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Investigation of different Damask rose (*Rosa damascena* Mill.) oil samples from traditional markets in Fars (Iran); Focusing on the extraction method

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Abstract

Bearing essential oil, Damask rose (Rosa damascena Mill.) is widely applied in pharmaceutical and perfumery industries. In Iran, the oil and hydrosol are obtained in two famous centers, Kashan and Fars. The process can be performed either traditionally or conventionally. Moreover, rose oil samples are produced synthetically by commercial industries. Current work outlines the differences between the collected samples. Eight samples yielded from those presentabove-mentioned methods were collected and analyzed using a gas chromatograph connected to a mass detector. Samples were prepared traditionally, conventionally, or synthetically. Results revealed that phenyl ethyl alcohol, β-citronellol and phenethyl acetate were the most detected component in synthetic sample (38.77, 15.73 % and 15.29 %, respectively). The synthetic sample involved two more major constituents (9.52 and 2.86 %). Traditionally and conventionally produced samples mainly contained hydrocarbons as nonadecane (17.42-40.38 %), heneicosane (17.26-26.17 %), 1-nonadecene (4.98-15.33%), heptadecane (3.96-10.33 %) aand eicosane (2.83-5.19%); but were lower in total rose alcohol content, from 0.00% in concentrated traditional samples to 30.24% in the samples prepared by a conventional method. High amounts of hydrocarbons in these samples might be related to prolonged and repeated distillation; thus nearly total amount of rose alcohol is transferred into the water phase.

Keywords: Essential oil, GC/MS, *Rosa damascena* Mill.

1. Introduction

Within the family Rosaceae, the genus Rosa is known as the main member with almost 200 different species (1). *Rosa damascena* Mill. (Commonly known as Damask rose) is a famous plant having 1-2 m high, with large and colorful flowers (2). Damask rose is an ornamental plant distributed in most parts of the northern hemisphere, including Iran (3). Apart from application of the Rose preparations in perfumery and cosmetic industries (4), its oil and aromatic water (hydrosol) as well as concrete, absolute and petals are more considerable for their pharmacological and nutritional properties (5-6). Damask rose is traditionally used for chest pain, constipation, depression, digestive disorders, inflammation, respiratory complications, menstrual bleeding, and tensions (7-9).

Rose essential oil is pharmacologically and cosmetically known as the main product. Studies showed that the oil exhibited antibacterial, analgesic, anti-inflammatory, antitussive, antispasmodic, and hypnotic effects (10-13). However, proved analgesic and antinociceptive, hypoglycemic, and laxative properties have also been reported from other rose preparations (8, 14-15).

The growth in use the usage of rose oil in pharmaceutical and perfumery industries creates an extensive demand to respective producers and also to search for more cost-benefit production

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processes. In Iran, rose flower is mostly cultivated in two main locations: Fars and Kashan provinces. Based on hydrodistillation, production of rose oil in Iran is performed via two main procedures as traditional and conventional. But the main extraction process is still the traditional one. In this method, an inhomogeneous rose oil sample with high viscosity will be obtained. It is very common in rose production centers of Fars province (Meymand region) to extract the oil in this way and obtain a concentrated and liquid form of the oil, which contains volatile constituents and fatty components. Rose water, an additive product for food industry, is also produced simultaneously (16). Due to the high demand for the oil, samples may also be prepared in poor quality or even in combination with artificial substances and synthetic compounds.

Several investigations have been performed on the phytochemical analysis of damask rose essential oil via different experimental extraction methods on various Rosa populations before (17-20). But no comprehensive evaluation has been done on rose oil samples currently in market-market until now.

In this regard, current paper aimed to analyze the different oil samples yielded by traditional and conventional methods as well as those produced synthetically and industrially.

2. Materials and Methods

2.1. Sample preparation

In this assessment, eight oil samples which are currently produced or used in related Fars province markets (Iran) were collected. These samples included those which were yielded via industrial, conventional and traditional procedures as well as a synthetic sample.

Samples produced traditionally are obtained during the production of rose water. Hydrodistillation is performed in large copper stills connected to a condensing apparatus. The hydrosol or rose water would be subsequently drained off and collected along with the oil to undergo to an additional distillation procedure. Oils from the first and second distillation steps are then mixed to make the final rose oil. The final oil is also separated to two phases and named the oil and the essence. In this process, the connections of appurtenance are not well sealed, thus parts of volatile constituents might be lost. On the contrary, in conventional method, the still is made of steel and connections are well sealed. The cohobation or repeated distillation is also omitted. Additional information of these samples is mentioned in the footnote of Table 1.

