

Evaluation of the Valuable Oxygenated Components in Iranian Rose Water

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Abstract: Rose water is one of the most important products obtained from *Rosa* genus (Rosaceae). Although Iran is one of the main producers of rose water, limited research has been done on it. A simple distillation and a Clevenger-type apparatus were used to obtain rose water and rose oil. All the volatile concentrates were investigated for their chemical composition by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Volatile concentrates from three commercial samples of rose water, purchased from the local market, were also studied.

The major component of laboratory prepared and commercial rose water samples was found to be phenylethyl alcohol (45.1%-85.4%), while aliphatic hydrocarbons were identified as the main constituents of the oily phase of rose water extracted by simple hydrodistillation and Clevenger-type distillation method (49.1%, 60.3%). Our results showed that the volatile obtained from the oily phase had lower economic value than rose water.

Keywords: Rose water, Gas chromatography-mass spectrometry, hydrodistillation, Phenylethyl alcohol.

Introduction and Experimental

The *Rosa* genus belongs to the Rosaceae family and is represented by about 200 species and also more than 18000 cultivars(1). The high numbers of cultivated form of the plant genus might be related to good medicinal properties of its various products(2-6). Recently, some biological activities, such as anti-HIV, antibacterial and antioxidant have been reported for the plants of this genus(5). It is also revealed that rose oil has some applications in aroma-therapy for the treatment of cardiac disease(7).

Rose water is one of the most common aroma concentrates of *Rosa* genus and is used in food as flavoring agent, soap, cosmetic, toiletrie and perfume because of its pleasant odor(8). In Asia and Europe, rose water is also applied as a perfume in religious ceremonies. The Persian name for rose water is Golab and Iran is one of the main producers of it by both traditional and industrial methods in the world. The distilled water has widely been applied in Iranian traditional medicine and also in food as a flavoring agent(9).

Rosa damascene Mill (damask rose) is one of the major rose species which is applied for making rose water/rose oil in all over the world as well as Iran(10,11). Fresh rose flowers (whole flowers or their petals) and their air-dried form has been used for the preparation of rose water/rose oil by different hydrodistillation methods(11-13). In some industrial houses, rose water is considered as a by-product in achieving rose oil and could be simply produced via dilution with distilled water(11,12).

The qualitative chemical compositions of different kinds of rose water/rose oil were studied previously(9,11-14). The quality of rose water/rose oil depends on the quantity of its components(15). For example, phenylethyl alcohol and its structural isomer (2-phenyl ethanol) are responsible for the pleasant smell of rose oil, but not always present in rose oil in high amounts(7). Such valuable oxygenated constituents may be dissolved in the water used for hydrodistillation methods. Therefore, the recovery of the water-soluble

compounds and/or setting up a suitable distillation method seems to be vital in the production of rose water(12,14,16).

Due to the fact that Iran is one of the main producers of rose water and rose oil, and also considering the wide consumption of rose water in Iranian traditional medicine, we tried to identify the chemical composition of rose water and rose oil and also compare their chemical constituents.

Hopefully, the results of this research could be useful for further studies on the optimal supplying conditions of traditional and industrial rose water.

Plant Material and Reagents

The air-dried rose flowers and three commercial samples of rose water were supplied from the Shiraz herbal market in Fars province in April 2011. All the chemicals were obtained from Merck Chemical Company (*Darmstadt, Germany*).

Isolation Procedure of Volatile Components

As Shown in figure 1, the air-dried rose flowers (50 g) were blended to produce powder. The powders were hydrodistilled for 4 h, using a simple distillation apparatus according to the standard method published by institute of standards and industrial research of Iran (ISIRI No.13697). The product of hydrodistillation consisted of two aqueous (A) and oily phases (B). The phases were separated from each other by decantation. The volatile of aqueous phase were extracted with hexane. The yield of oily phase was determined on the basis of dry plant weight and the yield of obtained aqueous phase was also calculated regarding to the water phase weight. In the other part of isolation procedure, the air-dried rose flowers (100g) were subjected to hydrodistillation using a Clevenger-type apparatus for 4 h according to the *European Pharmacopoeia*(17). The oil were dried over anhydrous sodium sulfate and stored in sealed vials at the temperature of 4-6°C in dark for further analysis.

