Effects of Locality, Flower Organ, and Ratio Flower to Distilled Water on Rose Water Quality of *Rosa* × *damascena* Herrm.

Reyhaneh Taebnia[1], Fatemeh Sefidkon[2*], Ali Mohammadi Torkashvand [1], Ali Ashraf Jafari[2] and Sepideh Kalate Jari[1]

[1] *Department of Horticultural Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2] *Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran*

Article Info

Article Type
Original Article

Article History
Received: 14 September 2024
Accepted: 17 November 2024

***Corresponding author**
Email: sefidkon@rifr-ac.ir

ABSTRACT

In order to study the optimized parameters for the production of rose water, a factorial experiment was conducted using three factors A) locality, B) flower organ, and C) flower-to-distilled water ratio (FDWR). The quality of rose water was assessed by essential oil, and phenyl ethyl alcohol content, pH, acid, ester, oxidation, and iodine number. The essential oils in rose water were extracted and analyzed by GC and GC/MS. There were significant differences between flowers and petals for all the traits except acid and iodine number. For FDWR, there was a significant difference between all traits except acid number. The amounts of essential oil, acidity, oxidation, and iodine numbers were decreased drastically by increasing FDWR. There was no significant difference between localities for traits, but the response of FDWR in both locations was not similar. The higher essential oil content (14.6 mg/100 mL) and iodine number (63.33) were obtained in 1:2 kg/L FDWR. The plant material by FDWR interaction was significant for oil content, pH, and acid number, i.e. the higher oil content (16.03 mg/100 mL) was obtained from petals in 1:2 kg/L FDWR. The higher values for essential oil, ester, oxidation and iodine number were obtained from the petals. A decreasing trend in the amount of methanol, ethanol and phenyl ethyl alcohol was observed with FDWR reduction. A comparison of the mean percentages of the main oil components in the rose water showed higher amounts of citronellol and phenyl ethyl alcohol and a lower percentage of geraniol in the Qamsar samples.

Keywords: Rose water, Hydro-distillation, Essential oil, Physicochemical characteristics

How to cite this paper
Taebnia R., Sefidkon F., Mohammadi Torkashvand A., Jafari A.A., Kalate Jari S. Journal of Medicinal Plants and By-products, 2025; 14(3):268-276. doi: 10.22034/jmpb.2024.367039.1764

INTRODUCTION

Rosa × *damascena* Herrm. recognized as gul-e-mohammadi (or gul-e-surkh), is one of the most popular species in the Rosaceae family. This is a perennial bushy shrub reaching approximately 1–2 m in height with large pale pink flowers [1]. This plant flowers only once annually, and its flowering period in Iran lasts for almost 35-45 days (from the second half of May to the end of June). During this period, a fully developed plant (4 years old and above) can produce around 500-600 flowers [2, 3].

Damask rose is the most important plant species for the production of rose water, which is commonly known as Golab in Iran. The origin of the damask rose is in the Middle East, and some evidence indicates that Iran is the origin of the rose water, but the origin of its fragrant oil and extracts is the flowers of Greece [1]. Due to the desirable climate for growing Damask rose in Iran, the produced essential oil possesses high quality. Iran is the largest producer of rose water in the world due to having suitable raw materials in terms of quantity and quality of the product. It can be used in foodstuffs, perfumery and cosmetics, and pharmaceuticals. Various parts, especially the petals of this versatile species are used for decorative, perfume, medicine, and food industry. Moreover, the essential oil of this plant is widely used in aromatherapy, perfumery and cosmetic industries [4].

The flowers of *R. damascena* contain various constituents including flavonoids, terpenes, polyphenols, anthocyanins and glycosides [5]. The volatile oil extracted from *R. damascena* flowers has analgesic, anticonvulsant, hypnotic, cardiovascular, laxative and antioxidant properties [6]. Rose water is one of the most famous and widely used products in traditional medicine, so many pharmacological studies show its potential therapeutic benefits against various diseases, and in cosmetics the results are similar to the standard drug, taking into account the many healing properties of rose water. It is used for relaxing the mind and anti-depression, treatment of old coughs, problems of the respiratory system, skin health [7] and in aching throat, puffy tonsillitis, slimming to women and uterine hemorrhages and urticarial healing of abdominal chest pain, strengthening the heart, cure of menstrual bleeding and digestive disorders and decrease inflammation used as a mild laxative [8]. More recent studies show that rose oil has anti-HIV properties [9] and can stop and kill some strains of Xanthomonas [10].

It has been reported that rose petals should be picked up in the early morning to obtain higher oil yield with high quality [3]. Some research has proven that the yield of rose oil decreases with increasing temperature and fully opened and ripe flowers are not appropriate for harvesting [11, 12]. Rose petals should be distilled immediately after harvest to stop the fermentation processes. The fermentation decreases the oil yield and increases the citronellol content [3].

2-Phenyl ethanol, generally known as phenyl ethyl alcohol, is a compound used in the food industry (drinks, candy, and cookies) and cosmetics. Its esters, especially phenyl ethyl acetate, are also worth fragrance compounds. Because of its feature fragrance and flavor 2-Phenylethanol may be found in the essential oils of many plants and flora. Due to phenyl ethyl alcohol, which is abundant in rose flowers, has a rose-like odor, being one of the dominant scents emitted by damask rose. Being hydrosoluble, this alcohol is the most useful thing in rose water [13]. The normal amount of 2-phenylethanol in rose oil was reported to be as much as 60% [13, 14].

