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Original research

Identification of volatile alcoholic compound in rosewater by GC-MS analysis: A method to differentiate original and artificial samples

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A B S T R A C T

In this study, an attempt was made to identify the differences between the two rosewater samples by identifying the chemical volatile compounds present in the original and artificial rosewater using GC and GC-MS. Then, to determine the authenticity of the studied rosewater samples, the amount of ethanol and methanol in the original and artificial samples was measured by GC-FID. Differences between phenyl ethyl alcohol, citronellol, geraniol, and citronellol/geraniol ratio of original, artificial samples and 10 industrial samples were tested by the current method. The results showed that the amount of phenyl ethyl alcohol in the artificial samples were much lower than the original sample ($p \leq 0.05$). The amount of citronellol in both samples was equal and did not have a statistically significant difference ($p > 0.05$). The geraniol content in the artificial samples was higher than the original sample while the citronellol / geraniol ratio showed a very low value in comparison to the original sample and other industrial samples. By examining the number of alcoholic compounds in rosewater, it was found that the original rosewater contained ethanol and methanol but no propylene glycol. By contrast, ethanol and methanol were not observed in the artificial samples, but the presence of propylene glycol alcohol. In addition to ethanol and methanol, some of the propylene glycols were observed in both samples. Consequently, this research showed that compounds such as ethanol, methanol, phenyl ethyl alcohol, geraniol, and citronellol/geraniol ratio can be used as markers in order to determine the originality of rosewater samples.

Keywords: Original rosewater; Artificial rosewater; GC-Mass; Phenyl ethyl alcohol; Citronellol/geraniol ratio

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1. Introduction

Medicinal plants have traditionally been used by people and today the tendency to consume them has increased. Changing people's attitudes towards chemical drugs has led to more attention and more research on medicinal plants, especially in the fields of production, processing, consumption, and evaluation of their chemical compounds (Mombeini et al., 2017). One of the most important products of herbal plants is their aromatic aqueous extract. The aromatic water is an aqueous solution of a volatile oil or other volatile substance prepared by distillation from a plant or a mixture of different plants (Miri et al., 2012). Rosewater (Golab) is one of the most famous aromatic water, which is the main product of the Rose flower with a scientific name of *Rosa damascene*. It has been used as a traditional product, and it has a global reputation (Moein et al., 2014). Rosewater (Golab) is one of the most common aroma extracts of the Rosa genus and is used in foods as a flavoring agent, soap, cosmetics, toiletries, and perfume because of its pleasant aroma (Safia et al., 2019).

Rosaceae is a family of flowering plants introduced commonly as roses that include near 3000 species of more than 90 genera (Potter et al., 2007). There are four species of a rose for producing essential oil. These are *Rosa damascena* Mill., *Rosa gallica* L., *Rosa moschata* Herrm. and *Rosa centifolia* L.

One of the important rose species is *Rosa damascena* Mill (damask rose) which is applied for making rosewater/rose essential oil around the world as well as in Iran (Verma et al., 2011; Rostami et al., 2018). The rosewater of damask rose (*Rosa damascena* Mill.) also used as materials for the perfume and cosmetic industries

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(Aycı et al., 2005). It is 1–2m in height with large colorful flowers (Boskabady et al., 2013). Besides its role as a perfume, this plant is cultivated in all parts of the world, including Iran (Dolati et al., 2011). Rose oil has nutritional, pharmacological, and industrial properties (Haghighi et al., 2008; Boskabady et al., 2011), expressed that rosewater (hydrosol) is known as Golab in Iran and has long been used in religious ceremonies (Haghighi et al., 2008).

