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Original Article

Evaluation of Chemical Compounds of Essential Oil in Damask Rose (*Rosa damascena* Mill.) Accessions

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Abstract

Damask Rose (*Rosa damascena* Mill.) is a valuable species whose various products are widely used in food, pharmaceutical, cosmetic and traditional medicine. This plant is cultivated in different countries such as Turkey, Italy, Bulgaria, Spain, India, and Iran. In this research, fifteen different genotypes were cultivated in a randomized complete block design with 3 replications. Within 2 years, Essential Oil (EO) was extracted by water distillation method and the chemical compounds were identified and determined by GC and GC/MS. Twenty chemical compounds were identified in essential oil, which has formed about 97.81% of the EO. The results showed that geraniol (21.89%), *n*-nonadecane (19.59%), *n*-henicosane (16.45%), geranial (13.30%), and *n*-tricosane (6.87%) had the highest share in the chemical compounds. A significant difference was observed for the compounds of Trans- rose oxide, *n*-undecanol, 2-6-E-farnesol, *n*-pentane at the level of 5% and for 1-hexyl hexanoate, γ-elements, α-cadinene, Butyl decanoate, *n*-hexadecanoic, and occidental acetate at 1%. There was a significant difference between the genotypes for all chemical compounds of essential oils. Also, the year/genotype interaction was significant for all EO compounds, except geraniol, *n*-hexadecanoate and *n*-henicosane. Genotypes were classified into 4 groups by cluster analysis (Ward's method) and Discriminant Function Analysis confirmed the results of cluster analysis. The results of the principal component analysis showed that the compounds *n*-heptane, *n*-pentane, *n*-hexadecanoic, and butyl decanoate had the most positive contribution and the compounds linalyl acetate, geranial, hexyl hexanoate and trans- rose oxide had the most negative share in the first component. *KEYWORDS: E*ssential oil, Gas chromatography, Gas chromatography with mass spectrometer, multivariate analysis.

Keywords: Essential oil, Gas chromatography, Gas chromatography with mass spectrometer, Multivariate analysis

Introduction

In Turkey, India, Bulgaria, Italy, Iran, and Spain, a variety of aromatic roses are grown and their aromatic compounds are used. Among these aromatic roses, the damask rose has the most valuable components of essential oil [1]. Essential oil of Damask Rose (*Rosa damascena* Mill.), is widely used in various pharmaceutical, food, perfume, and cosmetics industries [2-4]. Essential oil is extracted from the flowers of R. damascena and is full of linalool, geraniol, citronellol and nerol [2].

A great variety has been observed between the chemical compounds of the essential oil from different regions [5]. The main compounds in the essential oils of 2 genotypes of *R. damascena* were *n*-nonadecane (14.2%-25.5%), citronellol (17.7%-27%) and geraniol 13.3%-18-7% [6]. Amounts of chemical constituents of EO in several Damask rose accessions were *n*-nonadecane (34.2%), *n*-heneicosane (21.2%), *n*-hexadecanol (5.6%), *n*-tricosane (6.4%) and citronellol 8.5% [7].

In some different studies, the main compounds of EO have reported citronellol (34.7%), *n*-nonadecane (14.5%), and *n*-

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heneicosane 10.3% [8]. The main compound of essential oil in different *R. damascena* accessions was citronellol [9], *n*-nonadecane (39.73%), *n*-heneicosane (32.38%), *n*-docosane (7.34%), and citronellol 6.14% [10]. The main constituents of Bulgarian rose were citronellol (8.76-48.24%), geraniol (8.76-23.33%), *n*-nonadecane (8.78-18.84%) and nerol 12.9%-4.19% [11]. The most important compounds of EO in two damask rose genotypes were geraniol (21.8%), *n*-nonadecane (23.3%) and citronellol 12% [12]. The forty-one different compounds have been isolated and identified from *R. damascena* EO and citral, *n*-nonane, *n*-butyl acetate, and *n*-decane were the major compounds [13].

In a new study Ghavam *et al*. [14] reported that the most important compounds of the essential oil of *R. damascena* are nonadecane (24.72%), heneicosane (19.325%), oleic acid (17.63%), and citronellol 12.61%.

The result of a study showed a significant difference was observed between different *R. damasena* accessions for some EO compounds [15]. The 15 populations of *R. damascena* were clustered into 3 groups and the main compounds of EO have been reported [16].

Tabaei Aghdaei *et al*. [17] reported a significant mean square among landraces of *R. damascena* for essential oil. The essential oils of 44 *R. damascena* accessions had considerable variabilities for major and minor components [18].

Ghavam *et al*. [3] showed that the yield and chemical composition of essential oils obtained from *R.* × *damascena* were significantly affected by the collection area. A great variety of essential oil compounds has been reported among the 5 varieties of damascus roses from different parts of India [19].

Babaei *et al*. [20] observed genotypic polymorphism between different genotypes of *R. damascena*. Some other studies did not reveal any polymorphism among *R. damascena* genotypes collected from various regions in Turkey and Bulgaria [21, 22]. In another study, Yousefi & Tabaei Aghdaei [23] reported that there isn't a considerable genetic diversity among Iranian *R. damascena* landraces. Microsatellite genotyping demonstrated that *R. damascena* accessions from Bulgaria, Iran, and India and old European Damask rose varieties possess identical microsatellite profiles, suggesting a common origin [22].

