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Chemical composition, sensory evaluation and antimicrobial activity of Taif rose (*Rosa damascena* Mill.) essential oils

M. Kurkcuoglu[1], K. H. C. Baser[2], S. G. Aкterian[3], H. N. Fidan[4*], A. S. Stoyanova[3]

[1] *Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470, Eskisehir, Turkey*
[2] *Near East University, Faculty of Pharmacy, Department of Pharmacognosy, Nicosia, Turkish Republic of Northern Cyprus*
[3] *University of Food Technologies, Technological Faculty, Technology of Tobacco, Sugar, Vegetable and Essential oils, 4000, Plovdiv, Bulgaria*
[4] *University of Food Technologies, Faculty of Economics, Department of Nutrition and Tourism, 4000, Plovdiv Bulgaria*

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Rosa damascena Mill. is a traditional and promising agricultural plant used for manufacturing essential oil in Saudi Arabia. The present study aims at studying the chemical composition, sensory evaluation and antimicrobial activity of some samples of rose oils (*Rosa damascena* Mill.) from the region of Taif. The chemical composition was determined by GC and GC/MS. Main constituents in the studied essential oils were: citronellol, nerol, geraniol, and nonadecane. The monoterpene alcohols, citronellol, geraniol, and nerol, were the constituents responsible for the odor of the oils analysed. The antimicrobial activity was determined against Gram-positive bacteria (*Listeria monocytogenes* NCTC 11994, *Staphylococcus aureus* ATCC 25093, and *Bacillus cereus* ATCC 11778), Gram-negative bacteria (*Escherichia coli* ATCC 8739 and *Salmonella enterica* subsp. *enterica* serovar Abony NCTC 6017), and a fungal strain - *Aspergillus flavus*. The high antimicrobial effect of these rose oils was confirmed on six types of bacteria and fungi. The strongest antimicrobial effect was against the Gram-positive bacterium *S. aureus*. The specified characteristics of Taif rose oils indicate their potential implementation in perfumery, cosmetic, soap, household and personal care products manufactured in the Kingdom of Saudi Arabia.

Keywords: *Rosa damascena* Mill, Taif rose, chemical composition, sensory evaluation, antibacterial activity

INTRODUCTION

The genus *Rosa*, belonging to the Rosaceae family, contains hundreds of species that form a group of plants with a wide range of usage and application [1]. Bulgaria and Turkey are known as the main producers of *R. damascena* Mill. essential oil in the world. Different commercial products are produced from rose flowers such as rose essential oil, rose water, dried flowers, rose concrete, and rose absolute. Due to the antimicrobial, antioxidant, analgesic, anti-inflammatory, anti-diabetic and anti-depressant properties of *R. damascena*, there is a significant interest in the development of new innovative products with industrial and practical importance [2].

The roses have acquired cultural significance in the societies of Western Saudi Arabia, too. Especially, Taif rose (*R. damascena*) cultivation is a tradition in the Taif region that has contributed to making this city located in the South-west of Saudi Arabia a highly preferred tourist destination. Rose cultivation in the region of Taif started most probably in the 16th century. Today, the rose oil production in Saudi Arabia represents less than 5 % of the global world production. The main regions (fig. 1) for growing oil-bearing roses are Taif, Al Bahah, Abha and Khamis Mushait. The altitude of these regions is between 1800 and 2300 m above sea level. The annual temperature difference is 15-16 °C. Annual rainfalls are between 100 and 150 mm per square m. Therefore, full irrigation is needed for rose growing. The period for harvesting rose flowers is two months - from March to April [3]. Taif rose is used for essential oil and rose water production, having applications in medicine, food industry and perfumery.

A number of authors have conducted agro-bioassays, for example, describing diseases of rose plantations [4], investigating the effect of arbuscular *Mycorrhizal* fungus on the development of rose plantations [5], isolating bacteria, with phytase activity, growing in the soil on which roses have grown [6]. The rose processing in this region is traditionally done by water distillation.

The chemical composition and biological potential of the Taif rose essential oil has been the subject of several studies [7-15]. The content of the major constituents is one of the most important parameters which determine the quality of the rose oil.