The volatile of commercial rose water samples supplied from the herbal market (C-E) were also extracted with hexane according to the standard method published by institute of standards and industrial research of Iran (ISIRI No.13697) and the yield of the extraction was calculated based on the weight of water phase (Figure 1). All the products were dried over anhydrous sodium sulfate and stored in sealed vials at the temperature of 4-6°C in dark for further analysis.

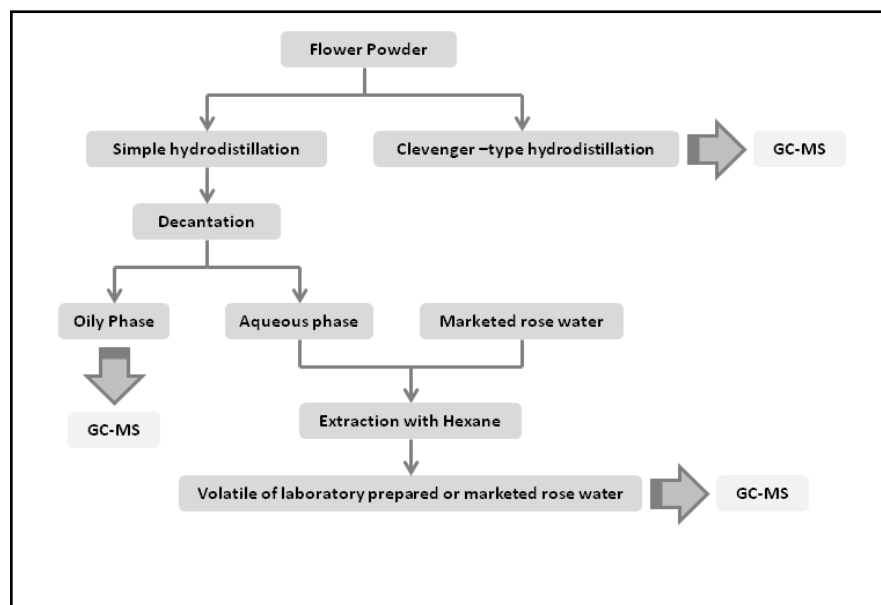


Figure 1. Hierarchical analysis of the volatile obtained from rose water samples (laboratory and marketed) and the essential oil.

Identification of the Oil Components

GC-FID analysis was carried out using an Agilent 6890N chromatograph (Agilent Technologies, USA) with an HP-5 capillary column (phenyl methyl siloxane, 30 m × 0.25 mm; 0.25 μm film thickness). The oven temperature increased from 60 to 240°C at a rate of 3°C/min, the injector and detector temperatures were 240°C and 250°C, respectively. Helium was used as the carrier gas with a flow rate of 0.9 ml/min. Relative percentage data were obtained from electronic integration of peak areas without the use of correction factor.

GC-MS analysis of the essential oil was performed using an Agilent 7890A chromatograph, coupled with an Agilent 5975C mass spectrometer (Agilent Technologies, USA), operating at 70 eV ionization energy, 0.5 s/scan and the mass range: 35-400 amu, equipped with a 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 0.9 ml/min and a split ratio of 1:50. Software adopted to handle mass spectra and chromatograms was ChemStation. In this study, GC-MS and GC-FID were used for qualitative and quantitative analysis of the chemical composition of the obtaining volatile.

Retention indices were determined by using retention times of normal alkanes that had been injected after the essential oil under the same chromatographic conditions, according to the Van Den Dool method(18). The compounds were identified by comparison of their mass spectra with the Wiley and Mass finder 3 libraries or with the published mass spectra(19).

Quantification of Phenylethyl Alcohol with GC-FID

For quantification of phenylethyl alcohol in volatile obtained from rose water, 10g of phenylethyl alcohol was dissolved in hexane and the volume of the solution was adjusted to 5ml (stock solution). Different concentration solutions were prepared by serial dilution of the stock solution for GC analyses. One μL of the solutions (2000, 1000, 500, 250 and 125 mg/ml), were injected to GC-FID with the same analytical conditions used for the GC-MS and calibration curve of the GC-FID area was plotted against the different concentrations of the standard compound. The linear regression equation for the standard compound was calculated using the Microsoft Excel program.

Data Analysis

The means of total components identified from three replications in all the samples were calculated using the Excel software and they were reported with standard deviation (SD).

Table 1. Chemical composition of the volatile obtained from laboratory prepared rose water, marketed rose water sampels and essential oil of rose flowers.