All purchased samples were subsequently diluted in dichloromethane (1:100) and injected to a gas chromatography-mass spectrometry system.

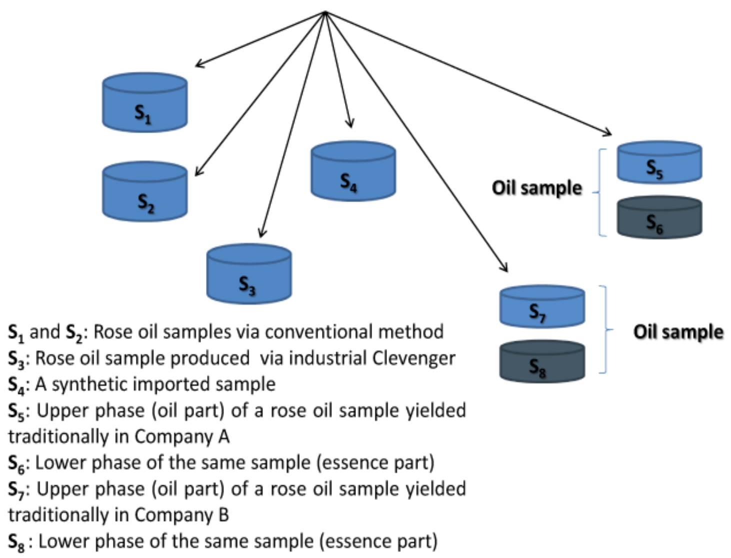

Figure 1. Description of collected rose oil samples.

Table 1. Composition of yielded Rosa oil (%) samples identified by GC/MS[1].

No.	Components2	RI3	Identi-fication	S1	S2	S3	S4	S5	S6	S7	S8
				Area (%)4							
1	α-pinene	935	MS-KI	-	0.93	0.96	-	0.32	1.36	-	-
2	β-pinene	980	MS-KI	-	-	0.25	-	-	0.33	-	-
3	β-myrcene	992	MS-KI	-	0.29	0.38	-	-	0.39	-	-
4	ρ-cymene	1024	MS-KI	-	-	-	3.98	-	-	-	-
5	cis-dihydrorose-oxide	1043	MS-KI	-	-	-	1.05	-	-	-	-
6	trans-β-ocimene	1048	MS-KI	-	-	-	1.75	-	-	-	-
7	unknown	1063	-	-	-	-	9.51	-	-	-	-
8	unknown	1088	-	-	-	-	2.86	-	-	-	-
9	linalool	1101	MS-KI	-	-	0.26	0.51	-	-	-	-
10	cis-rose oxide	1112	MS-KI	-	0.29	-	-	-	-	-	-
11	phenethyl alcohol	1119	MS-KI	-	0.44	-	38.77	-	-	-	2.37
12	isomenthone	1168	MS-KI	-	-	-	0.61	-	-	-	-
13	α-terpineol	1195	MS-KI	-	-	-	6.17	-	-	-	-
14	unknown	1201	-	-	-	-	0.73	-	-	-	-
15	unknown	1219	-	-	-	-	0.44	-	-	-	-
16	β-citronellol	1231	MS-KI	-	18.87	9.26	15.73	0.47	5.04	-	0.89
17	neral	1243	MS-KI	-	0.46	-	-	-	1.89	-	-
18	unknown		-	-	-	-	-	-	1.43	-	-
19	trans-geraniol	1255	MS-KI	-	10.93	4.47	-	-	0.28	0.52	-
20	cis-geraniol	1256	MS-KI	-	-	-	-	-	2.84	-	-
21	phenethyl acetate	1259	MS-KI	-	0.25	-	15.22	-	-	-	-
22	geranial	1272	MS-KI	-	0.54	-	-	-	-	-	-
23	citronellyl formate	1277	MS-KI	-	-	-	0.80	-	-	-	-
24	citronellyl acetate	1354	MS-KI	-	0.52	1.60	-	-	-	-	-
25	eugenol	1360	MS-KI	-	1.34	0.38	1.88	-	-	-	-
26	cis-geranyl acetate	1386	MS-KI	-	0.83	3.97	-	-	-	-	-
27	β-bourbonene	1389	MS-KI	0.61	-	0.29	-	-	0.35	0.52	-
28	β-elemene	1396	MS-KI	-	0.18	0.45	-	-	0.46	-	-
29	methyl eugenol	1406	MS-KI	-	1.90	0.27	-	-	-	-	-
30	trans-caryophyllene	1425	MS-KI	0.51	0.91	1.59	-	0.83	1.92	0.33	0.76
31	α-guaiene	1442	MS-KI	0.67	1.00	2.11	-	0.67	2.11	0.67	0.69
32	β-selinene	1482	MS-KI	0.60	0.74	1.39	-	0.59	1.56	0.40	-

