAHD) is a rapid, economical and environmentally-friendly extraction method by which the EO yield being extracted is higher than those extracted by hydrodistillation (HD). Moreover, the extracted oils using MAHD possess higher amounts of oxygenated constituents compared to classical HD extraction (Hashemi-Moghaddam et al., 2018; Heba et al., 2020).

3.2. Cluster analysis

The studied accessions were classified in 3 groups by cluster analysis based on the corresponding EO chemical profiles. As seen, the accessions of Isfahan1, Isfahan2, Isfahan3, Isfahan4, Isfahan6, Kermanshah1, Kermanshah4, Kermanshah5, Kermanshah8, Kermanshah9 and Kermanshah10 classified in the first group, Isfahan7, Isfahan8, Isfahan9, Isfahan10, Kermanshah3, Kermanshah11 in the second group and Isfahan5, Kermanshah2, Kermanshah6 and Kermanshah7 in the third group (Fig. 1). The maximum distance was also noted between cluster II and III. The results of the DFA statistical approach also confirmed the grouping of the accessions in cluster analysis and classified accessions in 3 groups (Fig. 2) and presented that 100% of the groups have been correctly classified by DFA (Table 3). Correlations between discriminating variables and standardized canonical discriminant functions showed that *n*-tricosane had the maximum positive correlation with first function and geranial, *n*-undecanol and methyl tetradecanoate had the maximum positive correlation with second function. Using the principal component analysis (PCA) approach, the first 5 components had Eigen values higher than 1. *n*-Eicosane, *n*-heneicosane, *n*-tricosane, *n*-nondecane and *n*-tetradecanal, had maximum portions in first component (Table 4) and accessions of Isfahan3, Isfahan4, Isfahan6, Kermanshah4 and Kermanshah5 had the highest amounts of these compounds (Fig. 3). Citronellyl acetate, geranial and *n*-undecanol had the maximum portions in the second component

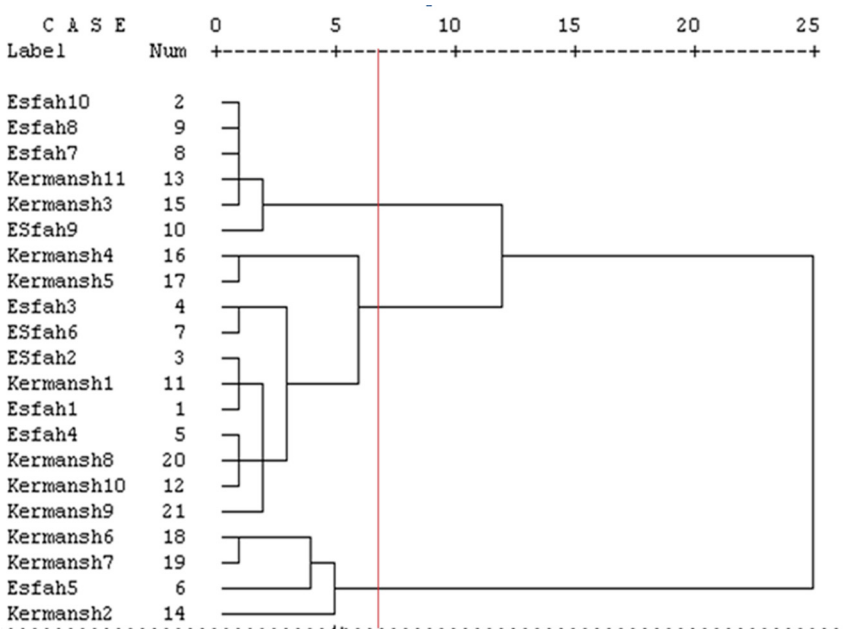

Fig. 1. Classification of *Rosa damascena* accessions by Ward method.