According to (Baser, 1992), the quality of rosewater/rose oil depends on the number of its ingredients. For example, phenyl methyl alcohol and its structural isomer (2-phenyl ethanol) are responsible for the nice smell of rose oil, but not always present in rose oil in high amounts (Loghmani-Khouzani et al., 2007). The main components of hydrosol in ethanol as a solvent are phenethyl alcohol (69.7–81.6%), citronella (1.8–7.2%), and geraniol (0.9–7%). These components have been also been reported using hydro distillation (phenethyl alcohol: 30.8%, citronellol: 15.6%, geraniol: 16.8%). Simultaneous distillation–extraction has also been utilized to describe the composition of rosewater, with phenethyl alcohol (81.27%), citronellol (5.72%), and geraniol (4.43%) known as the main constituents (Eikani et al., 2005). Some biological activities, such as anti-HIV, antibacterial, and antioxidant have been expressed for the plants of this genus (Jalali-Heravi et al., 2008). It was also reported that rose oil has some usages in aromatherapy for the treatment of cardiac disease. (Loghmani-Khouzani et al., 2007; Boskabady et al., 2011) have suggested that in Iran, rosewater is traditionally and industrially produced by distillation and usually contains 10–50% rose oil. Synthetic essences or essential oils of other aromatic plants are sometimes added to this product to decrease production costs (Loghmani-Khouzani et al., 2007).

Due to the popularity of rose by-products, especially rosewater, the possibility of fraud in the processing of this product has also increased. The purpose of this study was to investigate the volatile compounds in genuine and artificial rosewater to determine fraud in rosewater.

2. Material and Methods

2.1. Sample preparation

Rosewater samples (three samples) used in this research as original rosewater were produced by herbal factory (Kerman, Iran) from fresh petals (*Rosa damascena* Mill.) harvested between April to May 2020. Rosewater samples were achieved from a similar production batch and produced by the conventional water distillation method. Rosewater samples were collected during distillation from the compressed water in a Florentine flask.

Artificial rosewater sample (3 samples) was prepared by adding synthetic rose essential oil (25 mL) to distilled water (100 mL). Additionally, 10 samples of rosewaters belonging to different brands were collected from the commercial markets to investigate their originality and artificiality.

2.2. Chemical analysis

2.2.1. Measurement of ethanol and methanol by GC-FID

The required volume of methanol and ethanol standards was transferred to a 10 mL balloon and filled with deionized water. According to permissible amounts of methanol in rose water (100 mg/kg) and ethanol (250 mg/kg), the required standards from the initial standard of 100 mg/kg in 7 levels for methanol and 8 levels for ethanol were prepared and were then injected into the gas chromatography and a calibration curve was drawn (Fig. 1 and 2).

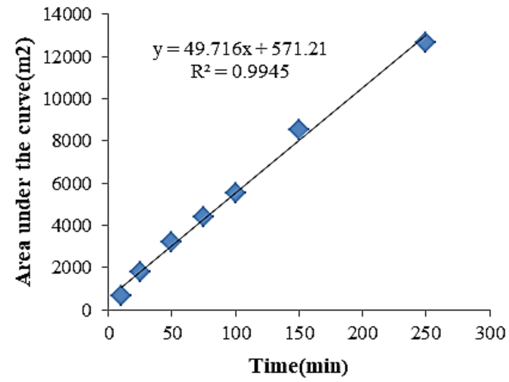

Fig. 1. Calibration curve of methanol.

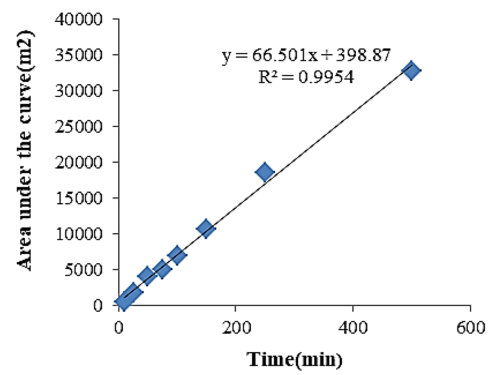

Fig. 2. Calibration curve of Ethanol.