Cluster analysis, based on morphologic and yield traits in some *R. damascena* genotypes revealed a racial and geographical dependence between them [24].

In Lentil (*Lens culinaris*) genotypes, Cluster analysis classified the genotypes into three groups and the maximum distance was between clusters I and III [25]. In a diverse set of 107 rice genotypes, the principal component analysis revealed the contribution of four PCs to maximum variability and hierarchical clustering grouped the genotypes into 18 divergent clusters [26]. Cluster analysis revealed that the 61 genotypes of breed wheat and 3 checks were grouped into eight clusters [27]. The result of factor analysis in 28 winter rapeseed cultivars showed three independent factors that explained 71% of the total variability [28]. We used multivariate analysis to reveal variation between damask rose accessions based on chemical components traits and introduce high accessions of damask rose for essential oil quality and breeding programs. Yousefi *et al*. [29] classified 22 Damask rose accessions in 4 groups by cluster analysis in terms of EO components.

Material and Methods

Experimental Materials

The 15 accessions of *R. damascene* from different provinces of Iran were collected. Specifications of Accessions are given in Table 1.

Table 1 Specifications of Accessions of *R. damascena* Mill.

Accession name	Abbreviation	Accession source	Accession no	Accession name	Abbreviation	Accession source	Accession no
Azarbaijan-gharbi1	AZRGH1	Khoi	2	Esfahan5	ESFA5	Kamoo	37
Esfahan9	ESFA1	Kamoo	4	Esfahan7	ESFA7	Ardahal	39
Fars1	FARS1	Ahiraz	16	Esfahan8	ESFA8	Kamoo	40
Kermanshah1	KERSH1	Kermanshah	21	Kermanshah3	KERSH3	Mian- darband	42
Khorasan2	KHOR2	Mashhad	23	Kermanshah8	KERSH8	Javanroud	47
Lorestan1	LOR1	Lorestan	26	Kermanshah9	KERSH9	Mahidasht	48
Arak1	ARAK1	Arak	18	Kermanshah10	KERSH10	Sahnah	49
Yazd1	YAZD1	Shirkoh	31	-	-	-	-

Table 2 Geographical, climatic, soil and water characteristics of the project site

Longitude	47.26°	Climatic condition	Cold temperate climate
Latitude	34.8°	Evaporation rate	1808.5 mm
Elevation	1346 M	Sunshine	2430.2 hours
The average annual rainfall	538 mm	soil texture	clay-silty
The average annual temperature	+10.5 °C	Soil EC	0.57 mM
Absolute maximum temperature	+41 °C	soil pH	7.5
Absolute minimum temperature	- 21.8 °C	water class	C2S1

Table 3 The abbreviation (symbol) for Chemical components of essential oil

Chemical component	Abbre.	Chemical component	Abbre
Geraniol	GEROL	*n*-heptane	NHPTA
Linalyle acetate	LINACE	*n*-pentane	NPEN
Geranial	GERAL	Hexyle decanoate	HXAD
Transrose oxide	THROS	Occidentalol acetate	OCCIACE
Citronellyl acetate	CITACE	N-nonadecane	NONADC
n-undecanol	UNDEC	N-icosane	EICO
α-Cadinene	ACAD	N-henicosane	HENICO
N-hexadecanoate	NHXDD	N-tricosane	TRICO
Hexyl hexanoate	HEXHEX	N-docosane	NDOCO
Butyl decanoate	BUDOD	N-hexadecane	NHEXDEC
Octadecanol	OCTD	Trans farensol	FARNS
γ-Elemene	YLMEM		

Experimental Conclusion

Seedlings were planted in the Islamabad-e-Gharb research farm of the Research and Education Center of Agricultural and Natural Resources of Kermanshah Province in 2014-2017 crop years with the following characteristics (Table 2).

The 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. The flowers were harvested early in the morning and transferred to the laboratory. 500 g of fresh petals were used for essential oil extraction. EO extracted by water distillation for 3 hours using the Cleavenger and British Pharmacopoeia system (1993), were dehydrated with dry sodium sulfate (Na_2So_4) and kept in a refrigerator (4 °C) until injection into a chromatographic apparatus.

Gas Chromatography

Samples were injected into gas chromatograph with specifications (Ultra Fast Model) Thermo-UFM and with Chrome- Card A/D data processor, cap-column Ph-5 (non-polar) made by the company with a length of 10 meters and an inner diameter of 0.1 and 0.4 μm thickness, when the inner surface of the device was coated with a stationary phase of 5% dimethylsiloxane phenyl. Column temperature program: Initial temperature 60 °C, start to final temperature 285 °C, which added every minute 80 °C to them, then stopped at this temperature for 3 minutes, detector type was FID with 290 °C. The temperature of the injection chamber was 280 °C, carrier gas was helium, and inlet pressure to the column is set at 0.5 kg/cm2.