* To whom all correspondence should be sent:
E-mail: hfidan@abv.bg

It has been reported that citronellol, geraniol, nonadecane, and nerol were among the main constituents of rose oil obtained from rose blossoms [8-10]. The investigation of the cytotoxic, genotoxic, antimutagenic and anticancer effect of the concrete and absolute rose oils from Taif rose showed that they displayed a cytotoxic effect towards normal human blood lymphocytes in a dose-dependent manner and anticancer activity against HepG2 and MCF7 cells [11].

Rose flower extracts were analyzed for their antioxidant [12-14] and antimicrobial activities [4, 8, 15], while the phytochemical study of the rose flower methanol extract led to isolation and identification of four new compounds [16].

The objective of the present study was to determine the chemical composition, odor evaluation and antimicrobial activity of some industrial samples of Taif rose essential oils.

Figure 1. Main regions with favorable conditions for growing oil-bearing roses in the Kingdom of Saudi Arabia: 1- Taif; 2- Al Bahah; 3- Abha; 4- Khamis Mushait; 5- Al Hada.

MATERIALS AND METHODS

Materials

Five samples of industrial essential oils (numbered from 1 to 5) were obtained from distilleries in the Taif region and were used for GC and GC/MS analyses. The oils were kept refrigerated at 4°C before analysis.

GC and GC/MS analyses of essential oils

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. (Agilent Technologies Inc., Santa Clara, CA). Innowax FSC column (Hewlett-Packard –HP, U.S.A.) (60 m × 0.25 mm, 0.25 μm film thickness) was used with helium as a carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted to 40:1. The injection temperature was 250°C. Electron impact mass spectra were generated at 70 eV with a mass range from *m/z* 35 to 450.

The GC analysis was carried out using an Agilent 6890N GC system. In order to obtain the same elution order with GC/MS, simultaneous injection was done by using the same column and appropriate operational conditions. FID temperature was 300°C.

The chemical constituents of the essential oils were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, MassFinder Library and confirmed by comparison of their retention indices. Straight-chain n-alkanes were used as reference points in the calculation of relative retention indices (RRI). Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Sensory evaluation of rose aroma products

Sensory evaluation of the samples was performed in a sensory laboratory according to ISO 8589 [17] at the University of Food Technologies, Plovdiv, Bulgaria. One professional perfumer and two aroma chemists (each having experience in the field over 10 years; 2 female) participated and independently evaluated the sensory characteristics. At the time of the procedure, each tester was in a normal healthy condition and mood. Three sniffs from the test strip (dipped in the rose product around 1 cm) were performed and fresh air was breathed between each product. The procedure was repeated three times daily within three days. Then, the testers were asked to give their evaluation in a form of descriptive analysis. The results were collected from each tester in a paper ballot. The odor descriptive characteristics of some individual compounds were based on the works of Bauer *et al.* [18], Ohloff *et al.* [19], and Bedoukian [20].

Antimicrobial activity

The antimicrobial activity of the rose oils was tested against test microorganisms provided by the National Bank for Industrial Microorganisms and Cell Cultures in Sofia, Bulgaria: Gram-positive bacteria: *Listeria monocytogenes* NCTC 11994, *Staphylococcus aureus* ATCC 25093, *Bacillus cereus* ATCC 11778; Gram-negative bacteria: *Escherichia coli* ATCC 8739, *Salmonella enterica* subsp. *enterica* serovar Abony NCTC 6017; and fungal strain: *Aspergillus flavus* (clinical isolate). The selective growth media were: Listeria Oxford

Agar Base /Merck/; Baird Parker Agar Base with Egg Yolk Tellurite emulsion supplement /Merck/, Rapid' *E. coli* 2 Agar /BioRad/ and Mac CONKEY Agar /Merck/, Mueller-Hinton agar (MHA) /Oxoid/, Sabouraud Dextrose Agar /Oxoid/, respectively. The media were inoculated with a 24-hour suspension of the bacterial species. The antimicrobial activity was determined by modification of the "agar diffusion" method by measuring the areas of inhibition of pathogen growth [mm] around rings in which a certain amount (0.05, 0.10, and 0.15 mL) of essential oil was applied [21].

RESULTS AND DISCUSSION

Chemical composition of Taif rose essential oils

The samples of essential oils were liquids with pale yellow color, having a low freezing temperature. The chemical composition of the Taif rose oils is shown in Table 1.