The samples were then centrifuged at 3000 rpm and passed through a 0.45 µm filter, then injected into the gas chromatography. GC analysis of the rosewaters was performed by applying a GC-2014 gas chromatograph (Shimadzu, Japan) fitted with a 30 m ×0.32 mm DB-ALC2 fused silica capillary column coated with 1.20 µm FFAP. The GC was operated at: injector temperature 240°C, oven temperature programmed from 40°C (5 min) to 250°C at 15 °C/min; carrier gas He at the flow rate of 1.0 mL/min; injection size 0.2 µL and detector temperature 280°C, chart speed 1 mm/min. The recognition of ingredients was done by comparing the retention time of peaks.

2.2.2. Sample preparation and operation to identify volatile compounds using GC-MS

Firstly, 210 mL of the sample was transferred to a 250 mL flask and 52 g of pure sodium chloride was added to it and stirred for 15 min until the complete dissolution of sodium chloride. Then 25 mL of n-pentane as solvent (Merck) was added and stirred for 3 h. The solution was then quenched to separate the oil and water phases. The organic phase was separated, and the solvent has evaporated at room temperature. Finally, 1 µL of the remaining plate, was mixed with 100 µL of pentane, and injected into the GC-MS.

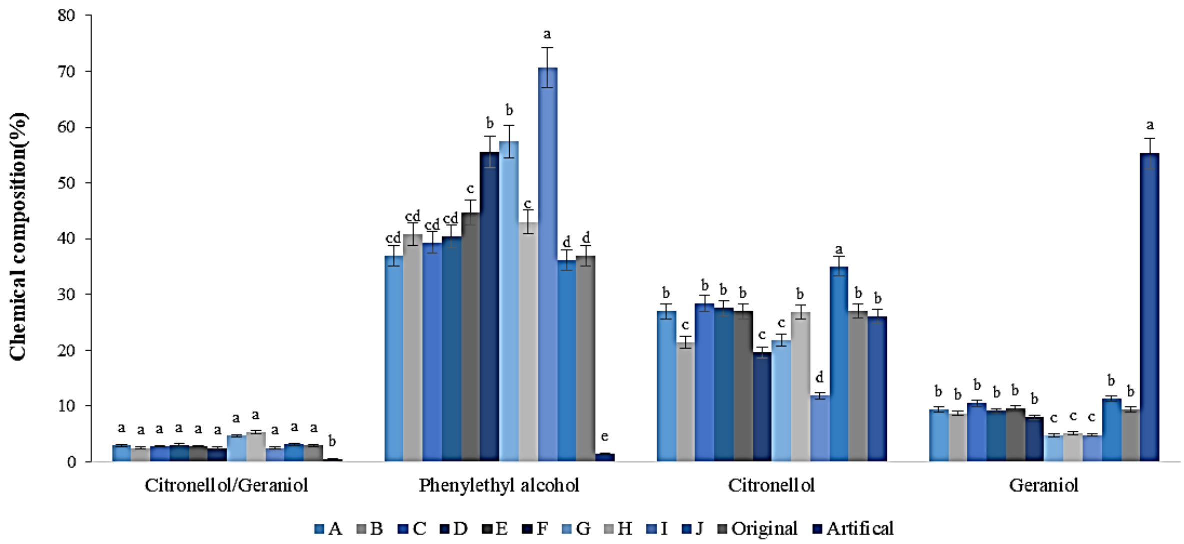

Fig. 3. Comparison of chemical composition (%) of rosewater volatiles. The letters A to J are commercial samples. The difference in letters indicates a statistically significant difference ($p \leq 0.05$).