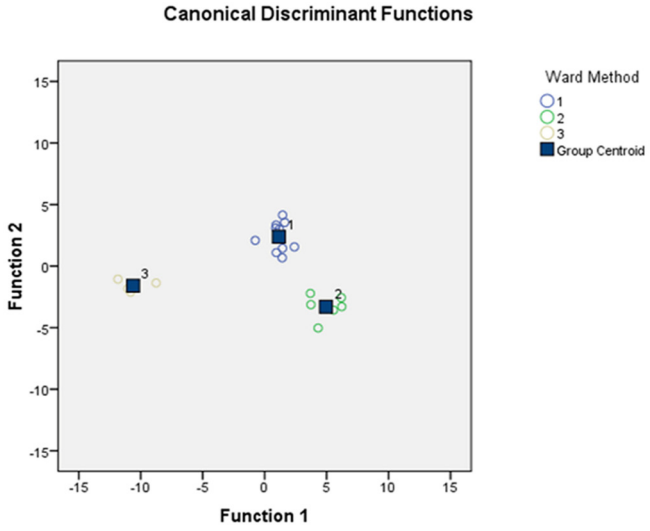

Fig. 2. Diagram of the discriminant function analysis for accessions groups.

Table 3
Classification Results by FDA.

Classification results	Predicted group membership				
	Ward method	1	1	3	Total
Original count	1	15	2	0	15
	2	0	3	0	4
	3	0	1	3	3
%	1	100	2	0	100
	2	0	3	0	100
	3	0	0	100	100

100.0% of original grouped cases correctly classified.

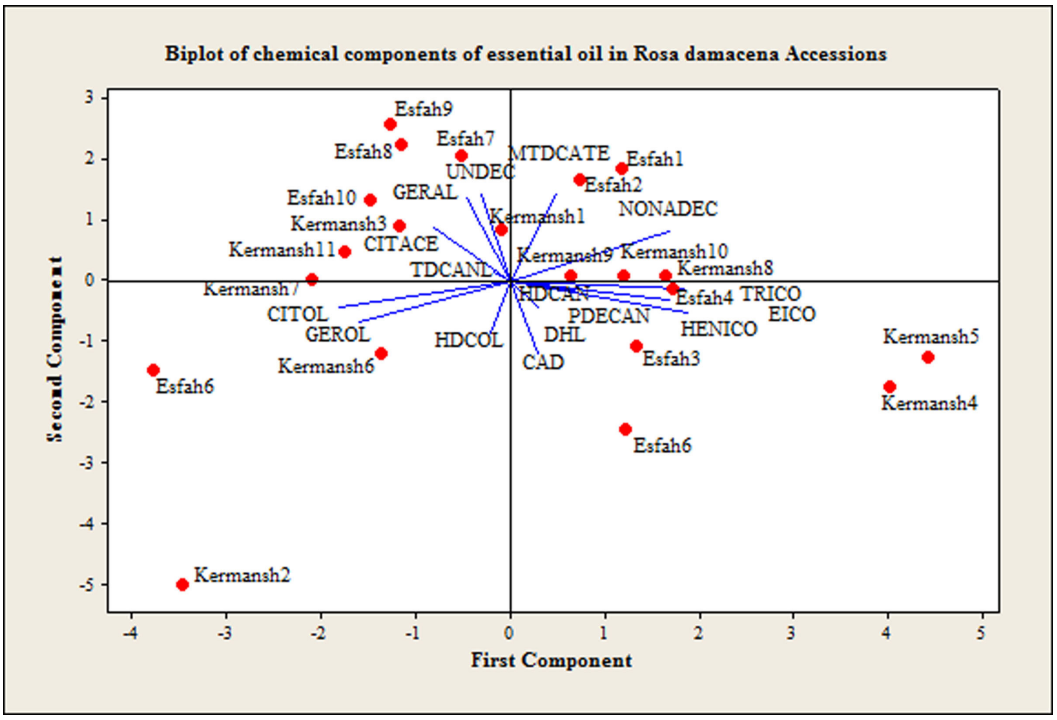

Fig. 3. Biplot of first and second component for chemical constituents of *Rosa damascena* EO accessions.

Table 4
Proportion of variable in components.