GC/MS device was Varian 3400 connected to a mass spectrometer (Saturn II), with an Ion telephoto system and ionization energy of 70 electron volts. It has a DB-5 column which is a semi-polar column (length 30 m, inner diameter 0.25 mm, and thickness of static phase layer equal to 0.25 microns). Column head gas pressure is set at 35 pounds per square inch, temperature 40 °C to 250 °C with increasing speed of 4 °C per minute, injection chamber temperature 260 °C and line transfer temperature 270 °C. The spectra were calculated by injection of normal hydrocarbons (C7-C25) under the same conditions as essential oil injection, by a computer program, and in BASIC language. The Chemical compounds of EO were identified via Compare spectra with different sources [30, 31].

Data Analysis

Analysis of variance, comparison of means by Duncan method (Univariate analysis), Pearson's Correlation coefficient, Cluster analysis using Euclidean distance following Ward's method, Discriminant Function Analysis and Principal Component Analysis (multivariate analysis) were performed by SPSS (ver.16) and MINITAB (ver.16)

software. The abbreviation symbol used for chemical compounds of EO is given in Table 3.

Results

The 23 different chemical compounds were identified, which accounted for about 97.81% of the EO. These compounds included geranial, a volatile terpenoid alcohol; geranial a terpenoid compound; *n*-icosane, *n*-henicosane, *n*-docosane and *n*-tricosane from the group of straight-line chain alkanes; linalyle acetate a terpene alcohol; citronellyl acetate and occidentalol acetate from monoterpene aldehydes; *n*-otadecanol and *n*-undecanol from long-chain fatty alcohols; *n*-hexadecane, *n*-nonadecane, *n*-pentane and *n*-heptane form alkanes; *n*-hexadecanoate, butyl decanoate and 1- hexyl hexanoate from carboxylic ester derivatives of fatty acids, trans-rose oxide (a monoterpene with pyran cycle); α-cadinene a two-ring sesquiterpene; trans -farnesol (an alcoholic compound of the non-binding sesquiterpene) and γ-elements that was also present in a few genotypes.

The results showed that the highest share in the chemical composition of essential oils was geraniol (21.89%), *n*-nonadecane (19.59%), *n*-heniocosane (16.45%), geranial (13.30%), and *n*-tricosane (6.87%) respectively.

Based on the results of analysis of variance between years, a significant difference (5%) was observed for trans-rose oxide, *n*-undecanol, 2-6-E-farnesol, and *n*-pentane and a significant difference at the level of 1%, for 1-hexyl hexanoate, γ-elements, α-cadinene, butyl decanoate, *n*-hexadecanoate, and occidental acetate, but not for other compounds (Table 4). There was a significant difference between the genotypes for all chemical compounds of EO. Also, the year/genotype interaction was significant for all EO compounds, except geraniol, *n*-hexadecanoate, and *n*-henicosane (Table 4).

Comparison of means by Duncan method (Table 5), showed that the highest amounts of geraniol there was respectively in Arak1 genotypes (29.09%) and Lorestan1 (27.97%), but the lowest percentage of geraniol was seen in Kermanshah1 (14.23%) and Isfahan8 (14.38%) genotypes. The highest amounts of geranial were observed in the EO of Kermanshah3 (22.05%), Fars1 (18.041%), and Lorestan1 (18.31%), and the lowest was present in Khorasan2 (6.29%). The highest levels of *n*-henicosane there was in Kermanshah1 (25.05%), *n*-diocesan in Kermanshah8 (3.34%), *n*-icosane in Arak1 (4.25%) and *n*-tricosane (18.23%) in Fars1 and the lowest amounts of these compounds respectively, there were in Fars1 (11.54%), Fars1 (0.18%), Fars1 (1.18%) and Kermanshah8 (3.77%).

The highest value of *n*-pentane and *n*-heptane respectively there was in Isfahan9 (0.87%) and Isfahan8 (2.76%) and the lowest value was seen in Azerbaijan-Gharbi1 (0.9%) and Arak1 (1.03 %). The highest amounts of *n*-undecanol and *n*-octadecanoic there were respectively, in the EO of Kermanshah10 (1.43%) and Kermanshah1 (0.041%). The highest and lowest values for linalyl acetate there were in Lorestan1 (8.43%) and Khorasan2 (2.55%), citronella acetate in Azerbaijan-Gharbi1 (0.52%) and Isfahan8 (0.09%) and occidental acetate in Khorasan2 (1.082%) and Lorestan1 (0.004%).

The highest and lowest percentage of butyl decanoate were observed in Isfahan8 (0.43%) and Azerbaijan-Gharbi1 (0.1%) respectively, *n*-hexadecane in Isfahan5 (4.28%) and Azerbaijan-gharbi1 (0.91%), *n*-nonadecane in Isfahan8 (28.45%), and Azerbaijan-gharbi1 (11.58%). Also, the highest amount of trans-rose oxide there was in Lorestan1 (0.45%) and α-cadinene in Azerbaijan-Gharbi1 (4.82%).