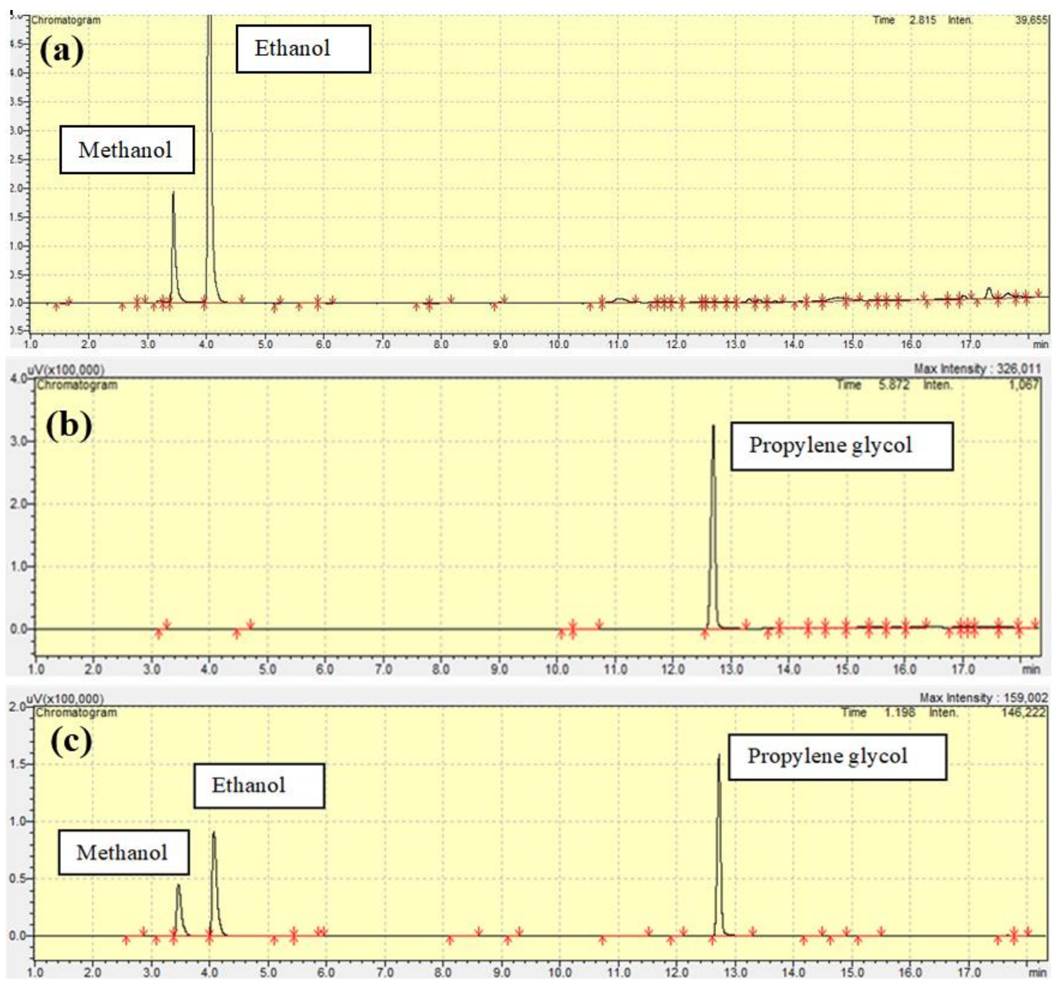

Fig. 4. GC-FID Chromatograms of (a) original, (b) artificial, and (c) mix of the original and artificial rosewater sample.

Table 1. Chemical composition of rosewater volatiles (%).