Increasing demand for rose water has led to some fraud and the production of inferior products containing the essential oils of other flowers or rose water diluted with water. Since there is no substituted natural alternative or synthetic essential oil to replace this precious flower, it is one of the most expensive oils in the entire world market [15]. Plant-derived essential oils are not true-to-type natural compounds; indeed, they are the complex product created during the distillation process with water due to the effect of high temperature, acidity, etc.., [16]. In addition, the production of essential oil as a secondary metabolite is affected by the different ecological and edaphic conditions of the zone where the plant grows [17]. Rose oil contains a complex mixture of different compounds. The most common compound obtained from flower blossoms is phenylethyl alcohol and its other main compounds include geraniol, citronellol and nerol alcohols, which are among the most important volatile substances used in the perfumery, cosmetic and soap-making industries. Phenyl alcohol is also one of the most important aromatic substances used in all types of perfumes [18]. Atanasova *et al.* [19] investigated the feasibility of creating new biomes for the growth and cultivation of rose plants in Bulgaria. The simulation of the climatic conditions of 2050 showed that with the general warming trend of Bulgaria's climate, the favorable areas for the cultivation of this plant have increased and the amount of essential oil production will also increase slightly compared to the current climate. Although Different parts of the rose hip are aromatic, usually the flowers are distilled for essential oil. Also, fresh and healthy flowers are used to produce Concrete and Absolute [20]. Analysis of the essential oil obtained from different parts of the flower showed that the petals had the highest quality and quantity of essential oil (more than 40% geraniol and citronellol and the lowest percentage of waxes). However, the amount of geraniol and citronellol in other flower parts was very low (about 2.5%) and the amount of waxes was significantly high [21]. The volatile compounds obtained from rose water (ex. *Rosa* × *damascena* flowers) by liquid-liquid extraction with dichloromethane consisted mainly of 2-phenylethanol (69.7-81.6%), linalool (1.5-3.3%), citronellol (1.8-7.2%), nerol (0.2-4.2%), geraniol (0.9-7.0%) along with rose oxides, and all other characteristic minor rose compounds [22]. Quality evaluation of 10 rose water samples from local markets in Shiraz, revealed that phenethyl alcohol, geraniol, and citronellol were the main constituents of most samples, but it seems that *Pelargonium* and *Dianthus* essential oils and synthetic essences had been added to some samples [23]. The purpose of this research is to investigate some factors influencing rose water quality. The results of this research could be used to modify the existing standards of rose water by the Iran National Standard Organization (INSO).

MATERIALS AND METHODS

Chemical Materials and Plant Materials

All chemicals and reagents were purchased from Merck and Aldrich. The flowers of *Rosa* × *damascena* were collected in the early morning from two localities, Qamsar (Kashan, Isfahan Province) and Safadasht (Alborz Province) in May 2021 and 2022. Whole flowers including petals, stamens and sepals (as traditionally used) were subjected to hydro-distillation to obtain rose water. In addition, the petals of some flowers were separated carefully and distilled separately (Fig. 1).

Fig. 1 Whole flowers and petals are used in the production of rose water

The rose water was obtained via hydro-distillation using two flower organs (whole flower and petals) and flower-to-water ratio (FDWR) in four levels (1:2, 1:4, 1:6 and 1:8 kg/L). The rose water was distilled as follows: 1.0 kg of the fresh rose petals or whole flowers, immediately after harvesting, were prepared to go through the water-distillation process. During the process, purified distilled water passes through fresh whole flowers and petals. All of the soluble elements and the water that condenses and drips into a collecting vessel are called rose water. Rose waters were collected in 2, 4, 6 and 8 liters from each sample, separately, at three replications.

Determination of Rose Water Quality

For assessment of rose water quality, the following chemical analyses were made: acidity (pH), acid number, ester number, oxidation number, iodine number, essential oil content and composition, methanol, ethanol and phenyl ethyl alcohol content. The methods of determination were as follows:

The acidity of the rosewater sample was recorded using a pH meter (model WTW InoLab Cond 720).

For determination of the acid number (INSO 5759, [24])**:** 50 mL of the rose water sample was transferred to a 250 mL Erlenmeyer flask [25]. Then a few drops of phenolphthalein indicator were added to it and titrated with sodium hydroxide solution 0.01 N until a stable pale pink color appeared. The acid number is calculated according to Equation (2):

$$A = \frac{V \times 0.6 \times 100}{50} \qquad (2)$$

Where:

V: Consumed volume of sodium hydroxide 0.01 N (mL);
A: Acid number in terms of acetic acid (mg/100 mL)

For the determination of ester number (INSO 5759, [24]): 100 mL of the rose water sample was transferred to a 250 mL Erlenmeyer flask [25]. Then a few drops of phenolphthalein indicator were added to it and titrated with sodium hydroxide solution 0.01 N until a stable pale pink color appeared for 30 sec. Next 10 ml of sodium hydroxide solution 0.1 N was added to it. The reflux system was placed on Erlenmeyer and then it was heated in a hot water bath at 90 °C for 2 h until the esters were saponified. After the end of reaction time, the contents of the Erlenmeyer were cooled to room temperature and then titrated with hydrochloric acid solution 0.02 N until a stable pale pink color appeared for 3 sec. Also, a control test with the same conditions (with distilled water) was carried out. The Ester number is calculated using equation (3):

$$S = 2 \times (B - A) \quad (3)$$

Where:
S: Ester number;
A: Consumed volume of hydrochloric acid 0.02 N for control test (mL);
B: Consumed volume of hydrochloric acid 0.02 N for sample test (mL)

For determination of oxidation number (INSO 5759, 2022):50 mL of the rose water was transferred to a 250 mL Erlenmeyer flask. Then, 10 mL of diluted sulfuric acid (1:3) and 15 mL of potassium permanganate 0.1 N were added to the flask. The Erlenmeyer lid was closed and kept in a dark place for 30 min. Then, 10 mL of potassium iodide solution 10% and a few drops of starch solution were added to the flask and titrated with sodium thiosulfate solution 0.1 N until it became colorless. Also, a control test with the same conditions (with distilled water) was performed. Oxidation number calculated using Equation (4):

$$X = 20 \times (B - A) \quad (4)$$

Where:
B: Consumed volume of sodium thiosulfate for control test (mL);
A: Consumed volume of sodium thiosulfate for sample test (mL);
X: Oxidation number

For the determination of iodine number (INSO 5759, [24]): 50 mL of the rose water was transferred to a 250 mL Erlenmeyer flask, and the sample was neutralized using sodium hydroxide solution 0.01 N and pH indicator paper (INSO, 2014). Then, 10 mL of sodium hydroxide solution 1.0 N and 10 mL of iodine 0.1 N were added to the flask. The Erlenmeyer lid was closed and kept in a dark place for 15 min. Then, 10 mL of diluted sulfuric acid (1:3) and a few drops of the starch solution were added to the flask and titrated with sodium thiosulfate solution 0.02 N until it became colorless. Also, a control test with the same conditions (with distilled water) was performed. The iodine number is calculated using Equation (5):

$$X = 4 \times (B - A) \quad (5)$$

Where:
B: Consumed volume of sodium thiosulfate for control test (mL);
A: Consumed volume of sodium thiosulfate for sample test (mL);
X: Iodine number