2.2. GC/MS analysis and compound identification

Diluted oil samples were subjected to gas chromatography/mass spectrometry (GC/MS) for analysis of the ingredients, using Agilent Technologies 7890 gas chromatograph linked with an Agilent Technologies model 5975C mass detector). The device was equipped with a HP-5MS capillary column (phenyl-methylsiloxane, I.D. 30 m×0.25 mm, Agilent Technologies 19091S-433, 60 to

Table 1. Continued.

33	α-humulene		MS-KI	-	-	-	-	-	-	-	0.61
34	germacrene D	1487	MS-KI	-	2.13	3.67	-	0.60	1.79	-	0.78
35	pentadecane	1499	MS-KI	0.95	0.59	1.39	-	0.54	1.60	1.24	0.56
36	α-bulnesene	1510	MS-KI	0.46	1.13	1.77	-	1.05	2.12	-	1.49
37	trans-Nerolidol	1566	MS-KI	-	-	0.26	-	-	0.24	-	-
38	caryophyllene oxide	1589	MS-KI	0.79	-	-	-	-	-	1.09	-
39	hexadecane	1600	MS-KI	0.52	-	0.67	-	-	0.59	1.14	-
40	heptadecane	1700	MS-KI	5.88	3.96	10.33	-	6.50	8.29	7.97	6.48
41	unknown	1746	-	0.38	0.30	0.41	-	1.48	1.81	0.02	1.99
42	octadecane	1799	MS-KI	1.22	0.26	0.63	-	0.93	1.00	1.29	-
43	1-nonadecene	1856	MS-KI	8.80	4.98	8.40	-	11.97	9.36	14.25	15.33
44	nonadecane	1903	MS-KI	37.87	17.42	23.81	-	29.44	22.66	31.43	40.38
45	1-eicosene	1974	MS-KI	0.42	0.24	-	-	0.47	0.54	0.68	-
46	eicosane	2003	MS-KI	5.19	3.02	3.63	-	4.44	4.82	4.62	2.83
47	unknown	-	-	-	-	-	-	-	0.43	-	0.59
48	heneicosane	2104	MS-KI	26.17	18.19	13.28	-	27.51	17.26	24.88	20.17
49	docosane	2199	MS-KI	0.95	0.58	0.40	-	1.38	0.78	1.04	0.42
50	tricosane	2300	MS-KI	-	-	-	-	-	6.39	7.58	3.66
51	tetracosane	2397	MS-KI	8.01	6.48	3.72	-	10.38	0.36	0.33	-
	Identification			99.62	99.70	99.97	87.64	97.76	98.14	99.98	99.41
	Monoterpenes			-	36.90	21.80	31.87	0.79	12.13	0.52	0.89
	Sesquiterpenes			3.64	6.09	10.08	-	4.85	10.55	3.01	4.33
	Hydrocarbons			95.98	55.72	67.65	-	93.56	73.65	96.45	89.83
	Total rose alcohol			-	30.24	13.73	54.50	0.47	8.16	0.52	3.26

1- Description for the samples: S1: A sample of rose oil prepared via conventional method in Dārāb (a county in eastern part of Fars province, 28 °45′N 54 °32′E); S2: A sample of rose oil prepared via conventional method in Meymand (a county in western part of Fars province, 28 °52′N 52 °45′E); S3: A sample of rose oil which is produce in industrially in Meymand (the procedure is similar to that which is obtained via Clevenger- apparatus); S4: A synthetic imported sample; S5: The upper phase (oil part) of a rose oil sample from company A (yielded traditionally) in Meymand; S6: The lower phase of the same sample (essence part); S7: The upper phase (oil part) of a rose oil sample from company B (yielded traditionally) in Meymand; S8: The lower phase of the same sample (essence part)

2- Components are listed in accordance with their elution from a HP5-MS column.