Compound	A	B	C	D	E	F	G	H	I	J	Original	Artificial
Benzyl Alcohol	0.55±0.01	0.24±0.01	ND	0.33±0.01	0.02±0.00	0.56±0.02	ND	0.56±0.02	0.63±0.03	ND	0.508±0.02	ND
Cis linalool oxide	0.03±0.00	0.06±0.00	ND	0.01±0.00	0.05±0.00	0.04±0.00	0.02±0.00	0.03±0.00	0.05±0.00	ND	0.038±0.01	ND
Linalool	8.4±0.06	11.85±0.09	7.9±0.06	6.93±0.08	6.27±0.07	3.5±0.09	3.33±0.10	4.69±0.07	1.24±0.02	6.5±0.07	8.407±0.003	2.753±0.001
Trans rose oxide	0.17±0.01	ND	0.26±0.00	0.05±0.00	0.24±0.02	0.24±0.01	0.11±0.01	0.37±0.01	0.14±0.02	ND	0.178±0.002	ND
Phenylethyl alcohol	36.91±0.33	40.81±0.31	39.27±0.27	40.41±0.59	44.6±0.82	55.49±1.02	57.4±1.12	43.01±1.22	70.64±1.41	36.2±1.32	36.913±0.01	1.402±0.001
Terpene 4-ol	0.67±0.03	0.93±0.01	0.18±0.00	0.36±0.00	0.31±0.02	0.16±0.01	0.4±0.01	1.15±0.02	0.34±0.08	0.1±0.00	0.678±0.006	ND
Terpineol	5.28±0.02	8.79±0.08	4.23±0.02	5.72±0.03	3.27±0.01	6.27±0.06	2.5±0.05	1.86±0.02	2.59±0.11	3.06±0.02	5.289±0.003	ND
Citronellol	27.01±0.40	21.3±0.12	28.36±0.31	27.55±0.22	26.97±0.12	19.56±0.27	21.8±0.21	26.83±0.11	11.86±0.32	35±1.82	27.019±0.001	26.085±0.001
D-Carvon	0.13±0.00	0.3±0.00	0.4±0.00	0.45±0.00	0.1±0.00	0.22±0.01	0.2±0.02	0.36±0.04	0.32±0.03	0.4±0.02	0.132±0.002	2.39±0.01
Geraniol	9.39±0.02	8.68±0.22	10.44±0.42	9.1±0.28	9.58±0.12	7.95±0.13	4.7±0.16	5.11±0.12	4.8±0.12	11.3±0.42	9.393±0.01	55.251±0.01
Eugenol	7.98±0.01	4.48±0.10	5.8±0.12	5.66±0.11	5.19±0.14	4.88±0.12	6.5±0.09	10.9±0.32	4.92±0.22	5.2±0.12	7.984±0.003	ND
Methyl Eugenol	3.46±0.07	2.43±0.03	2.91±0.02	3.06±0.01	3.22±0.03	0.83±0.02	2.8±0.01	4.86±0.11	2.16±0.08	1.8±0.01	3.461±0.001	ND
Citronellol/Geraniol	2.876±0.04	2.45±0.03	2.716±0.02	3.027±0.02	2.815±0.01	2.46±0.05	4.638±0.08	5.25±0.07	2.47±0.05	3.097±0.08	2.876±0.001	0.472±0.001

*The results are average of three repetitions ± SD.

GC-MS analysis of the rosewater sample was done by an 7990 A gas chromatograph couples with an Agilent 5975 C mass spectrometer (Agilent Technologies, USA), operating at 70 eV ionization energy, 0.5 s/scan and the mass range: 35-400 μm, attached with an HP-5MS capillary column (phenyl methyl siloxane, 30 m × 0.25 mm; 0.25 μm film thickness) programmed as above with helium as the carrier gas with the flow rate of 1 mL/min and a split ratio of 1:50. The software adapted to handle mass spectra and chromatograms was Chem Station. Sequencing of the compounds was performed using the GC-MS library.

2.3. Statistical analysis

Analysis of test data was performed by SPSS software, version 21. The mean was compared with Duncan test at 95% confidence level ($p \leq 0.05$).

3. Results and Discussion

The chemical composition of rosewater volatiles of 10 commercial samples, original and artificial samples are presented in Table 1. The major components of rosewater volatiles of all the samples except the artificial sample were phenyl ethyl alcohol (36.2–57.4%), citronellol (11.86–35%), and geraniol (4.7–55.25%). The rosewater volatiles obtained from the artificial sample showed considerable variation in the concentration of major and minor components. In artificial sample, the phenyl ethyl alcohol and citronellol had the lowest quantity and geraniol had the highest amount among samples. In addition, compounds such as terpene 4-ol, terpineol, eugenol, methyl eugenol, ethanol and methanol, were not detected in the artificial samples, while the citronellol/geraniol ratio was the lowest. The highest ratio of citronellol/geraniol was found for sample H, followed by A and original sample with 5.3 % and 2.87, respectively.