Table 1. Composition of Taif rose oils (%)

	RRI	Compound	1	2	3	4	5
1	942	Ethanol	tr	tr	tr	tr	tr
2	1032	α-Pinene	1.0	0.7	2.0	1.6	0.5
3	1118	*β*-Pinene	0.2	0.2	0.4	0.4	0.1
4	1132	Sabinene	0.6	0.1	0.1	0.2	0.4
5	1174	Myrcene	0.1	0.5	1.0	0.9	-
6	1203	Limonene	0.1	0.1	0.1	0.1	0.1
7	1246	(*Z*)- *β*-Ocimene	-*	-	-	tr	-
8	1255	*γ*-Terpinene	-	-	-	0.1	-
9	1266	(*E*)- *β*-Ocimene	0.1	tr	0.1	0.1	-
10	1280	*p*-Cymene	0.1	tr	0.1	0.1	-
11	1290	Terpinolene	0.1	-	-	tr	-
12	1362	*cis*-Rose oxide	0.3	0.2	0.3	0.2	0.1
13	1376	*trans*-Rose oxide	0.1	0.1	0.1	0.1	tr
14	1500	Pentadecane	0.3	0.3	0.3	0.3	0.3
15	1535	*β*-Bourbonene	0.1	0.2	0.1	0.1	0.1
16	1553	Linalool	3.5	2.8	5.6	3.7	6.1
17	1596	*α*-Guaiene	0.5	0.5	0.4	0.4	0.4
18	1611	Terpinen-4-ol	0.7	0.5	0.7	0.7	0.6
19	1612	*β*-Caryophyllene	0.4	0.4	0.4	0.5	0.5
20	1668	Citronellyl acetate	0.4	0.2	0.3	0.4	0.2
21	1687	*α*-Humulene	0.4	0.4	0.3	0.4	0.4
22	1694	Neral	0.4	0.5	0.5	0.6	0.4
23	1700	Heptadecane	1.2	2.0	1.5	1.3	2.2
24	1706	*α*-Terpineol	1.0	0.9	1.6	1.0	2.1
25	1726	Germacrene D	0.6	0.9	0.7	1.0	0.7
26	1730	*δ*-Guaiene (=α-Bulnesene)	0.4	0.3	0.3	0.4	0.3
27	1733	Neryl acetate	0.2	0.1	-	0.2	0.1
28	1740	Geranial	0.9	1.0	1.0	1.0	0.6
29	1758	(*E,E*)-*α*-Farnesene	-	-	-	-	tr
30	1765	Geranyl acetate	0.7	0.8	1.1	2.2	1.1
31	1772	**Citronellol**	**38.0**	**24.2**	**24.6**	**23.1**	**21.1**
32	1808	**Nerol**	**10.8**	**13.8**	**12.5**	**13.5**	**11.0**
33	1838	2-Phenylethyl acetate	0.1	0.1	0.1	0.2	0.1
34	1857	**Geraniol**	**18.2**	**25.7**	**24.7**	**28.0**	**24.7**
35	1900	**Nonadecane**	**7.2**	**10.8**	**7.4**	**7.0**	**10.7**
36	1915	Nonadecene	2.7	1.4	3.2	1.9	2.4
37	1937	Phenylethyl alcohol	2.3	3.0	2.2	2.4	3.1
38	2000	Eicosane	0.6	0.7	0.5	0.6	0.7
39	2030	Methyl eugenol	1.3	1.0	0.8	1.0	1.0
40	2050	(*E*)-Nerolidol	-	-	0.1	-	0.1
41	2100	Heneicosane	2.2	2.7	2.0	2.2	2.6
42	2186	Eugenol	0.9	1.1	1.1	0.6	1.8
43	2187	*γ*-Eudesmol	-	-	0.1	-	0.3
44	2250	*α*-Eudesmol	-	-	-	-	0.1