Essential Oil Content

Chemical tests to determine the essential oil content were carried out according to the Pentane method (INSO 5759, [24]). So, 50 g of sodium chloride and 33 mL of *n*-pentane were added to 250 mL of the sample and stirred for 15 min. After the exhaust gases had been released, the mixture was left stilled until two phases of water and oil were well separated. Then, the water phase discharged, and the amount of essential oil was calculated according to the weight difference, as shown in the following equation:

$$S = (A - B) \times 0.53 \times 1000 \quad (6)$$

Where:
A: weight of Erlenmeyer with essential oil (g);
B: weight of Erlenmeyer without essential oil (g);
S: The amount of essential oil (mg /100 mL)

Measurement of Methanol, Ethanol, and Phenyl Ethyl Alcohol Content

Methanol, ethanol and phenyl ethyl alcohol concentrations of each sample were measured by Gas Chromatography (GC), according to ISO (No. 22448) [26, 27]. Calibration curves are presented in

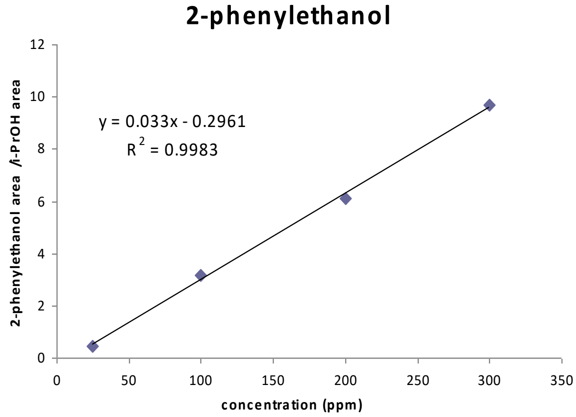
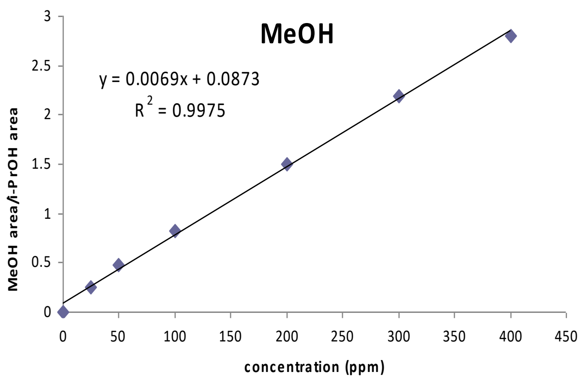
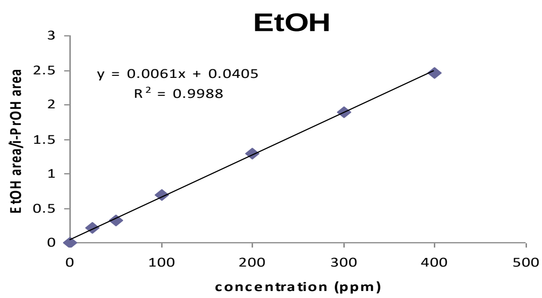


Fig. *2*. The GC instrument was Agilent Technologies (7890A) equipped with a DB-5 Fused silica column (30 m × 0.32 mm i.d., Film thickness 1.0 µm) and a flame ionization detector (FID).





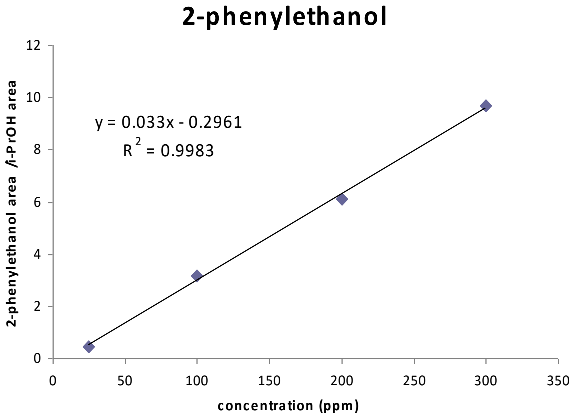


Fig. 2 Calibration curve for determination of methanol, ethanol, and phenyl ethyl alcohol (2-phenyl ethanol) contents in rose waters

The initial temperature of the column oven was 40ºC, held for 5 min, then increased to 200 ºC at a rate of 20 ºC/min and held at this temperature for 2.5 min. The split ratio of injections was 10:1. The FID detector temperature was 200 °C. The injection port temperature was 200 °C. The carrier gas was Nitrogen with a flow rate of 1 mL/min and 1 µL of each sample was injected into the device after adding the internal standard.

Essential Oil Analysis

The oils were analyzed by a combination of capillary gas chromatography, using a flame ionization detector (GC-FID) and mass spectrometric detection (GC/MS). The components of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature [28-30]. The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes.

GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm). The Oven temperature was held at 50 °C for 5 min. and then programmed to 240 °C at a rate of 3 °C/min. The Detector (FID) temperature was 265 °C and the injector temperature was 250 °C. Helium was used as a carrier gas with a linear velocity of 32 cm/s.

Gas chromatography-mass spectrometry (GC/MS) analysis was conducted with a Varian 3400 GC connected to a mass spectrometer Saturn model, equipped with a DB-5-fused silica capillary column (30 m length, 0.25 mm internal diameter, and 0.25 μm film thickness); the injection chamber temperature and the transfer line were set at 260 and 270 °C, respectively. The carrier gas was helium with a linear velocity of 31.5 cm/s, a split ratio of 1 to 60, 1 s of scan time, and a mass range of 40–300 a.m.u. The oven temperature adjustment program was the same as the one mentioned for GC. The FID data was used and the percentages of compounds were calculated by the area normalization method, without considering response factors.

Statistical Analysis

The collected data were analyzed statistically using a factorial experiment based on a completely randomized design with three replications. The Mean comparison was made using Tukey's test at 5% confidence level.

RESULTS

There were significant differences between localities for acid number (P<0.01), essential oil content and iodine number (P<0.01) (Table 1). The main effect of the flower organ was significant for Essential oil, pH, ester number and oxidation number (P<0.01). The effect of flower to distilled water ratio (FDWR) was significant for all the traits except acidity (P<0.01). The locality by FDWR interaction effect was significant for all traits except oxidation number. Similarly, the flower organ by FDWR interaction effect was significant for essential oil acidity and acid number. Finally, the three-way interaction effect of location by flower organ by FDWR was significant for pH and acid number (Table 1).