The high citronellol/geraniol ratio (H) can be due to the high weight ratio of rose flower to water during distillation.

For the rose water to be introduced as an original sample, the sample must be free of propylene glycol solvent and contain phenyl ethyl alcohol, and the ratio of citronellol/geraniol should be 2 to 2.5, although this ratio can vary depending on the dosage of rose. In the original rose, some compounds must be present, including linalool, terpineol, 4-terpineol, eugenol, and methyl eugenol, as well as ethanol and methanol. A comparison amount of phenyl ethyl alcohol, citronellol, geraniol, and citronellol/geraniol among samples has been done and presented in Fig.3 and Table 1.

According to Fig. 1, the highest and lowest ratio of citronellol/geraniol was for sample H and artificial, respectively. The difference between samples: A, B, D, E, F, H, and original was not significant ($p \geq 0.05$). Phenyl ethyl alcohol content of sample I was the highest among the samples and the artificial sample had the lowest amount of phenyl ethyl alcohol. Citronellol in sample J had the most quantity and citronellol in the artificial sample was higher than the others.

To better identify the differences in volatile compounds in Table 1, phenyl ethyl alcohol, citronellol, geraniol, and citronellol/geraniol ratio were first investigated in the artificial and original samples.

According to the results in Fig. 1, it can be stated that the amount of phenyl ethyl alcohol in the artificial sample was about 26.3 times less and the difference was statistically significant. The amount of citronellol in both original and artificial samples was about 27% and the difference was not significant. Moreover, the amount of geraniol in the artificial sample was about 5.9 times higher than the original sample being also significantly different. The last parameter was the citronellol /geraniol ratio, which was about 6.7 times lower in the artificial sample. Therefore, compounds such as phenyl ethyl alcohol, geraniol, and citronellol/geraniol ratio can be effective in distinguishing the original rosewater sample from the artificial. Regarding the commercial samples, those with the highest similarity in the amounts of phenyl ethyl alcohol, citronellol, geraniol, and citronellol/geraniol ratio with the original sample more than 95% were more likely to be the original.

For this purpose, the values between 2 to 3% of phenyl ethyl alcohol were not significantly different from the original sample. Citronellol levels between 24 and 29% were not significantly different from the original sample and values outside this range were less likely to be original. The amount of geraniol in the range of 8 to 10.5 was not significantly different from the original sample ($p > 0.05$). Moreover, the citronellol/geraniol ratio was the most important factor for discriminating the original rosewater from the artificial one, which states that if the citronellol/geraniol ratio is between 2.4 and 3, the rose product is more likely to be original and by reducing this amount, the probability being artificial of rosewater is much higher. Artificial rosewater is produced by adding rose essential oil to water and according to Fig. 1, the amount of geraniol in the rose essential oil is an indicator compound. Therefore, high levels of geraniol will be a sign of adding essential oil to the water (Moein et al., 2014).

Also, phenyl ethyl alcohol is the main ingredient in rose and rosewater, but its amount is much lower in synthetic roses because phenyl ethyl alcohol is soluble in water and polar, and most of it dissolves in distilled water. For this reason, the aroma and composition of natural rose oil is different from natural rosewater

Different percentages of phenyl ethyl alcohol were found in the samples. Samples of rose essential oil usually include scrimpy quantities of phenyl ethyl alcohol or none at all. This compound is highly soluble in water and alcoholic and aldehyde monoterpenes are insoluble in water, thus, the proportion of phenyl ethyl alcohol usually increases during the production of rosewater (Kurkcuoglu & Baser, 2003).

Another difference in the artificial and original samples is the ethanol and methanol content shown in Fig. 2 (a,b,c).

Moein et al. (2014) studied the chemical composition of 10 rose water samples and reported that in most samples, phenethyl alcohol, geraniol and b-citronellol were the main constituents, and the geraniol was found in large amounts in half of the samples, and it was concluded that pelargonium and dianthus essential oils and synthetic essences had been added to some samples.