Pearson's Correlation coefficient (Table not provided), revealed significant positive correlation between linalyle acetate with geranial (r=0.97**) and trans-rose oxide (r=0.81**); geranial with trans-rose oxide (r=0.83**); hexyl hexanoate with α-cadinene (r=0.80**) and trans-farensol (r=0.65**); γ-elemene with butyl decanoate (r=0.53*) and *n*-nonadecane (r=0.53*); α-cadinene with trans-farensol (r=0.88**); *n*-hexadecanoate with butyl decanoate (r=0.52*); *n*-heptane with butyl decanoate (r=0.66**) and *n*-nonadecane (r=0.65**); hexyl hexanoate with *n*-hexadecanoate (r=0.59*) and *n*-nonadecane (r=0.83**); *n*-hexadecanoate with *n*-nonadecane (r=0.68**). Also there were negative correlation between geraniol with trans Farnesol (r= -0.59*) and geraniol with *n*-henicosane (r=-0.59*); linalyle acetate with *n*-hexadecanoate (r=-0.63*), *n*-nonadecane (r=-0.73**) and *n*-henicosane (r=-0.84**); geranial with *n*-hexadecanoate (r=-0.65**) and *n*-nonadecane (r=-.0.74**); trans-rose oxide with *n*-henicosane (r=-0.57*); *n*-undecanol with *n*-henicosane (r=-0.58*); hexyl hexanoate with Butyl decanoate (r=-0.55*), *n*-hexadecanoate (r=-0.76**) and *n*-nonadecane (r=-0.59*) and finally a significant negative correlation between α-cadinene with *n*-hexadecanoate (r=-0.52*).

In cluster analysis (Ward's method), based on EO chemical compounds, the genotypes were classified into 4 groups. Kermanshah1, Isfahan8, and Khorasan2 classified in the first group had the maximum distance with others and the high differences with other genotypes. Fars1 and Azerbaijan-Gharbi1 were placed in the second group and had a relatively high distance from other genotypes. Arak1, Lorestan1, Kermanshah3, and Isfahan7 are classified in the third group. The other genotypes were in the fourth group and had relatively moderate similarities with each other (Fig. 1). In Discriminant Function Analysis, the genotypes were classified into 4 groups and confirmed the results of cluster analysis (Table 7).

Table 4 Analysis of variance for chemical components of essential oil of *R. damascena* Mill. Genotypes

Source of variations	df	Mean Square						
		Geraniol	Linalyle acetate	Geranial	Trans roseoxide	Citronellyl acetate	*n*- un decanol	*n*- nona decane
YEAR	1	0.004	0.352	1.224	0.014*	0.140	0.141*	0.214
YEAR/REP	4	3.423	0.119	0.411	0.001	0.020	0.0110	0.471
GEN	14	2.28*	0.715**	2.24**	0.01**	0.027**	0.089**	1.812**
GEN/YEAR	14	1.506	1.16**	2.40**	0.031**	0.015**	0.158**	1.578**
Error	56	0.908	0.152	0.330	0.004	0.006	0.021	0.281
CV		20.75	16.22	16.02	7.21	8.42	11.95	12.04

*, **- Significant at the 0.05 level and 0.01 level, Other without significant difference

Table 4 (continue) Analysis of variance for chemical components of essential oil of *R. damascena* Mill. Genotypes

Source of variations	df	Mean Square						
		1- hexyl hexanoate	*n*- pentane	α- cadinene	N- heptanes	2-6-E- farensol	Butyl decanoate	*n*-hexa decanoate
YEAR	1	0.354**	0.38*	6.03**	0.07	0.899*	0.018**	23.52**
YEAR/REP	4	0.0090	0.03	0.06	0.01	0.09	0.001	0.084
GEN	14	0.131**	0.123**	0.17**	0.153**	0.17*	0.032**	0.133*
GEN/YEAR	14	0.102**	0.066**	0.18**	0.152**	0.38**	0.015**	0.083
Error	56	0.006	0.013	0.01	0.04	0.09	0.003	0.069
CV		8.89	11.81	7.64	14.07	17.38	6.19	14.59

*, **- Significant at the 0.05 level and 0.01 level, Other without significant difference

Table 4 (continue) Analysis of variance for chemical components of essential oil of *R. damascena* Mill. Genotypes

Source of variations	df	Mean Square					
		1- hexyl hexanoate	*n*- pentane	α- cadinene	*n*- heptanes	2-6-E- farensol	Butyl decanoate
YEAR	1	0.354**	0.38*	6.03**	0.07	0.899*	0.018**
YEAR/REP	4	0.0090	0.03	0.06	0.01	0.09	0.001
GEN	14	0.131**	0.123**	0.17**	0.153**	0.17*	0.032**
GEN/YEAR	14	0.102**	0.066**	0.18**	0.152**	0.38**	0.015**
Error	56	0.006	0.013	0.01	0.04	0.09	0.003
CV		8.89	11.81	7.64	14.07	17.38	6.19

*, **- Significant at the 0.05 level and 0.01 level, Other without significant difference

Table 4 (continue) Analysis of variance for chemical components of essential oil of *R. damascena* Mill. Genotypes