45	2255	α-Cadinol	-	-	0.1	-	0.4
46	2257	β-Eudesmol	-	-	-	-	tr
47	2300	Tricosane	0.3	0.4	0.3	0.3	0.4
48	2314	(2*E*,6*Z*)-Farnesol	0.1	0.1	0.1	tr	0.1
49	2349	Geranic acid	0.1	0.1	0.1	0.1	0.1
50	2369	(2*E*,6*E*)-Farnesol	0.7	1.2	1.0	1.1	1.9
51	2500	Pentacosane	-	-	0.1	-	0.1
		Total, %	99.9	100	100	100	100
		Aliphatic hydrocarbons,%	14.2	18.3	15.3	13.6	19.4
		Monoterpene hydrocarbons,%	2.2	1.6	3.7	3.4	1.1
		Oxygenated monoterpenes,%	75.7	70.9	73.2	74.8	68.3
		Sesquiterpene hydrocarbons,%	2.4	2.7	2.2	2.8	2.4
		Oxygenated sesquiterpenes,%	0.8	1.3	1.3	1.1	2.8
		Phenyl propanoids,%	4.7	5.2	4.3	4.3	6.0

RRI: Relative retention indices were calculated against straight-chain n-alkanes; %: calculated from FID data; tr: Trace (< 0.1 %); -: not detected.

Table 2. Comparison between the chemical compositions of Taif rose essential oils of different (%) origins

Component	Saudi Arabia Taif Rose Oil		Bulgaria*		Morocco*		Turkey*		Turkey* peasant type	
	min	max	min	max	min	max	min	max	min	max
Ethanol			-	2.0	-	3.0	-	7.0	-	2.0
Citronellol	21.1	38.0	20.0	34.0	30.0	47.0	34.0	49.0	26.0	40.0
Nerol	10.8	13.8	5.0	12.0	3.0	11.0	3.0	11.0	6.0	12.0
Geraniol	18.2	28.0	15.0	22.0	6.0	23.0	8.0	20.0	12.0	29.0
β-Phenyl-ethanol	2.2	3.1	-	3.5	-	3.0	-	3.0	-	3.0
Heptadecane	1.2	2.2	1.0	2.5	0.6	4.0	0.8	3.0	0.7	3.0
Nonadecane	7.0	10.8	8.0	15.0	7.0	16.0	6.0	13.0	6.0	8.5
Heneicosane	2.0	2.7	3.0	5.5	2.0	5.5	2.0	4.0	1.5	4.0

*ISO 9842 [22]

In sample 1, 41 constituents representing 99.9 % of the total content of the oil were identified. The main compounds were citronellol (38.0 %), geraniol (18.2 %), and nerol (10.8 %). The other compounds characterized were nonadecane (7.2 %), linalool (3.5 %), nonadecene (2.7 %), phenylethyl alcohol (2.3 %), and heneicosane (2.2 %).

Thirty-eight constituents were identified in sample 2, representing 100 % of the total content of the oil. The major compounds were- geraniol (25.7 %), citronellol (24.2 %), nerol (13.8 %), and nonadecane (10.8 %). The third sample contained 43 compounds, representing 100 % of the total content of the essential oil. The main constituents in sample 3 were citronellol (24.6 %) and nerol (12.5 %), geraniol (24.7 %), nonadecane (7.4 %), and linalool (5.6 %).

Sample 4 consisted of 40 constituents representing 100 % of its total content. The main components in the essential oil were geraniol (28.0 %), citronellol (23.1 %), nerol (13.5 %), and nonadecane (7.0 %).

The essential oil obtained from sample 5 was composed of 41 components, representing 100 % of the total essential oil content. Geraniol (24.7 %), citronellol (21.1 %), nerol (11.0 %), nonadecane (10.7 %), and linalool (6.1 %) were the major constituents detected. The distribution of the identified components by groups of compounds is presented in Table 1. Oxygenated monoterpenes (68.3-74.8 %) and aliphatic hydrocarbons (13.6-19.4%) were the dominant groups in the rose oils, followed by phenyl propanoids (4.3-6.0 %), monoterpene hydrocarbons (1.1-3.7 %), sesquiterpene hydrocarbons (2.2-2.8 %), and oxygenated sesquiterpenes (1.1-2.8 %).

According to the requirements of ISO 9842 [22], ethanol content was found to be low and the contents of monoterpene alcohols citronellol, geraniol and nerol determining the rosy odor of the samples comply with the ISO standard. The content of the hydrocarbons, acting as odor fixers and determining the viscosity of the oils at low temperature, are also within the standard limits, while the content of phenylethyl alcohol was found to be higher [22].