Compound	RI _{cal.} ^a	Relative peak area [%] ^c						Identification ^d
		A	B	C	D	E	F	
<i>n</i> -Octane	802	-	1.5±0.2	0.2±0.0	-	t	-	RI, MS
2-Methyl octane	860	0.1±0.0	-	-	-	-	-	RI, MS
3-Methyl octane	867	0.1±0.0	-	-	-	-	-	RI, MS
<i>n</i> -Nonane	898	1.1±0.1	14.8±0.5	2.8±0.3	0.1±0.0	0.2±0.0	-	RI, MS
Cumene	928	-	-	-	t	t	-	RI, MS
α -Pinene	936	0.1±0.0	1.6±0.1	-	-	-	-	RI, MS
Benzaldehyde	961	t	-	-	-	-	-	RI, MS
<i>n</i> -Decane	1001	0.3±0.0	2.3±0.1	0.7±0.0	t	0.1±0.0	-	RI, MS
1,8-Cineol	1029	t	-	t	-	t	t	RI, MS
Benzene	1041	0.1±0.0	-	-	-	-	-	RI, MS
acetaldehyde								
<i>cis</i> -Linalool oxide	1071	-	-	-	t	-	t	RI, MS
<i>trans</i> -Linalool oxide	1087	t	-	t	t	-	t	RI, MS
Linalool	1098	2.2±0.2	-	1.4±0.1	3.1±0.3	4.4±0.4	3.2±0.4	RI, MS
<i>cis</i> -Rose oxide	1107	0.3±0.0	-	0.5±0.0	0.3±0.0	0.4±0.0	3.9±0.4	RI, MS
Phenylethyl alcohol	1108	50.6±1.3	-	85.4±1.5	45.1±1.1	69.6±1.7	-	RI, MS

<i>trans</i> -Rose oxide	1123	-	-	-	-	-	1.2±0.1	RI, MS
Terpinene-4-ol	1175	-	-	-	-	-	t	RI, MS
α -Terpineol	1190	1.3±0.1	-	-	1.2±0.1	2.6±0.2	-	RI, MS
Citronellol	1223	7.0±0.5	-	7.4±0.3	35.7±1.3	12.6±0.9	18.7±0.5	RI, MS
Carvone	1240	1.4±0.1	-	-	-	-	-	RI, MS
Piperitone	1253	0.4±0.0	-	-	-	-	-	RI, MS
Geraniol	1254	7.4±0.6	-	-	9.0±0.8	t	1.2±0.1	RI, MS
Geranial	1269	0.3±0.0	-	-	-	-	-	RI, MS
(<i>E</i>)-Anethole	1287	0.2±0.0	-	-	-	-	-	RI, MS
Thymol	1291	9.6±0.8	-	-	-	-	-	RI, MS
Carvacrol	1300	5.9±0.6	-	-	-	-	-	RI, MS
Piperitenone	1243	3.2±0.2	-	-	-	-	-	RI, MS
Eugenol	1360	3.3±0.2	-	-	1.4±0.2	1.4±0.2	1.7±0.1	RI, MS
(<i>E</i>)- β -Damascenone	1382	t	-	-	-	-	-	RI, MS
<i>n</i> -Tetradecane	1399	t	-	-	-	-	-	RI, MS
Methyl eugenol	1401	2.4±0.1	-	0.3±0.0	2.1±0.2	0.4±0.0	2.1±0.1	RI, MS
<i>n</i> -Pentadecane	1500	t	-	0.1±0.0	-	-	-	RI, MS
Caryophyllene oxide	1581	0.2±0.0	-	-	-	-	-	RI, MS
Salvial-4(14)-en-1-one	1590	-	-	-	-	-	t	RI, MS
<i>n</i> -Hexadecane	1601	t	1.6±0.1	-	-	-	t	RI, MS
Humulene epoxide II	1604	-	-	-	-	-	0.7±0.1	RI, MS
Junenol	1618	-	4.1±0.2	-	-	-	-	RI, MS
Valeranone	1675	t	-	-	-	-	-	RI, MS
α -Bisabolone oxide A	1686	0.5±0.0	-	-	-	-	-	RI, MS
1-methyldecyl benzene	1698	-	-	-	-	-	-	RI, MS
<i>n</i> -Heptadecane	1702	t	2.9±0.2	-	-	-	1.7±0.1	RI, MS
(<i>2E,6Z</i>)-Farnesol	1717	t	-	-	-	-	-	RI, MS
<i>n</i> -Octadecane	1801	t	2.4±0.2	-	-	-	t	RI, MS
9-Nonadecene	1879	-	-	-	-	-	4.5±0.2	RI, MS
1-Nonadecene	1897	-	5.6±0.3	-	-	-	-	RI, MS
<i>n</i> -Nonadecane	1902	0.4±0.0	21.9±0.0	-	-	t	25.7±1.0	RI, MS
Unknown	1908	-	14.2±0.5	-	-	-	-	RI, MS
<i>n</i> -Eicosane	2000	t	2.9±0.2	-	t	t	2.4±0.3	RI, MS
1-Heneicosene	2081	-	-	-	-	-	t	RI, MS
<i>n</i> -Heneicosane	2100	0.2±0.0	9.4±0.4	-	t	t	21.2±0.7	RI, MS
<i>n</i> -docosane	2200	-	-	-	t	t	0.6±0.0	RI, MS
(<i>Z</i>)-9-Tricosene	2283	-	-	-	-	-	t	RI, MS
<i>n</i> -Tricosane	2301	t	3.3±0.2	-	t	t	6.2±0.2	RI, MS
<i>n</i> -Tetracosane	2400	-	-	-	t	t	t	RI, MS
<i>n</i> -Pentacosane	2503	t	8.0±0.5	-	-	-	-	RI, MS
<i>n</i> -Hexacosane	2600	-	-	-	t	t	2.5±0.1	RI, MS
Total		98.6	96.5	98.8	98.0	91.7	97.5	