Source of variations	df	Mean Square					
		Occidentalol acetate	*n*-eicosane	*n*-diocesan	*n*-henicosane	*n*-tricosane	*n*-octadecanol
YEAR	1	0.412**	0.494	0.343	0.003	0.197	0.002
YEAR/REP	4	0.015	0.169	2.928	0.045	1.246	0.002
GEN	14	0.17**	0.27**	1.63**	0.28*8	1.71**	0.028**
GEN/YEAR	14	0.247**	0.31**	1.866**	0.089	1.522**	0.004**
Error	56	0.008	0.102	0.623	0.072	0.194	0.001
CV		9.50	19.32	19.67	26.34	17.03	

*, **- Significant at the 0.05 level and 0.01 level, Other without significant difference

Table 5 Means comparison for chemical components of essential oil in different genotypes of Damask rose

Genotype	Geraniol	Linalyle acetate	Geranial	Trans roseoxide	Citronellyl acetate	N-undecanol	1-hexyl hexanoate	N-pentane	α-cadinene	N-heptane
ARAK1	29.09 a	6.42 abcd	15.15 bcd	0.28 bcd	0.298 bcd	1.30 ab	0.06 def	0.13 d	0 f	1.03 e
AZARGH	17.1b c	7.46 ab	17.08 bc	0.26 cd	0.52 a	0.83 bcd	1.36 a	0.09 d	4.82 a	1.66 bcde
FARS1	19.46 abc	7.71 ab	18.41 ab	0.32 abc	0.44 abc	1.037 ab	0.43 bc	0.12 d	0.56 de	1.86 bcde
ISFA5	26.01 ab	4.95c de	11.7 de	0.27 bcd	0.368 abcd	1.075 ab	0.24 cde	0.56bc	1.16 bc	2.09 abcd
ISFA7	25.84 ab	6.35 abcd	15.12 bcd	0.31 bcd	0.335 abcd	1.16 ab	0.33 bcd	0.38 cd	0.77 cde	2.23 abc
ISFA8	14.38 c	3.26 ef	7.14f g	0.26 cd	0.09e	0.82 bcd	0.0 d	0.38 cd	1.27 b	2.76 a
ISFA9	26.94 ab	4.60 def	10.79 def	0.18 cd	0.48ab	1.1 ab	0.41 bc	0.87 ab	1.28 b	2.33 ab
KERSH1	14.23 c	3.08 ef	6.93f g	0.169 c	0.37abcd	1.03 ab	0.0 d	0.56 bc	1.17 bc	1.58 bcde
KERSH10	19.28 abc	5.62 bcd	12.60 de	0.295 bcd	0.425abc	1.43 a	0.27 cde	0.34 cd	0.9 bcde	2.27 ab
KERSH3	22.79 abc	8.15 a	22.05 a	0.41 ab	0.27cde	1.12 ab	0.29 cd	0.53 c	0.93 bcd	1.16 e
KERSH8	19.33 abc	6.99 abc	15.15 bcd	0.275 bcd	0.25cde	1.24 ab	0.19 cde	1.06 a	1.18 bc	1.29 de
KERSH9	24.59 abc	4.30 def	9.13 efg	0.185 cd	0.47ab	0.92bc	0.15 cde	0.6b c	1.21 b	1.81 bcde
KHOR2	21.23 abc	2.55 f	6.29 g	0.187 cd	0.36abcd	0.52 cd	0.2 cde	0.65 bc	1.24 b	1.35 cde
LORS1	27.96 a	8.43 a	18.31 ab	0.447 a	0.27cde	1.27 ab	0.06 def	0.41 cd	0 f	2.13 abcd
YAZD1	20.01 abc	5.1 cde	13.59 cd	0.30 bcd	0.21de	0.46 d	0.57 b	0.16 d	0.5 e	1.45bcde

Items with common letters without significant difference

Table 5 (continue)- Means comparison for chemical components of essential oil in different genotypes of Damask rose