As seen from Fig. 4a, ethanol and methanol are the only detected compounds in the original sample. These alcohols were not present in artificial rosewater obtained by adding rose flavour to water and the only peak presented in the chromatogram belongs to propylene glycol (Fig. 4b). Some samples contained ethanol, methanol, and propylene glycol, which indicate both original and synthetic rosewater has been used in their chemical composition.

Various factors affect the amount of ethanol and methanol in plant extracts. The most important reasons are the origin of plant tissue, genetic and racial differences of plants, climatic conditions, plant life used, and extraction method. Gazani et al. (2015) reported that rosewater samples had a methanolic content of 79 mg/L. Since in industrial processes of rose production, usually, a large volume of flowers is used along with petioles and sepals (photosynthetic green tissues), it can be a reason for the amount of methanol and ethanol in the rosewater resulted from the process. Also, in the process of producing rosewater and other herbal extracts, the plant has picked and used after 48 h of dehydration, and this stress leads to an increase in the concentration of ethanol and methanol in the rosewater (Gazani et al., 2015). Since the extraction is done 24 to 48 h after picking the flowers up, therefore, the fermentation process during picking up and storage is not the main reason for the production of alcohol in rosewater. Because this fermentation process requires at least a few weeks and the most important factor in alcohol production can be the presence of pectin methyl esterase in other tissues than petals (Gazani et al., 2015). It should be noted that the amount of alcohol in the extract, especially methanol, can never be reduced to zero, so the presence of methanol determines the authenticity of the plant extract. In confirmation of our results, Sharafati Chaleshtori et al. (2018) studied on chemical composition, antioxidant and antibacterial activity of *Bunium persicum*, *Eucalyptus globulus*, and rosewater on multidrug-resistant listeria species and reported the chemical components of rosewater as followed linalool (6.6%), terpineol (3.3%), carvone (0.31%), citronellol (6.85%), trans-geraniol (7.11%), phenyl ethanol (66.84%), eugenol (4.53%), citronellol, hydroxyl (1.15%), and geranic acid (1.2%). Mahboubifar et al. (2014) also showed that the major component of laboratory prepared, and commercial rose water samples were found to be phenyl ethyl alcohol (45.1%-85.4%), while aliphatic hydrocarbons were identified as the main constituents of the oily phase of rose water extracted by simple hydro distillation and Clevenger-type distillation method (49.1%, 60.3%). Also, Miri et al. (2012) studied on chemical composition of the essential oils and aromatic water from Teucrium *persicum* Boiss and they showed that the main components of essential oil were α-cadinene (9.7%), 1,4-cadinadiene (9.2%), and α-terpinyl acetate (7.9%); and the predominant compounds in the aromatic water (rosewater) were linalool (10.4%), α-cadinene (7.5%), and γ-terpineol (7.3%). Most of the compounds identified from different oils were similar, but their amounts differed. The oil revealed a higher content of total phenolics than the aromatic water (1.71 ± 0.12 mg GAE/g DW and 1.36 ± 0.11 mg GAE/g DW, respectively).

4. Conclusion

In this study, samples of original and artificial rosewater (obtained by adding rose essential oil to water) and 10 samples of industrial rosewater, were studied and compared in terms of volatile compounds. According to our results, the main indicators for detecting differences between origin and artificial rosewater are as follows: phenyl ethyl alcohol, geraniol, and citronellol/geraniol together with eugenol/methyl eugenol ratios.

For instance, artificial samples were mainly characterized by a lower citronellol/geraniol ratio and a lower amount of phenyl ethyl alcohol than original rosewater; and the artificial rosewaters contained mainly polypropylene glycol while in the original samples this alcohol was not observed. This study revealed that industrial rosewater samples were similar to the original rosewater sample. Finally, the presence of ethanol and methanol confirms an original rosewater sample, while propylene glycol confirms an artificial one. Moreover, when propylene glycol is present with ethanol and methanol alcohols, it indicates that the original and artificial samples were mixed. For the accurate selection of rose water, the presence of all identified compounds in specific proportions is essential.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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