Table 1 Analysis of variance of the effects of localities, flower organ, and flower/distilled water ratio (FDWR) on Rose water of *R. damascena*

Source of variation	DF	MS					
		Essential oil	Acidity (pH)	Acid number	Ester number	Oxidation number	Iodine number
Locality (L)	1	12.93 *	0.025	3.52 **	0.001	1813	375.2 *
Flower organ (F)	1	62.3 **	0.130 **	0.083	2.52 **	17366 **	23.8
FDWR (R)	3	127.0 **	0.421 **	0.294	2.20 **	37707 **	1635.1 **
L× F	1	0.203	0.025	0.053	0.021	1131	78.5
L×R	3	43.0 **	0.329 **	0.74 *	2.15 **	855	911.1 **
F×R	3	12.3 **	0.038 *	1.31 **	0.29	620	81.9
L×F×R	3	2.85	0.055 **	0.48*	0.28	1296	175
Error	32	2.13	0.011	0.172	0.32	1248	101.3
Total	47						

*, ** significance at 0.05 and 0.01 probability levels, respectively.

The means of locations are presented in **Error! Not a valid bookmark self-reference.**. The higher numbers of many traits were obtained in Safadasht. There was a significant difference between whole flowers and petals for all the traits except acid and iodine numbers (P<0.01). The lower amount of pH and the higher values of essential oil content, ester and oxidation numbers were obtained in petal water. For example, the essential oil of the petals was 27% higher than whole flowers (Table 3).

For the flower/distilled water ratio (FDWR), the trends of essential oil, pH, oxidation, and iodine numbers, were negative and their values were decreased drastically by increasing of rose water volume. In contrast, for acid and ester numbers, the trend was positive and the higher numbers were obtained in 1:8 liter FDWR (Table 4).

Table 2 Mean comparison between two localities (Qamsar and Safadasht) for rose water traits in *R. damascena*

Locality	Essential Oil (mg/100 mL)	Acidity (pH)	Acid number (mg CH_3COOH /100 mL)	Ester number	Oxidation number	Iodine number
Qamsar	8.987 b	5.808 a	1.542 b	1.517 a	167.50 a	33.417 a

Safadasht	10.025 a	5.854 a	2.083 a	1.525 a	155.21 a	27.825 b

Means with the same letter are not significantly different at 0.05 probability level by Tukey's test

Table 3 Mean comparison between whole flowers and Petals for rose water traits in *R. damascena*

Flower Organ	Essential Oil (mg/100 mL)	Acidity (pH)	Acid number (mg CH_3COOH/100 mL)	Ester number	Oxidation number	Iodine number
Flower	8.366 b	5.883 a	1.771 a	1.29 b	142.33 b	29.917 a
Petals	10.646 a	5.779 b	1.854 a	1.75 a	180.38 a	31.325 a

Means with the same letter are not significantly different at 0.05 probability level by Tukey's test

Table 4 Mean comparison between Flower/distilled water ratio (FDWR) levels in rose water traits in *R. damascena*

FDWR (kg/L)	Essential Oil (mg/100 mL)	Acidity (pH)	Acid number (mg CH_3COOH/100 mL)	Ester number	Oxidation number	Iodine number
1: 2	13.613 a	5.908 ab	1.817 a	1.41 b	235.83 a	47.51 a
1: 4	9.518 b	5.983 a	1.625 a	1.40 b	169.83 b	29.16 b
1: 6	9.233 b	5.875 b	1.811 a	1.13 b	133.42 bc	24.08 b
1: 8	5.658 c	5.558 c	2.008 a	2.13 a	106.33 c	21.73 b

Means with the same letter are not significantly different at 0.05 probability level by Tukey's test.

Two-way Interaction Effects

There were no significant differences between locations by flower organ interaction effect, indicating that in both locations, the responses of both flower and petals were similar and a higher amount of essential oil was obtained in petals (Table 5).

The locations by FDWR interaction effect was significant for all the traits except oxidation number (P<0.01) (Table 1), indicating the response of FDWR in both locations was not similar, since the higher essential oil content (14.6 mg/100 mL) and the higher iodine number (63.33 mg/100 mL) were obtained in 1:2 kg/L ratios that were significantly higher than all the numbers of both locations (Table 6).

The flower organ by FDWR interaction effect was significant for essential oil content, pH, and acid number (P<0.01) (Table 1). Indicating the response of FDWR in the flower organ was not similar, since the highest amount of essential oil (16.03 mg/100 mL) was obtained from petals in 1:2 Kg/l ratio that was significantly higher than all the other samples in both whole flower and petals (Table 7).

Table 5 The effects of locality and rose organ interactions on rose water traits

Locality	Flower Organ	Essential Oil (mg/100 mL)	Acidity (pH)	Acid number (mg CH_3COOH/100 mL)	Ester number	Oxidation number	Iodine number
Qamsar	Flower	7.78 c	5.88 a	1.46 c	1.26 b	153.33 ab	31.43 a
	Petals	10.19 a	5.73 b	1.61 bc	1.76 a	181.67 a	35.41 a
Safadasht	Flower	8.95 bc	5.88 a	2.07 ab	1.31 b	131.33 b	28.41 a
	Petals	11.11 a	5.82 ab	2.092 a	1.73 a	179.08 a	27.25 a

Means with the same letter are not significantly different at 0.05 probability level by Tukey's test.

Table 6 The effects of locality and flower/distilled water ratio (FDWR) interactions on rose water traits in *R. damascena*

Locality	FDWR	Essential oil (mg/100 mL)	Acidity (pH)	Acid number (mg CH3COOH/100 mL)	Ester number	Oxidation number	Iodine number
Qamsar	1: 2	14.67 a	6.06 a	1.28 c	1.06 b	252.6 a	63.33 a
	1: 4	9.98 bc	6.11 ab	1.55 bc	1.16 b	178.1 bc	27.46 b
	1: 6	6.01 d	5.85 c	1.367 c	1.11 b	130.5 cd	21.86 b
	1: 8	5.26 d	5.31 e	1.967 b	2.73 a	108.8 d	21.11 b
Safadasht	1: 2	12.55 ab	5.75 d	2.35 a	1.76 ab	219.1 ab	31.66 b
	1: 4	9.05 c	5.96 b	1.71 b	1.63 b	161.6 bcd	30.86 b
	1: 6	12.45 ab	5.91 bc	2.23 ab	1.16 b	136.3 cd	26.31 b
	1: 8	6.05 d	5.81 cd	2.05 b	1.53 b	103.8 d	22.46 b

FDWR= Flower (Kg) to water (Liter) ratio

Means with the same letter are not significantly different at 0.05 probability level by Tukey's test.