The results for the five oil samples do not differ much from the literature data for oils obtained from this region. The differences in quantities are possibly caused mainly by the time of harvest, and the processing conditions.

The chemical composition of the Taif rose essential oil has been studied by several researchers [1, 7-10]. Table 2 represents the comparison between the content of the main constituents of Taif rose oil and *R. damascena* essential oils growing in different countries, such as Saudi Arabia, Bulgaria, Turkey and Morocco. The differences between the chemical compositions of Taif rose essential oils predominantly depend on the rose genotype but are also influenced by the environmental and climatic conditions, flower processing and oil distillation practices. The differences found in the content of the main constituents with the data in the literature on roses growing in other countries, such as Bulgaria, Turkey, India, China, *etc.*, may be explained by the differences in agrobiological conditions and processing conditions [1, 23-36]. Aliphatic hydrocarbons (alkanes and alkenes) play a significant role in the aroma products as compounds responsible for odor stability. The content of heptadecane and nonadecene/ nonadecane is considered to be of particular importance [37]. Bazaid [7] examined the chemical composition of essential oil obtained from four sites in Taif under laboratory conditions. Essential oils not stored and stored for 12 months were analyzed. According to the author, during the storage, the contents of α-pinene, β-pinene, limonene, citronellol, and eugenol were reduced, while that of rose oxide increased. Halawani [8] found that the main components of rose oil, obtained by water distillation of rose blossoms, harvested from Al-Hada and Al-Shafa farms were citronellol (14.8-29.0 %), geraniol (11.3-16.2 %), and nerol (11.6 %). According to the author, the differences in the composition of the analyzed oil and that of the literature were due to specific geographical conditions under which they were cultivated.

Kurkcuoglu *et al.* [9] examined the composition of commercial oil samples sourced from retailers in Riyadh, Saudi Arabia. The major components of the oils were citronellol (22.8 and 27.5 %), geraniol (19.9 and 13.5 %), nonadecane (10.9 and 15.7 %), nerol (10.5 and 6.4 %), respectively. The authors compared the results obtained with the data of the ISO standard and according to them the amount of monoterpene compounds was in the range, while that of nonadecane and heneicosane was above the limits.

According to Abdel-Hameed *et al*. [10] citronellol (17.6 %), geraniol (11.4 %), nonadecane (6.5 %), nerol (6.4 %), linalool (5.9 %), α-pinene (4.5 %) and phenylethyl alcohol (3.6 %) were the main components of the Taif rose oil. In addition, the *in vitro* cytotoxic, genotoxic and anticancer effects of the oil were investigated toward normal human peripheral blood lymphocytes and two kinds of human cancer cell lines. The essential oil of Taif rose was suggested to be used as an effective therapeutic natural agent after further toxicological *in vitro* and *in vivo* studies.

Sensory evaluation of rose aroma products

According to the sensory characteristics of the investigated Taif rose essential oils, sample 1 was characterized by very intense and typical rose odor with citrus- and balsamic-like undertones. Sample 2 was described with a floral odor reminiscent of rose, while sample 3 had floral odor reminiscent of rose with spice undertones. It was determined that sample 4 had a balsamic odor, with floral and citrus-like undertones, whereas the last sample (sample 5) had an intense and typical rose odor with citrus-like undertones.

The monoterpene alcohols citronellol, geraniol, and nerol are the main constituents responsible for the odor of the oils tested [8-10]. Citronellol is characterized by a pleasant rose-like odor, geraniol with a flowery rose-like odor (different from that of citronellol), and nerol with a pleasant rose-like odor (different from that of geraniol). Other monoterpene alcohols, such as linalool with a flowery-fresh odor; terpinen-4-ol with a spicy, woody-earthy, and also lilac-like odor, and α-terpineol with a characteristic lilac aroma are contributing for the denser characteristic odor of rose oil.

The characteristic rose-like odor of the rose oils is due to the phenylethanoid β-phenylethyl alcohol, eugenol (with spicy, clove-like odor), methyl eugenol (with mild-spicy, slightly herbal odor), *trans*-rose oxide (with a strong odor reminiscent of geranium oil and carrot leaf odor) regardless of the amount involved in the formation of the bouquet of the smell [2].