Genotype	2-6-EFARNES	BUTDODEC	NHEXADEC	NNONADEC	OCCIACE	NEICO	NDOCO	NHENI	NTRIC	NOCTADECA
ARAK1	1.87 b	0.2 cde	2.628 bc	19.285 bc	0.09 e	4.25 a	0.417 b	11.77 de	5.175 de	0 g
AZARGH	4.58 a	0.1 ef	0.91 d	11.58 c	0.46 cd	2.15 bc	0.68 b	14.76 cde	7.77 de	0 g
FARS1	1.93 b	0 f	2.661 bc	11.57 d	0.22 cde	1.177 c	0.175 b	11.54 e	18.23 a	0 g
ISFA5	2.51 b	0.31 abc	4.377 a	20.70 bc	0.11 e	2.358 bc	0.425 b	15.26 cde	4.87 de	0.06f g
ISFA7	2.42 b	0.37 ab	2.415 cd	22.73 ab	0.04 e	2.274 bc	0.44 b	13.76 cde	4.63 de	0.1efg
ISFA8	2.87b	0.43 a	4.255ab	28.45 a	0.22 cde	2.132 bc	0.558 b	21.68 abc	6.573 cde	0.12 ef
ISFA9	2.59 b	0.27 bcd	3.194 abc	21.67 b	0.13 e	2.246 bc	0.388 b	14.62 cde	4.93de	0.14 ef
KERSH1	2.86 b	0.36 a	3.792 abc	22.24 b	0.49 c	3.482 ab	0.934 b	25.05 a	10.987 b	0.14 ef
KERSH10	3.24 ab	0.41 a	3.398 abc	23.19 ab	0.22 cde	2.56 abc	0.427 b	17.02 bcd	5.228 de	0.2 de
KERSH3	2.83 b	0.18 de	2.342 cd	14.96 cd	1.08 b	1.672 bc	0.375 b	14.24 cde	4.416 de	0.21 cde
KERSH8	2.86 b	0.2 cde	2.632 bc	18.55 bc	0.18 cde	2.76 abc	3.335 a	13.66 cde	3.765 d	0.27 bcd
KERSH9	2.2 b	0.32 ab	3.414 abc	21.09 bc	0.06 e	2.697 abc	0.495 b	19.7abc d	6.137 cde	0.28 bcd
KHOR2	2.18 b	0.2 cde	3.773abc	20.675 bc	1.82 a	2.46 abc	0.837b	24.55bc	9.17bc	0.31abc
LORS1	1.97 b	0.31 abc	3.643abc	16.97 bcd	0 e	1.8 bc	0.253b	11.8de	3.69e	0.36bc
YAZD1	2.07 b	0.11 ef	2.907 abc	20.196 bc	0.16de	1.903 bc	0.673b	20.36abc	7.44cd	0.41a

Items with common letters without significant difference

Table 6 Pearson's Correlation coefficient between OE chemical compounds

	GEROL	LINACE	GERAL	THROS	CITACE	UNDEC	HEXHEX	YELE	NPEN	ACAD	HXAD	NHPTA	EFARN	BUDOD	NHXDD	NNODEC	OCCIACE	NEICO	NHENI	NDOCO
GEROL	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LINACE	0.31	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GERAL	0.30	0.97**	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
THROS	0.28	0.81**	.83**	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CITACE	0.15	0.06	0.03	-0.37	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UNDEC	0.36	0.49	0.40	0.36	0.13	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HEXHEX	-0.18	0.34	0.36	0.002	0.48	-0.3	1	-	-	-	-	-	-	-	-	-	-	-	-	-
YELEM	0.18	-0.48	-0.48	-0.49	0.22	0.01	-0.11	1	-	-	-	-	-	-	-	-	-	-	-	-
NPEN	0.08	-0.28	-0.31	-0.33	-0.01	0.14	-0.38	0.34	1	-	-	-	-	-	-	-	-	-	-	-
ACAD	-0.43	0.002	-0.02	-0.31	0.45	-0.28	.80**	0.10	-0.09	1	-	-	-	-	-	-	-	-	-	-
HXAD	-0.27	-0.148	-0.12	0.16	-0.26	0.24	-0.20	0.32	-0.06	-0.04	1	-	-	-	-	-	-	-	-	-
NHPTA	0.16	-0.26	-0.33	-0.01	-0.19	0.28	-0.44	0.4	0.01	-0.37	0.47	1	-	-	-	-	-	-	-	-
EFARN	-0.52*	0.09	0.06	-0.15	0.29	0.02	0.65**	0.08	-0.04	.88**	0.28	-0.25	1	-	-	-	-	-	-	-
BUDOD	0.01	-0.45	-0.51	-0.18	-0.21	0.33	-0.55*	0.53*	0.3	-0.19	.52*	.66**	0.06	1	-	-	-	-	-	-
NHXDD	0.02	-0.63*	-.65**	-0.22	-0.34	-0.05	-0.76**	0.37	0.33	-.52*	0.38	.59*	-0.46	.59*	1	-	-	-	-	-
NNODEC	-0.10	-.73**	-.74**	-0.4	-0.43	-0.04	-0.59*	0.53*	0.29	-0.31	0.44	.65**	-0.16	.83**	.68**	1	-	-	-	-
OCCIACE	-0.23	-0.28	-0.16	-0.16	0.023	-0.45	0.04	-0.29	0.16	0.18	-0.02	-0.50	0.09	-0.22	0.002	-0.13	1	-	-	-
NEICO	0.12	-0.35	-0.4	-0.46	0.01	0.26	-0.35	0.17	0.12	-0.19	-0.11	-0.09	-0.04	0.32	0.11	0.36	-0.08	1	-	-
NHENI	-0.59*	-.84**	-.80**	-.57*	-0.25	-.58*	-0.33	0.16	0.19	-0.02	0.15	0.02	-0.03	0.32	.55*	.56*	0.41	0.19	1	-
NDOCO	-0.29	0.004	-0.07	-0.18	-0.22	0.06	-0.07	-0.11	.61*	0.11	-0.18	-0.38	0.19	-0.08	-0.12	0.02	0.03	0.24	0.09	1

* and **, Correlation is significant at the 0.05 level and 0.01 level.