Table 7 The effects of flower organ and flower/distilled water ratio (FDWR) interactions on rose water traits in *R. damascena*

Flower Organ	FDWR	Essential oil (mg/100 mL)	Acidity (pH)	Acid number (mg CH_3COOH/100 mL)	Ester number	Oxidation number	Iodine number
Flower	1: 2	11.19 b	5.98 ab	2.15 a	1.11 b	207.33 ab	47.83 a
	1: 4	9.37 bc	6.03 a	1.51 ab	1.13 b	149.33 bc	25.33 b
	1: 6	7.78 cd	5.98 ab	1.35 b	1.11 b	118.67 c	22.51 b
	1: 8	5.17 d	5.53 d	2.08 ab	1.83 ab	94.11 c	24.11 b
Petals	1: 2	16.03 a	5.83 bc	1.48 ab	1.73 ab	264.33 a	47.16 a
	1: 4	9.67 bc	5.93abc	1.75 ab	1.66 ab	190.33 b	33.11 ab
	1: 6	10.63 b	5.76 c	2.25 a	1.16 b	148.17 bc	25.66 b
	1: 8	6.21 d	5.58 d	1.93 ab	2.43 a	118.67 c	19.46 b

FDWR= Flower (Kg) to water (Liter) ratio

Means with the same letter are not significantly different at 0.05 probability level by Tukey's test.

Three-way Interaction Effect

The three-way interaction effect of locations by flower organ by FDWR was significant for pH and acid number. For all the traits the three-way interactions are presented in Fig. ***3***. The higher essential oil content with one exception was always obtained on petals in both locations (Fig. ***3***a). The higher rose water pH with few exceptions was obtained in the whole flower organ (Fig. 3b). For acid numbers, the trends of variations were not stable (Figure 3c), but for ester, oxidation and iodine numbers with few exceptions, the higher values were always obtained in petals (Fig. 3d, 3e and 3f), respectively.

The Effects of Locality and FDWR on Alcohol Content

Determination of methanol, ethanol and phenyl ethyl alcohol in all distilled waters from whole flowers and petals of *Rosa* × *damascena* in two localities and different FDWRs showed a decreasing trend in the amount of the alcohols with reducing FDWR in both localities and flower organs (
Table ***8***). For distilled waters from whole flowers, the average amount of phenyl ethyl alcohol was the same in Safadasht and Qamsar, but samples from Qamsar contained a higher amount of ethanol and a lower amount of methanol than Safadasht. For distilled waters from petals, samples from Safadasht contained higher amounts of ethanol and methanol, but lower phenyl ethyl alcohol than Qamsar.

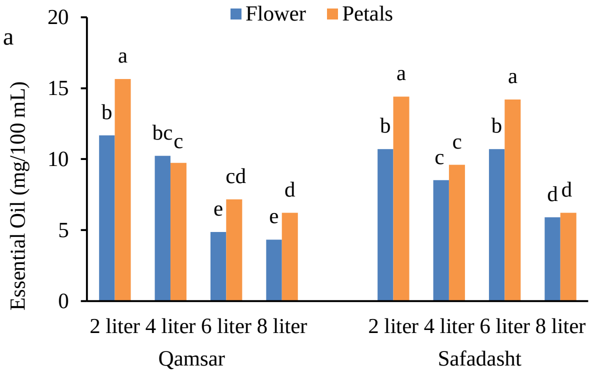
A comparison of the average amount of alcohol in rose water obtained from whole flowers and petals in two locations showed that there was not much difference between whole flowers and petals in Qamsar, but in Safadasht, the amount of methanol and ethanol in petal distilled water was higher than in whole flowers.

Essential Oil Composition (in Rose Water)

The essential oils were extracted from all rose water samples by liquid-liquid extraction with n-pentane. The oils were analyzed by a combination of capillary gas chromatography, using a flame ionization detector (GC-FID) and mass spectrometric detection (GC/MS). Two samples of GC chromatograms of the essential oils extracted from whole flower (**Error! Reference source not found.**) and petal rose water are presented (Fig. 5).

The Effects of Locality and FDWR on Essential Oil Compound

Fourteen constituents were identified in essential oils extracted from whole flower (Table 9) and petal (Table 10) rose water. The major components of the essential oils extracted from whole flower distilled water in different FDWRs were citronellol (27.6 to 47.5% in Safadasht and 34.9 to 46.4% in Qamsar), geraniol (16.9 to 30.3% in Safadasht and 3.4 to 11.4% in Qamsar) and phenyl ethyl alcohol (13.5 to 24.8% in Safadasht and 22.5 to 46.6% in Qamsar).