3- Retention indices are in elution from the mentioned column and determined for each constituent with reference to the retention times of normal alkanes.

4- Area (%) represents the relative percentage of each component on the HP5-MS column which is derived by using GC-MS detector.

5- The appearance of some data in bold is to represent them as major constituent (>2%).

325/350 °C). Oven temperature program was adjusted to be increased from 60 °C (0 min) to 220 °C in increments of 5 °C/min and subsequently held for 10 min. The selected carrier gas was selected as Helium, and the flow rate was adjusted at the rate of 1 ml/min. Mass spectrometer operated in EI mode at 70 eV. The interface temperature was set at 280 °C and the mass scan ranged from 30 to 600 m/z.

Constituents of oil samples were identified by determination of their relative retention indices with reference to the retention time of injected normal alkanes (C8-C20) under the same GC/MS program. To confirm the identification, the derived mass spectra as well as calculated retention indices for each constituent was compared to those of the internal reference libraries, Wiley (nl-7) and Adams as well as to those of the related literatures (21).

2.3. Cluster analysis

Cluster analysis was carried out by using multivariate statistical package. To determine the relationship between (S1-S9) populations based on percentage compositions of the essential oil, unweighted pair-group method (UPGMA) was used for cluster definition, selecting Euclidean distance as a measure of similarity.

3. Results and discussion

In this study, eight different rose oils involving two conventionally, one synthetically, one industrially, and two traditionally obtained samples (these two samples were individually separated to upper phase or oil and lower phase or essence). Figure 1 schematically outlines the samples' descriptions.

Table 1 represents the identified ingredients and relative amounts for the studied samples. In total, 44 compounds related to 8 defined samples (11-29 constituents) were identified (87.64-99.98%). Major compounds for all samples were saturated and unsaturated hydrocarbons including nonadecane with the highest abundant (17.42-40.38%), followed by heneicosane (17.26-26.17%), 1-nonadecene (4.98-15.33%), heptadecane (3.96-10.33%), and eicosane (2.83-5.19%), except for the synthetic sample (S4), which was appeared with no trace of hydrocarbons. Tetracosane was also determined as a major constituent for samples S1-S3. While S5 (3.72-10.38 %) had no tricosane, it was found as a main ingredient in S6-S8 (3.66-7.58).

The profile of constituents in S1 (obtained via conventional method) was found similar to that of the S5 and S7 (upper phase of rose oil yielded via traditional method). On the other hand, the S2 (obtained conventionally) was much similar to S3 (obtained via industrial Clevenger apparatus) in regard to the amounts of monoterpenes, sesquiterpenes, and hydrocarbons.

With reference to Table 1, no main variations were found either in major constituents or in general profiles of S5 and S6 as well as S7 and S8, respectively. However, β-citronellol (5.04%) and cis-geraniol (2.84%) were found as other major constituents of S6 (lower phase of rose oil sample, essence) which were not detected in S5.

On the other side, findings showed that mostly the major and the minor compounds of S4 (synthetic sample) were significantly different to those of others. As presented in Table 1, Phenethyl alcohol (38.77%), β-citronellol (15.73%), phenethyl acetate (15.22%), α-terpineol (6.17%),

Table 2. Major constituents of studied oil samples.

No.	Components	S1	S2	S3	S4	S5	S6	S7	S8
1	ρ-cymene	-	-	-	3.98	-	-	-	-
2	phenethyl alcohol	-	-	-	38.77	-	-	-	-
3	α-terpineol	-	-	-	6.17	-	-	-	-
4	β-citronellol	-	18.87	9.26	15.73	-	5.04	-	-
5	trans-geraniol	-	10.93	4.47	-	-	-	-	-
6	cis-geraniol	-	-	-	-	-	-	-	-
7	phenethyl acetate	-	-	-	15.22	-	-	-	-
8	cis-geranyl acetate	-	-	3.97	-	-	-	-	-
9	germacrene D	-	-	-	-	-	-	-	-
10	heptadecane	5.88	3.96	10.33	-	6.5	8.29	7.97	6.48
11	1-nonadecene	8.8	4.98	8.4	-	11.97	9.36	14.25	15.33
12	nonadecane	37.87	17.42	23.81	-	29.44	22.66	31.43	40.38
13	eicosane	5.19	3.02	3.63	-	4.44	4.82	4.62	2.83
14	heneicosane	26.17	18.19	13.28	-	27.51	17.26	24.88	20.17
15	tetracosane	8.01	6.48	3.72	-	10.38	-	-	-