Table 7 Classification Results of DFA (summary table)

Original Count	Ward Method	Predicted Group Membership				
		1	2	3	4	Total
	1	4	0	0	0	4
	2	0	2	0	0	2
	3	0	0	6	0	6
	4	0	0	0	3	3
%	1	100	0	0	0	100
	2	0	100	0	0	100
	3	0	0	100	0	100
	4	0	0	0	100	100

100.0% of original grouped cases correctly classified

Table 8 Share of chemical components of essential oil for 3 first components

Variables	Components		Variables	Components	
	PC1	PC2		PC1	PC2
GERO	-0.067	0.289	NHEPTA	0.184	0.273
LINACE	-0.370	0.174	FARNES	-0.110	-0.243
GERAL	-0.373	0.150	BUDOD	0.277	0.187
THIROS	-0.243	0.263	NHEXDC	0.336	0.142
CITACE	-0.120	-0.166	NNODC	0.361	0.112
NUNDEC	-0.091	0.330	OCIACE	0.060	-0.290
HEXHEX	-0.272	-0.274	NEICO	0.162	0.030
YELEM	0.161	-0.240	NHENI	0.307	-0.263
NPENT	0.171	0.022	NDOC	0.029	-0.075
ACADEN	-0.124	-0.344	NTRIC	-0.046	-0.212
Eigen value	5.979	4.386	Eigen value	5.979	4.386
Proportion of variances	0.299	0.219	Proportion of variances	0.299	0.219
Cumulative variances	0.299	0.518	Cumulative variances	0.299	0.518

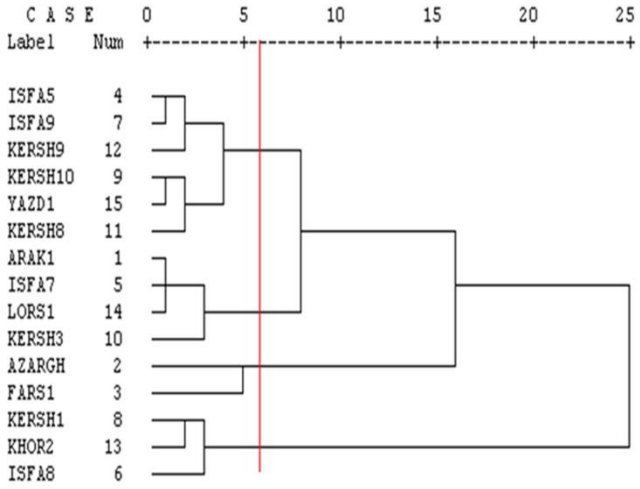

Fig. 1 Classification of *R. damascena* Mill. Genotypes by Ward method

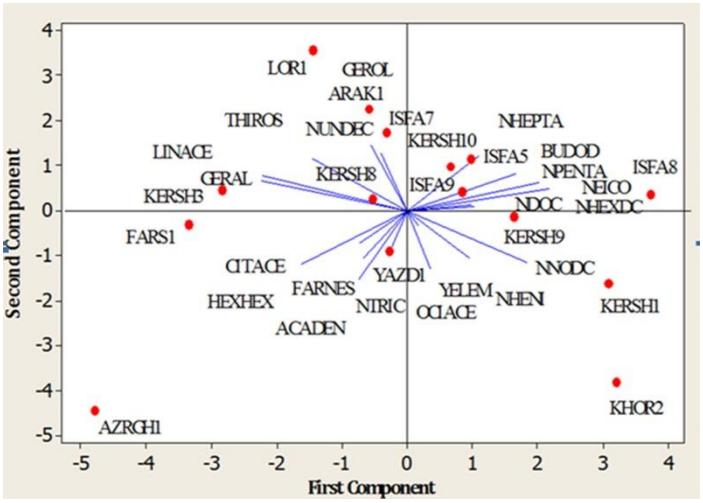

Fig. 2 Diagrams of the first and second components for chemical compounds of essential oil and Damask rose genotypes

The results of the principal component analysis showed that the compounds *n*-heptane, *n*-pentane, *n*-hexadecanoic, and butyl decanoate had the most positive contribution and the compounds linalyl acetate, geranial, hexyl hexanoate and trans- rose oxide had the most negative share in the first component. Also, the compounds *n*-undecanol and geraniol had the most positive portion, and the compounds α-cadinene, occidental acetate, *n*-nonadecane, and *n*-henicosane had the most negative role in the second component. The genotypes of Isfahan8, Isfahan9, Isfahan5, and Kermanshah10 had the highest amount of *n*-icosane, *n*-dicosane, *n*-pentane, *n*-heptane, *n*-hexadecanoic, and butyl decanoate. Kermanshah1, Kermanshah9, Yazd1 and Khorasan2 had the more abundant of *n*-henicosane, *n*-nonadecane, γ-elemene and Occidentalol acetate. Isfahan7, Arak1, Lorestan1, Kermanshah8, and Kermanshah3 had the highest amounts of geraniol, geranial, *n*-undecanol, trans-rose oxide, and linalyle acetate. *n*-tricosane, α-cadinene, farnesol, and hexyl hexanoate were observed to a greater extent in the genotypes of Yazd1, Fars1, and Azerbaijan-Gharbi1 (Fig. 2). The values of components for the EO chemical compounds, eigen value, the proportion of variances, and the cumulative variances are presented in Table 8.