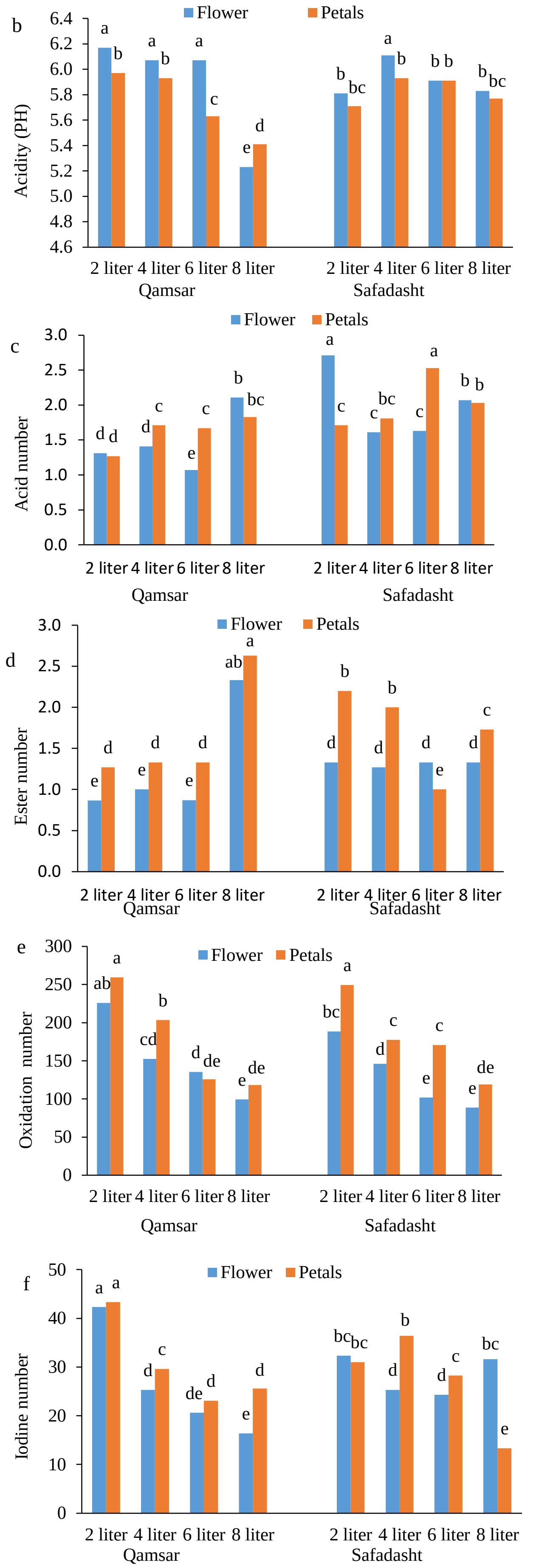


Fig. 3 The three-way interaction effects of location by flower organ by flower/distilled water ratio (FDWR) on rose water traits in *R. damascena* Means of column with the same letter are not significantly different at 0.05 probability level by Tukey's test.

There was no clear trend in changing the number of compounds in the oil of whole flower rose water at different FDWRs, but the comparison of the mean percentage of main components showed higher amounts of citronellol (41.1%) and phenyl ethyl alcohol (32.2%), and a lower percentage of geraniol (8.8%) In the Qamsar samples (Table 9).

Table 8 The effects of locality and flower/distilled water ratio (FDWR) on rose water from whole flowers and petals of *Rosa damascena*

Location	FDWR	Whole flower			Petal		
		Methanol (ppm)	Phenyl ethyl alcohol (ppm)	Ethanol (ppm)	Methanol (ppm)	Phenyl ethyl alcohol (ppm)	Ethanol (ppm)
Safadasht	1: 2	138.5 a	61.3 a	20.9 c	226.8 a	91.7 a	166.7 a
	1: 4	108.7 b	54.2 b	21.2 c	151.7 a	50.5 b	135.0 a
	1: 6	69.1 c	49.7 b	18.9 c	56.0 b	33.1 c	31.2 b
	1: 8	44.8 d	47.3 b	14.1 d	35.3 c	31.8 c	21.7 b
	Mean	90.2	53.1	18.8	117.5	51.8	88.7
Qamsar	1: 2	135.3 a	89.3 a	153.0 a	122.9 a	85.9 a	146.8 a
	1: 4	82.6 c	55.9 b	73.3 b	63.1 b	50.4 b	140.6 a
	1: 6	67.2 c	34.5 c	68.8 b	54.6 b	50.3 b	45.8 b
	1: 8	33.3 d	31.4 c	54.1 b	52.1 b	47.9 b	29.9 b
	Mean	79.6	52.7	87.3	73.2	58.6	90.8

Means with the same letter in each column are not significantly different at 0.05 probability level by Tukey's test.

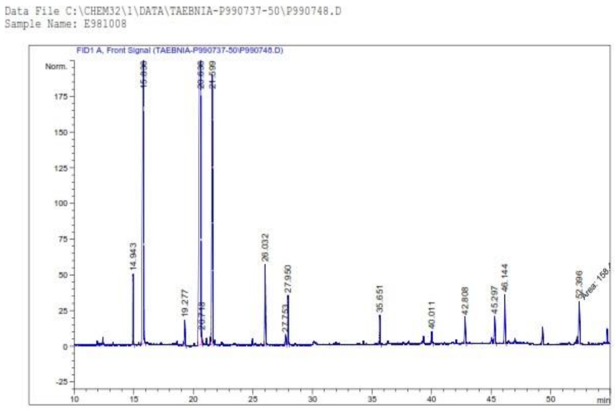


Fig. 4 GC chromatogram of the essential oil extracted from the whole flower distillate of *Rosa* × *damascena*

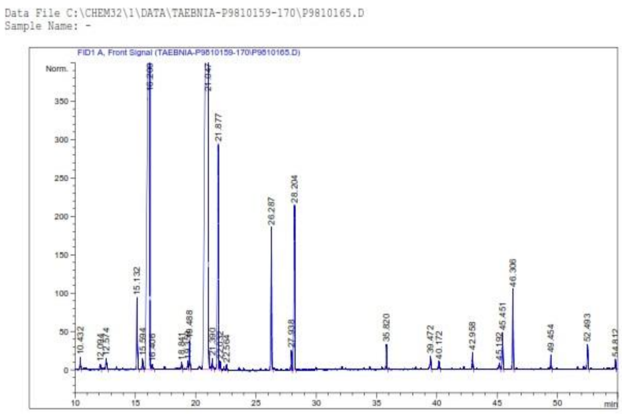


Fig. 5 GC chromatogram of the essential oil extracted from petal distillate of *Rosa* × *damascena*

The major components of the essential oils extracted from petal distilled water in different FDWRs were citronellol (32.9 to 38.9% in Safadasht and 25.8 to 49.9% in Qamsar), geraniol (12.7 to 33.1% in Safadasht and 8 to 13.3% in Qamsar) and phenyl ethyl alcohol (17.4 to 42.4% in Safadasht and 27.5 to 33.6% in Qamsar).

There was also no clear trend in the changing number of compounds in the oil of petal rose water at different FDWRs, but the comparison of the mean percentage of main components showed higher amounts of citronellol (44.1%) and phenyl ethyl alcohol (30.5%), and a lower percentage of geraniol (9.2%) in Qamsar samples (**Error! Reference source not found.**).

DISCUSSION

As the results showed, there were significant differences between localities for acid and iodine numbers and essential oil content in rose waters. In addition, the interaction effect of locations by FDWR was significant for most of the traits. The rose waters from Safadasht samples had higher amounts of acid and iodine numbers, and essential oil content. The essential oils extracted from rose water samples from Qamsar contained higher levels of citronellol and phenyl ethyl alcohol and lower levels of geraniol than Safadasht. Effects of climatic factors such as altitude, temperature, and rainfall on the quantity and quality of secondary metabolites of medicinal and aromatic plants were reported in some previous investigations. *Rosa* × *damascena* Herrm. has been used for essential oil production in some areas.

A study on essential oils of seven cultivars of *R. damascena* from different locations in Lebanon showed differences in the identified components [31]. In addition, the essential oil yield varied between 0.033 and 0.065%. The results of other studies showed that the factors that influence the chemical composition of *R. damascena* essential oil varied [3]. Some of these factors are varieties or accessions of Damask rose, agronomic practices, method of propagation, cultivation date, harvest procedures, time and level of pruning, storage of plant material, and method of distillation.