^a calculated retention indices.

^b Mean \pm SD (n=3)

^c RI, retention indices; MS, mass spectra in electronic impact mode.

A: Volatile of laboratory prepared rose water,

B: Essential oil obtained by simple hydrodistillation method,

C-E: Volatile of marketed rose water samples,

F: Essential oil obtained by Clevenger-type distillation method.

t: trace (< 0.05%). RI: retention indices relative to C8–C28 *n*-alkanes on HP5-MS. The components are listed in order of their elution from the HP-5MS column.

Results and Discussion

The chemical compositions of the obtained volatile, were analyzed by GC and GC/MS techniques. The relevant data are summarized in Table 1, listed in order of their elution on the HP-5 column.

The yields of volatile from laboratory prepared rose water and the obtained oily phase were 0.01% (w/w) and 0.02% (w/w), respectively. The extraction yields were found to be 0.03%, 0.05% and 0.02% (w/w) for marketed rose water samples (C, D and E). The yield of essential oil obtained by Clevenger-type hydrodistillation method was 0.07 % (w/w).

Thirty-nine compounds representing 98.6% of the total oil were identified in laboratory prepared rose water. The main compounds were phenylethyl alcohol (50.6%), thymol (9.6%), geraniol (7.4%) and citronellol (7.0%). Among the identified components, the volatile oil contained three alcohols (53.9%), fourteen monoterpenoids including thirteen oxygenated monoterpenes (39.2%) and one monoterpene hydrocarbon (0.1%), two esters (2.6%), thirteen alkanes (1.8%), three oxygenated sesquiterpenes (0.5%) and two aldehydes (0.1%).

Fourteen components comprising 96.5% of the total oil were identified in the oily phase. The main compounds were nonadecane (21.9%), nonane (14.8%), heneicosane (9.4%) and pentacosane (8.0%). The volatile oil contained 11 aliphatic hydrocarbons, including ten alkanes (49.1%) and one alkene (5.6%), one oxygenated sesquiterpene (4.1%) and a monoterpene (1.6%).

Eleven, nineteen and twenty compounds comprising 98.8%, 98.0% and 91.7% of the total oil were also identified in volatile that was extracted from purchased rose water samples (C, D and E). The oxygen containing components, phenylethyl alcohol and citronellol, were reported as the major constituents in these samples.

Twenty-six components were identified in the essential oil of air-dried rose flowers obtained by Clevenger-type distillation method, which contained 97.5% of the oil. Nonadecane (25.7%), heneicosane (21.2%), citronellol (18.7%) and tricosane (6.2%) were found to be the major constituents of the oil. The obtained essential oil contained 13 aliphatic hydrocarbons, including ten alkanes (60.3%) and three alkenes (4.5%), followed by 6 oxygenated monoterpenes (28.2%).