Variable	PC1	PC2	Variable	PC1	PC2
DHL	0.07	-0.13	HDCAN	0.1	-0.03
CITOL	-0.4	-0.01	PDECAN	0.15	-0.05
GERO	-0.33	-0.2	HDCOL	-0.05	-0.26
GERAL	-0.1	0.42	MTDCATE	0.11	0.437
CITACE	-0.18	0.27	NONADC	0.37	0.25
UNDEC	-0.07	0.43	EICO	0.37	-0.1
CAD	0.07	-0.36	HENICO	0.41	-0.16
TDCANL	-0.04	0.05	TRICO	0.4	-0.03
Eigen value	4.62	3.03	-		
Proportion of variance	0.387	0.176	-		
Cumulative variance	0.39	0.56	-		

(Table 4) and accessions of Isfahan7 Isfahan8, Isfahan9, Kermanshah1, Kermanshah3, Kermanshah10 and Kermanshah11 had the highest amounts of these compounds. Accessions of Isfahan1, Isfahan2, Kermanshah8, Kermanshah9 and Kermanshah10 had the highest percent of *n*-nonadecane and methyl tetradecanoate. Accessions of Isfahan5, Kermanshah2, Kermanshah6 and Kermanshah7 had the highest percent of geraniol and citronellol, the two key chemical components, responsible for the quality of rose oil, and *n*-hexadecanol (Fig. 3). Pearson's correlation estimation between variable (Table not provided) showed a significant positive correlation between dihydrolinallol and α-cadinene (r = 0.71**), citronellol with geraniol (r = 0.45**), and a significant negative correlation between citronellol with *n*-nonadecane (r = -0.72**), citronellol with *n*-eicosane (r = -0.58**), citronellol with *n*-heneicosane (r = -0.66**), citronellol with *n*-tricosane (r = -0.73**), citronellol with *n*-nonadecane (r= -0.81**). A significant negative correlation between geraniol with *n*-nonadecane (r = -0.81**), geraniol with *n*-tricosane (r = -0.60**), geraniol with *n*-undecanol (r = -0.77**), and a significant negative correlation (r = -0.56**) between *n*-undecanol with methyl tetradecanoate was seen. Even under the same conditions, *e.g.*, soil, irrigation, altitude, climatic and nutritional conditions, significant variation between different accessions was expected for the chemical composition of EOs because these accessions have geographical distances as well as