Table 9 Composition of the essential oil extracted from whole flower rose water in different FDWRs

No	Compound	RI	Safadasht (%)					Qamsar (%)				
			1:2	1:4	1:6	1:8	Mean	1:2	1:4	1:6	1:8	Mean
1	Linalool	1100	0.2	0.3	0.2	-	0.2	-	-	-	-	-
2	Phenyl ethyl alcohol	1112	13.5	16.1	19.9	24.8	18.6	22.5	32.7	27.0	46.6	32.2
3	Decanal	1203	-	0.5	0.4	-	0.2	-	-	-	-	-
4	Nerol	1233	-	-	-	-	-	0.3	-	0.3	-	0.2
5	Citronellol	1228	47.5	39.0	27.6	35.6	37.4	45.8	37.1	46.4	34.9	41.1
6	Neral	1241	0.3	10.3	-	9.5	5.0	0.3	-	-	-	0.1
7	Geraniol	1257	30.3	20.7	28.6	16.9	24.1	11.4	9.8	10.6	3.4	8.8
8	Eugenol	1356	3.2	4.1	5.5	4.4	4.3	3.3	4.1	2.8	2.4	3.2
9	Methyl eugenol	1405	1.3	2.3	0.9	2.4	1.7	1.4	1.3	1.7	1.8	1.6
10	n-Hexadecane	1600	-	-	2.6	-	0.7	0.6	-	1.1	1.4	0.8
11	Nonadecene	1880	0.3	0.7	1.7	1.0	0.9	1.9	-	1.1	-	0.8
12	n-Nonadecane	1902	0.3	2.0	2.2	2.3	1.7	1.9	-	1.9	1.4	1.3
13	n-Eicosane	2003	-	-	1.4	-	0.4	0.4	-	-	-	0.1
14	n-Heneicosane	2104	-	0.7	0.5	0.8	0.5	3.8	-	1.9	2.2	2

Table 10 Composition of the essential oil extracted from petal rose water in different FDWRs

No	Compound	RI	Safadasht					Qamsar				
			1:2	1:4	1:6	1:8	mean	1:2	1:4	1:6	1:8	mean
1	Linalool	1100	tr	3.5	0.5	0.6	1.4	tr	tr	-	-	
2	Phenyl ethyl alcohol	1112	17.4	19.7	42.4	21.2	25.1	27.5	28.6	33.6	32.1	30.5
3	Decanal	1203	-	1.4	0.1	0.3	0.5	-	-	-	-	
4	Nerol	1233	tr	tr	-	-	tr	0.3	0.5	-	-	0.2
5	Citronellol	1228	32.7	38.9	34.6	32.9	34.8	43.4	49.9	39.0	43.9	44.1
6	Neral	1241	11.4	-	0.1	-	2.9	0.4	-	0.2	0.3	0.2
7	Geraniol	1257	31.0	31.4	12.7	33.1	27.1	13.3	6.1	8.0	9.3	9.2
8	Eugenol	1356	2.3	0.9	3.8	1.4	2.1	5.6	2.9	3.6	2.4	3.6
9	Methyl eugenol	1405	0.8	0.6	2.0	0.4	0.9	2.1	1.5	2.1	2.3	2
10	n-Hexadecane	1600	-	-	-	-	-	0.8	tr	1.0	1.2	0.8
11	Nonadecene	1880	0.5	0.6	0.4	1.0	0.6	0.2	0.4	1.3	0.4	0.6
12	n-Nonadecane	1902	1.3	2.0	1.4	4.7	2.4	0.4	0.9	2.4	1.1	1.2
13	n-Eicosane	2003	-	-	0.1	0.4	0.1	0.4	0.3	0.4	0.2	0.3
14	n-Heneicosane	2104	0.4	-	0.7	1.6	0.7	-	-	0.9	0.6	0.4

There was also a significant difference between the rose water samples obtained from whole flowers and petals for all the traits except acid and iodine numbers. Rose waters from petals had lower pH, and higher essential oil content, ester and oxidation numbers. In addition, higher amounts of ethanol, methanol, and phenyl ethyl alcohol were also found in petal water. The essential oils extracted from petal waters contained a higher percentage of phenyl ethyl alcohol, citronellol, and geraniol than whole flowers. Ahmadi *et al.* [32] showed that using rose petals for essential oil production could raise the quantity and quality of the oil (high percentage of citronellol and geraniol). Furthermore, geranylacetone, hexadecanol, methyl eugenol, and heneicosane, traced in the petal oil, were found high in the essential oil of other parts of the rose flower. So, they suggested the essential oil should be prepared using only the petals without other rose flower organs [32].

This issue, that is, the amount of rose water production by distillation of 1 kg of rose flowers, has always been a matter of disagreement among rose water producers. Some believe that the proper ratio of rose water to flowers FDWR must be 1:1, which cannot be economical. By summarizing the results, efficient FDWR to rose water production with an acceptable quality could be a 1:4 ratios.

CONCLUSION

This study was the first on the quality of rose water in Iran. The higher essential oil contents (14.6 and 16.03 mg/100 mL) were obtained in 1:2 kg/L FDWR. The higher amounts of ester, oxidation and iodine numbers were obtained from petals. A decreasing trend in the amount of methanol, ethanol and phenyl ethyl alcohol was observed with FDWR reduction. A comparison of the mean percentage of the main oil components in the rose waters showed higher amounts of citronellol and phenyl ethyl alcohol and a lower percentage of geraniol in Qamsar samples.

According to this research, 2 to 4 liters of distilled water could be produced from 1 kg of the whole flower to be affordable for producers. Of course, the distillation of rose petals instead of the entire flower leads to a better quality of rose water. Nowadays, the cultivation of *R. damascena* is expanding in many regions of Iran with different climates. It should be noted that climatic conditions could affect the quality of rose flowers, essential oil, and rose water. Based on these results, four liters of distilled water from 1 kg of whole flowers was recommended to increase rose water quality.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Declaration of Funding

This research was financially supported by the Research Institute of Forests and Rangelands (RIFR) in Tehran, Iran

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available in the article.

Acknowledgment

We would like to extend our sincere thanks to Dr. Mahshid Rahimifard, Dr. Razieh Azimi, and Somayeh Fekri, from the Department of Medicinal Plants, Research Institute of Forests and Rangelands, Tehran, Iran who generously shared their time, experiences, and insights with us. Their willingness to engage with our research was essential to the success of this project, and we are deeply grateful for their participation.