Antimicrobial activity

The antimicrobial activity of different Taif rose essential oils against five bacterial strains and one fungus is presented in Table 3.

Table 3. Growth inhibition zones (mm) of Taif rose oils against pathogenic bacteria and a fungus

Test micro-organism	Sample 1			Sample 2			Sample 3			Sample 4			Sample 5		
	Concentration of the essential oil, mL														
	0.15	0.10	0.05	0.15	0.10	0.05	0.15	0.10	0.05	0.15	0.10	0.05	0.15	0.10	0.05
E. coli	18	16	15	16	12	10	6	6	6	20	17	16	16	11	6
S. enterica	6	6	6	25	24	22	15	6	6	10	6	6	16	6	6
L. mono-cytogenes	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
S. aureus	26	24	20	14	12	11	14	13	11	29	22	20	10	9	8
B. cereus	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
A. flavus	6	6	6	6	6	6	6	6	6	6	6	6	11	10	6

Results were evaluated according to the diameter of the growth inhibition zone including the diameter of the ring (6 mm). A broad spectrum and variation in the antimicrobial properties of the oils were shown in the study. Sample 1 showed antibacterial activity against *S. aureus* and *E. coli*, as the inhibition zone ranged between 26 to 20 mm for *S. aureus* and 18 to 15 mm for *E. coli* according to the applied amount of essential oil. *S. enterica* is a foodborne pathogenic bacterium with high significance due to the safety of the foods and showed the highest sensitivity on the action of sample 2 with a diameter of zone of inhibition ranged between 25 and 22 mm. Sample 4 showed the strongest antibacterial activity against *S. aureus*, followed by sample 2 which possessed potential against *S. enterica*. Clearly, the *L. monocytogenes* and *B. cereus* were most resistant to the tested essential oils. Sample 3 showed a moderate level of activity only against *S. aureus*, while sample 5 showed the lowest effect against the pathogenic bacterium *S. aureus* and antifungal activity against *A. flavus*.

In terms of antimicrobial activity, the samples tested do not differ from the literature data [8, 25, 28-30].

Taif rose essential oils are complex mixtures comprising many constituents. Each of these constituents contributes to some effects of these oils. Therefore, we suggest that the antibacterial activity of the tested samples could be due to the high amount of monoterpene alcohols and concurrent presence of hydrocarbons.

The higher antibacterial effect of sample 4 was due to the higher content of geraniol. Aridogan *et al*. [38] evaluated the *in vitro* antibacterial activities of *R. damascena* and their components. They found that the antimicrobial activity of *R. damascena* essential oil was determined only against *S. aureus* strains (the inhibition zone was 8 mm), whereas the antimicrobial activity of geraniol and nerol was found against both *S. aureus* and *E. coli* strains (with inhibition zone diameters ranged between 21 mm and 12 mm). They found that citronellol, geraniol and nerol had more potent antimicrobial activity individually than in the oil. Our results are not in agreement with that reported by Aridogan *et al.* [38] and it could probably be related to the synergistic effect between some of the minor components occurring in the essential oils and the major components citronellol, geraniol and nerol. Similar to our results Shohayeb *et al*. [14] reported that rose oil and its different petal extracts exerted a broad spectrum of antimicrobial activities against three fungi and eleven Gram-positive, Gram-negative bacteria. Mahboubi *et al*. [39] also determined the antimicrobial activity of *R. damascena* oil against a large number of microorganisms including Gram-positive and Gram-negative bacteria, and yeast by micro broth dilution assay.

CONCLUSIONS

The results of chemical analysis and sensory evaluation show that Taif rose oils are typical rose oils with their own specific features. Monoterpene alcohols citronellol, geraniol, and nerol were the major constituents and these oils were in compliance with the specifications of ISO 9842 [22]. The variations in chemical composition and odor characteristics of different samples can be explained by specific agro-climatic conditions of sub-regions, the botanical specificity of rose bushes grown and the technological diversities in local distilleries. The antimicrobial studies showed high activity of the rose oils studied, the highest being against the Gram-positive bacterium *S. aureus*. The characteristics of Taif rose oils show that they can be successfully applied not only in perfumery and cosmetic products, but also in soap, household and personal care products. Presently, these applications are limited in the domestic market due to higher prices of these rose oils.

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