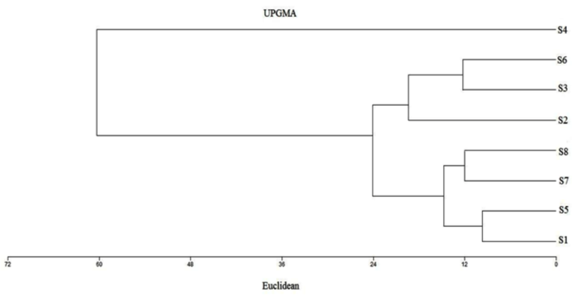

Figure 2. Dendrogram obtained by clustering analysis of the percentage composition oil of 8 populations of *Rosa damascena*.

and ρ-cymene (3.98%) were identified as main components for S4, while two additional major constituents (9.51 and 2.86%) were remained unidentified. However, the only major constituent that was common between S4 and other studied samples was β-citronellol (Table 1). The amount of rose alcohol in S4 was found as 54.50%, which is considerable; whereas the synthetic rose oil costs nearly 1/1000th as much as the sample prepared naturally. Cheap Rose oil samples are usually prepared by different combinations of rose alcohol ingredients, mostly containing of 30% of synthetic citronellol (22). Rose alcohol content generally refers to the total amounts of citronellol, geraniol, and nerol, together with phenethyl alcohol (23).

Phenethyl alcohol is an aromatic alcohol with a pleasant floral odor. It is reported as the main component of rose hydrosols which is used in perfumery, when desiring the smell of rose is considered (6). Despite the appearance of the compound in S4 and S8, howeve with lower amounts (2.37%), none of the others contained this constituent. Table 2 represents the main ingredients found in studied samples.

To make a better comparison between those samples, cluster analysis was performed in this study (Figure 2). According to cluster analysis results, two groups (clade) were identified for 8 populations of Rosa damascena essential oil samples. The first group is included S1-S5 populations as a sister group to S7-S8 populations. Second, S6-S3 populations are nested within S2 population, and S4 population is separated from all other populations. These results confirmed that the sample, S4 which has been made synthetically has less relationship with other samples.

Until now, numerous studies have been performed on damask rose essential oil analysis. It is well accepted that the extraction method and growth location profoundly affects the composition of rose essential oil (24). Geraniol and β-citronellol as well as nonadecane (24-40.50%) and heneicosane (7-14.50%) were reported as the main constituents of samples collected from the central Iran (18). Another investigation has remarked the compound geraniol (15.37-26.50%) as the main ingredient. The mentioned study also showed that the amount of rose alcohol was increased from 55.25% to 83.41%, with increasing of the pressure and temperature (13). However, in our research, geraniol was not detected in samples yielded traditionally. Moreover, the total rose alcohol in studied samples was varied from 0.00 to 30.24% (except for the synthetic samples) which were found with a much higher alcohol content in previous studies (25-26). In other words, it was found scant in most traditionally yielded samples (Table 1). It is notable that in traditional distillation procedure, focusing on the quality of rose water leads to high appearance of these components in the water part (6).

On the other hand, the total hydrocarbon content in our samples (except in synthetic S4) was considerable (55.72-96.45%). In one study, the total hydrocarbon content of a rose oil sample from northern Iran was reported in high amount (63.21%) (27). However, in previous studies, the amount of main hydrocarbons was reported lower (25-26, 28-29). It is concluded that for samples obtained traditionally, prolonged and repeated distillation resulted higher amounts of hydrocarbons.

4. Conclusion

In the present work, rose oil samples which are currently supplied in markets of Fars province were evaluated regarding the type of production. As most prevalent oil samples of damask rose are prepared traditionally, outlining a report on profiles compared to those of conventional and synthetic procedures was performed. Due to the repeated and prolonged distillation, most abundant groups of compounds hit for these sample were hydrocarbons with higher amounts compared to the prior investigations. However, the total rose alcohol in all selected samples yielded in Fars province, was considerably lower than that of the previous reports.

Acknowledgements

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Conflict of Interest

None declared.

5. References

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