For quantification of phenylethyl alcohol in the rose water, the linear regression equation for the standard compound was calculated to be $Y=2E+06X-3E+07$, $R^2=0.9988$. The concentration of phenylethyl alcohol in the volatile obtained by the simple hydrodistillation method in our laboratory (A) and volatile of the marketed rose water samples (C-E) were calculated to be 595.8, 908.9, 501.1 and 733.7 mg/ml, respectively. As indicated in Table 1, although the volatile that has been extracted of the laboratory prepared and commercial rose water samples exhibited some differences in their chemical compositions, but the most abundant component of the laboratory prepared and marketed rose water samples is phenylethyl alcohol, that it can be consider as the marker compound of the water phase, followed by oxygenated monoterpenes, which resembled the finding in other rose water samples(11-14). The oxygen-containing monoterpenes; citronellol and geraniol, have also been reported as the main constituents of rose water samples(11-14).

The variation between the chemical compositions of the volatile extracted of the laboratory prepared and marketed rose water samples might be attributed to the different sources used for preparation of them(12). Literature review showed that the amount of the valuable water-soluble components in rose water could be increased by applying different extraction methods(12-16).

According to Table 1, several aliphatic hydrocarbons such as nonadecane, heneicosane and nonane have been found in considerable amounts in the oily phase obtained by the simple hydrodistillation method and in the volatile oil obtained by the Clevenger-type method.

The main chemical compounds percentage of the volatile obtained from rose water and oily phase has been prepared in our laboratory by simple hydrodistillation method, has been compared in Figure 2.

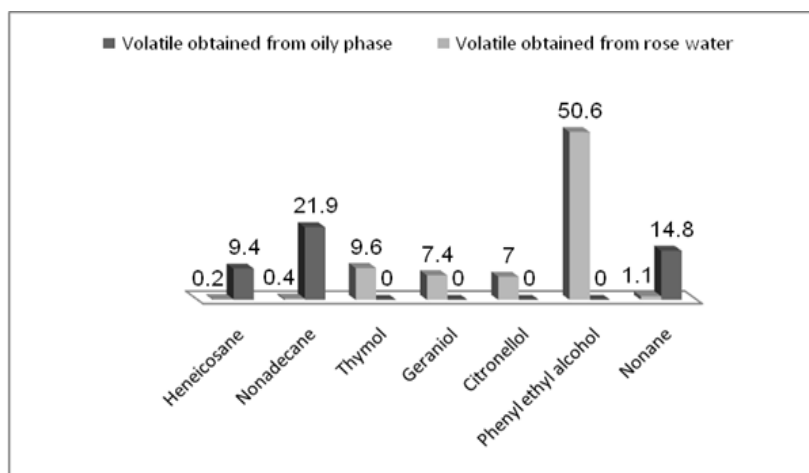


Figure 2. The percentage of major constituents of the volatile obtained from rose water sample and oily phase prepared in our laboratory by simple hydrodistillation method.

The absence of phenylethyl alcohol and other oxygenated monoterpenes in the oily phase could be a good evidence for complete recovery of water-soluble components in the aqueous phase (rose water), and it seems that the oily phase of hydrodistillation has no or less commercial or economic value than the rose water samples.

According to the previous studies, the quality of rose water could be determined by the amount of phenylethyl alcohol, monoterpenoid components and alkanes(11,15). High amounts of phenylethyl alcohol and oxygenated monoterpenes and low amounts of aliphatic hydrocarbons could be responsible for good quality of obtaining rose water(14). Moreover, the procedure of herbal distilled water production has been used in Iranian traditional and industrial methods resembles the simple distillation method used in this study. The high amounts of oxygen-containing components in the rose water could be related to the polarity and solubility of the constituents, and the amount of these compounds might be changed according to the using extraction method. Therefore, research on the effect of the extraction methods could help to obtain the optimal supplying conditions of traditional and industrial rose water for getting the best percentage composition.

Conclusions

Our study revealed that phenylethyl alcohol and the other water-soluble compounds such as citronellol, geraniol and thymol are the major constituents of rose water. None of these compounds could be found in the oily phase of hydrodistillation. The main compounds of the oily phase are aliphatic hydrocarbons. It seems that the oily phase obtained from hydrodistillation method has no or lower commercial/economic importance when compared to the rose water. Thus, for obtaining potentially valuable constituents of rose oil, it might be better to produce its distilled water initially and then extract the valuable compounds from it. We hope the results of this study provide a scientific viewpoint at the potentially valuable constituents of rose water.

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