REFERENCES

1. Boskabady M.H., Vatanprast A., Parsaee H., Boskabady M. Possible mechanism of inotropic and chronotropic effects of *Rosa damascena* on isolated guinea pig heart. DARU J. Pharm. Sci. 2013;21:38.
2. Shakeri F., Boskabady M.H. A review of the relaxant effect of various medicinal plants on tracheal smooth muscle, their possible mechanism(s) and potency. J. Ethnopharmacol. 2015;175:528-548.
3. Nunes H.S., Miguel M.G. *Rosa damascena* essential oils: a brief review about chemical composition and biological properties. Trends Phytochem. Res. 2017;1:111-128.
4. Safia A., Aamir Z., Iqbal A., Rafi S., Zafar M. Assessment of rose water and evaluation of antioxidant and anti-inflammatory properties of a rose water-based cream formulation. Int. J. Pharm. Clin. Res. 2019;11:43-48.
5. Wang H. Beneficial medicinal effects and material applications of rose. Heliyon 2024;10:e23530.
6. Hamedi B., Pirbalouti A.G., Rajabzadeh F. Manures, vermicompost, and chemical fertilizer impact on the yield and volatile compounds of the damask rose (*Rosa damascena* Mill.) flower petals. Ind. Crops Prod. 2022;187:115470.
7. Momeni T., Shahrokhi N. Essential oils and their therapeutic actions. Tehran, Iran (In Persian). 1991.
8. Andalib S., Vaseghi A., Vaseghi G., Naeini A.M. Sedative and hypnotic effects of Iranian traditional medicinal herbs used for the treatment of insomnia. Excli J. 2011;10:192.
9. Mahmood N., Piacente S., Pizza C., Burke A, Khan A.I., Hay A.J. The Anti-HIV activity and mechanisms of action of pure compounds isolated from *damascena*. Biochem. Biophys. Res. Commun. 1996;229:73-79.
10. Basim E., Basim H. Antibacterial activity of *Rosa damascena* essential oil. Fitoterapia. 2003;74:394-396.
11. Baydar H., Baydar N.G. The effects of harvest date, fermentation duration and Tween 20 treatment on essential oil content and composition of industrial oil rose (*Rosa damascena* Mill.). Ind. Crops Prod. 2005;21:251-255.
12. Dobreva A., Kovatcheva N., Astatkie T., Zheljazkov V.D. Improvement of essential oil yield of oil-bearing (*Rosa damascena* Mill.) due to surfactant and maceration. Ind. Crops Prod. 2011;34:1649-1651.
13. Daneshfar A., Babaee S. Determination of 2-phenyl ethanol in rose water using dispersive liquid-liquid microextraction with gas chromatography flame ionization detection. Anal. Bioanal. Chem. Res. 2016;3:131-138.
14. Chen X.M., Kobayashi H., Sakai M. Functional characterization of rose phenylacetaldehyde reductase (PAR), an enzyme involved in the biosynthesis of the scent compound 2-phenylethanol. J. Plant Physiol. 2011;168:88-95.
15. Yousefi B., Fadakar A. Effect of environmental variation on the flower and essential oil yield of superior genotypes of *Rosa damascena* Mill. (case study: Kurdistan province). Iran. J. Hortic. Sci. 2022;52:779-788 (In Persian).
16. Machado C.A., Oliveira F.O., de Andrade M.A., Hodel K.V.S., Lepikson H., Machado B.A.S. Steam distillation for essential oil extraction: An evaluation of technological advances based on an analysis of patent documents. Sustainability. 2022;14:7119.
17. Toluei Z., Hosseini Tafreshi S.A., Arefi Torkabadi M. Comparative Chemical Composition Analysis of Essential Oils in Different Populations of Damask Rose from Iran. J. Agric. Sci. Technol. 2019;21:423-437.
18. Jalali Chime Z., Gandomkar A., Khodagholi M., Batooli H. Comparison of the *Rosa damascena* Mill's essence composition in climate change condition case: Kashan, Kamoo, Barzok, Qamsar, Niasar and Natanz Garden. Geogr. Res. 2019;34:41-50.
19. Atanasova T., Kakalova M., Stefanof L. Chemical composition of essential oil from *Rosa damascena* mill., growing in a new location in Bulgaria. Ukrain. Food J. 2016;5:492-498.
20. Scarman J. Gardening with Old Roses. Collins. 1996.
21. Nedkov N., Dobreva A., Kovacheva N., Bardarov V., Velcheva A. Bulgarian rose oil of white oil-bearing rose. Bulg. J. Agric. Sci. 2009;15:318.
22. Agarwal S., Gupta A., Kapahi B., Baleshwar Thappa R., Suri O. Chemical composition of rose water volatiles. J. Essent. Oil Res. 2005;17:265-267.
23. Moein M., Zarshenas M.M., Delnavaz S. Chemical composition analysis of rose water samples from Iran. Pharmacol. Biol. 2014;52:1358-1361.
24. INSO. Rose Water-Test Methods, I.R.Iran: Iranian National Standard Organization (INSO 5759). 2022.
25. INSO. Rose Water-Test Methods. In. 2nd ed. I.R.Iran: Iranian National Standard Organization (INSO). 2014.
26. Shirani K., Hassani F.V., Azar-Khiavi K.R., Moghaddam Z.S., Karimi G. Determination of methanol in Iranian herbal distillates. J. Altern. Comp. Med. 2016;13:123-127.
27. Shirani K., Bostan H.B., Baroti A. Ethanol and methanol concentration in commonly used brands of Ma-al-shaeer in Iran: Estimation of dietary intakes and risk assessment. J. Pharmacopunct. 2018;21:168-176.
28. Adams R.P. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. [3rd ed.] ed. Carol Stream, Ill.: Allured Pub. Corp. 2004.
29. Shibamoto T. Retention indices in essential oil analysis (Vol. 259). Huethig Verlag, New York. 1987.
30. Davies N. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20M phases. J. Chromatogr. A. 1990;503:1-24.
31. Najem W., Marc El Beyrouthy M., Wakim L.H., Neema C., Ouaini N. Essential oil composition of *Rosa damascena* Mill. From different localities in Lebanon. Acta Bot. Gallica. 2011;158:365-373.
32. Ahmadi K., Sefidkon F., Asareh M. Comparison of quantity and quality of the essential oils of different parts of rose flower (petals, sepals and other parts) in two genotypes of *rosa damascena*. Pajouhesh & Sazandegi. 2008;79:62-71. (In Persian).