genetic differences that can affect different biosynthetic pathways of the chemical composition of EOs. In some reports, the obtained results confirmed a great variety of EO compounds in samples from different regions (Jaimand et al., 2005b; Rezaee et al., 2004). Verma et al. (2011) showed that the major components of rose water volatiles obtained from different stages of flowering and different cultivar are diverse. By the use of the cluster analysis based on the chemical composition of the *Rosa damascena* EO, the interpretation of the classification of accessions regarding the origin and geographical distances is complex and ambiguous. Most accessions are in the first group and their EOs had large amounts of hydrocarbon compounds, including heneicosane, docosane, pentadecane, heptadecane and nonadecane. These accessions may have a common origin and it seems that the sampling origins of Kermanshah1 (Mehregan), Kermanshah4 (Harsin), Kermanshah5 (Kandoleh), Kermanshah8 (Javanrood), Kermanshah9 (Mahidasht) and Kermanshah10 (Sahnah) has been Isfahan1 (Camoo), Isfahan2 (Camoo), Isfahan4 (Camoo) or Isfahan6 (Qamsar) accessions. The common feature of Isfahan7 (Mashhad Ardehal), Isfahan10 (Isfahan), Kermanshah3 (Kermanshah) and Kermanshah11 (Sahnah) with the accessions of Isfahan8 (Camoo) and Isfahan 9 (Camoo) in the second group, was the presence of large amounts of geranial, citronellyl acetate and *n*-undecanol. These accessions may have a genetic affinity and a common origin. However, for confirmation, it is necessary to check these accessions with genetic marker experiments and molecular analyses. Kermanshah2 (Kermanshah), Kermanshah6 (Malavi) and Kermanshah7 (Mian-Darband) genotypes have a genetic affinity with Isfahan5 genotype and their common feature is the presence of high amounts of 2 basic compounds of rose EOs, geraniol and citronellol, which showed that the EOs of these accessions are qualitatively better than those obtained in other studied areas (Yousefi, 2009; Yousefi et al., 2016). In a recent study, 15 populations of Damask rose were clustered into 3 groups (Toluei et al., 2019). Some genotypes of *Rosa damascena* from different regions of Iran, based on EO chemical compounds have been classified into 4 clusters (Yousefi et al., 2016). This conclusion is consistent with the results of other researchers (Tabaei-Aghdaee et al., 2004b; Yousefi, 2009). It should be noted that the EO profiles are largely influenced by environmental and physiological factors that may not be a very accurate indicator of genetic similarities and differences. Also, different methods of extracting may affect the type and percent of EOs components. According to some reports, EO extracted with dichloromethane contains higher amounts of 2-phenyl ethyl alcohol (Babu et al., 2002). Principal component analysis (PCA) revealed that the same groups of chemical compounds of EO had the same trends between accessions. Two alkane compounds, namely heptadecane and pentadecane and three hydrocarbonic elements, namely *n*-icosane, *n*-heneicosane and *n*-tricosane, had the same trend. The two key chemical components in rose EO, responsible for quality of rose oil, geraniol and citronellol showed the same trend. Citronellyl acetate, geranial and *n*-undecanol had the same trend, methyl tetradecanoate and *n*-nonadecane were found to be unique and finally *n*-tetradecanal, dihydrolinalol, hexadecanol and α-cadinene showed the same behavior (Fig. 1). The existence of genetic variation is the primary base for breeding programs; therefore, selection for EO compounds traits could be possible. Some reports have implied significant differences among landraces EOs of *Rosa damascena* (Babaei et al. 2004a; Tabaei Aghdaei et al. 2007; Tabaei Aghdaei et al., 2018). Babaei et al. (2007) observed genotypic polymorphism between 40 Iranian *R. damascena* genotypes, while some other studies did not reveal any polymorphism among *R. damascena* genotypes collected from various regions of Turkey and Bulgaria (Baydar et al., 2004; Rusanov et al., 2005). Some authors believe that because of asexual reproduction (through cuttings), there is a low genetic variation among Damask rose accessions and landraces (Yousefi and Tabaei Aghdaei, 2018). In Iran, unlike other countries such as Bulgaria and Turkey, the main goal of rose cultivation isn't for its EO and instead for flower and rose water production. Thus, the Damask rose landraces haven't been subjected to genetic improvement for EO and there isn't a considerable genetic diversity among Iranian landraces (Yousefi and Tabaei Aghdaei, 2018). Modern industrial oil rose cultivation is based on a very narrow gene pool and the rose collections contain many genetically identical accessions (Rusanov et al., 2005). In some *Rosa* accessions, a significant difference has been observed in yield and flower yield components (Kodori and Tabaei-Aghdaei, 2007). The great variety of traits increases the chances of choosing superior accessions. It should be noted that a part of the observed diversity is genetical and refers to the real difference between the accessions that have been collected from different geographical areas with different climatic conditions and another part related to the difference between ecological conditions of the region (Tabaei-Aghdaei et al., 2004c). The existence of rich genetic diversity for traits can provide a suitable ground for breeding criteria such as different selection methods and hybridization in Damask rose (Tabaei-Aghdaei et al., 2007). Due to the existence of genetic diversity, it is suggested to use the studied accessions to modify and produce cultivars. Significant positive correlation between geraniol and citronellol; two important compounds for quality of EO, must be considered in genetic improvement for EO, because increasing each compound is accompanied by increasing the other compound. Also, significant negative correlation between geraniol with *n*-nonadecane, *n*-tricosane, *n*-undecanol and citronellol with *n*-nonadecane, *n*-eicosane, *n*-heneicosane, *n*-tricosane and *n*-nonadecane (hydrocarbonic compounds) that reduce the quality of EO, must be considered in genetic improvement of an EO.

4. Concluding Remarks

Accessions of Isfahan5, Kermanshah2, Kermanshah6

and Kermanshah7 had the highest percent of geraniol and citronellol and had the best quality of EO. In order to improve EO quality in *Rosa damascena*, it is necessary to select and study genotypes with higher amounts of geraniol, citronellol and 2-phenyl ethyl alcohol. Of course, to increase the quantitative and qualitative production of EO, it is necessary to consider the yield of flowers per hectare and the yield of EO at the same time.

Conflict of Interest

The authors declare that there is no conflict of interest.

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