Discussion

Significant variation between different genotypes for the chemical compounds of EO Was expected because these genotypes have geographical distances as well as genetic differences that can affect different biosynthetic pathways of the chemical composition of EO. In some studies, the results showed a great variety of EO compounds in *R. damascena* genotypes from different regions [18, 32, 33, 5, 34, 35]. In a study, quantitative and qualitative differences were shown for the EO chemical composition of *R. damascena* growing in Lebanon [36].

One of the important points in cluster analysis was the placement of some genotypes with a long geographical distance in a group, which shows that despite long geographical distances, these genotypes may have a common origin and may have been imported for cultivation. In the first group, Kermanshah1, Isfahan8, and Khorasan2 genotypes are in the same group and their most important common denominator is large amounts of long-chain alkanes (*n*-henicosane and *n*-tricosane). Fars1 and Azerbaijan-Gharbi1, despite the longest geographical distance, are in the same group and the important feature of their commonality is the high values of geranial. The genotypes of Arak1, Lorestan1, Kermanshah3, and Isfahan7 were located in the one group and their common feature is large amounts of the main and basic composition of rose EO (geraniol), which shows the EO quality of these genotypes is better than others. Among genotypes of Isfahan origin, continuous and purposeful selections may be the reason for their genetic similarity. The results of the present study are consistent with the results of other researchers that reported genetic similaritis in terms of essential oil components between Isfahan landraces of *R. damascena* [37] and different accessions of *R. damascena* from Isfahan [38]. Also, the results of cluster analysis showed that in some cases, there is not much agreement between the geographical origin of genotypes and their clustering. The reason may be the transfer of some genotypes from a common origin, such as Isfahan to other areas. There is a similarity between many of these genotypes that are grown or cultivated in different parts of Iran and the origin of many of them may be the same. It also seems that in some cases the differences between the genotypes of different geographical areas have not been enough to be in separate branches. Therefore, according to the results, it can be concluded that these genotypes can't be grouped for the chemical composition of EO based on geographical origin. For example, the genotypes of Azerbaijan-Gharbi1 and Fars1, despite the great geographical distance, are in the same group in terms of the chemical composition of EO. Some authors believed that these two genotypes seem to have different origins with most of the damask rose cultivated in Iran and may even be the result of a different hybridization [39]. Some other researchers have provided similar results [16, 37, 38, 39]. It should be noted that the amount of EO compounds is largely influenced by environmental and physiological factors, which may not be a very accurate indicator of genetic similarities and differences between genotypes. Also, different methods of extracting EO are effective on the type and the number of chemical compounds in EO. Karami *et al*. [18] reported Cluster analysis, was shown that the 44 accessions of damask rose had five distinct chemotypes namely citronellol, geraniol, neral, dihydro-linalool and *n*-nonadecane.

In damask rose essential oil, 4 main compounds affecting the quality of EO are citronellol, geraniol, nerol and 2-phenyl ethyl alcohol. However, in most of the essential oils extracted by water distillation, 2-phenyl ethyl alcohol (an important compound) cannot be separated [40]. According to some reports, damask rose essential oil extracted with dichloromethane contains higher amounts of 2-phenyl ethyl alcohol [1].

The results of the PCA revealed to some extent the relationships between the chemical compounds of EO. Four different trends were observed for EO chemical compounds among the genotypes. On the other hand, it showed the

relationships between each genotype and each group of genotypes with the EO chemical compounds.

A positive correlation between linalyle acetate and geraniol shows that the increases of one of these compounds lead to increasing another compound. Therefore, breeding for the synthetic direction of one of these compounds increases the other compound as well. Some researchers believe that it is necessary to consider the positive relationship between the geraniol and citronellol with geranial and citronellyl acetate in breeding programs of damask rose [41]. A negative correlation there was between the amounts of *n*-docosane, *n*-henicosane, *n*-tricosane, and *n*-icosane (long-chain alkanes), and increasing the percentage of a compound was associated with decreasing the amounts of others.

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 *R. damascena* [17].

Although Yousefi and Tabaei Aghdaei [23] believe that because of asexual reproduction (through cuttings) there is a low genetic variation among Damask rose accessions and landraces [23], Due to the existence of genetic diversity, it is suggested to use the studied accessions to modify and produce cultivars for high quality and quantity rose oil.

Conclusion

There are differences between different genotypes of damask rose that is grown in the same climatic and agronomic conditions for EO chemical compounds. In order to improve and produce the quality of essential oil, it is necessary to select and study genotypes with higher amounts of geraniol, citronellol, and 2-phenyl ethyl alcohol. Of course, in order to increase the quantitative and qualitative production of essential oil, it is necessary to consider the yield of flowers per hectare and the EO yield at the same time.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Data availability statement

The data that support the findings of this study are available from the author, [B. Y.], upon reasonable request.

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