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Tocopherol, Carotene, Phenolic Contents and Antibacterial Properties of Rose Essential Oil, Hydrosol and Absolute

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Abstract The antioxidant and antibacterial activities, and total phenolic contents of *Rosa damascena* Mill. flower extracts (absolute, essential oil and hydrosol) were investigated. The chemical compositions of these extracts were analysed by GC-MS. Phenylethyl alcohol (78.38%) was found to be the main constituent of rose absolute, while citrenellol and geraniol were the major compounds (>55%) of rose essential oil and hydrosol. Tocopherol and carotene levels were determined by high performance liquid chromatography (HPLC) analysis. The levels of beta carotene (422.3±35.6 ppm), alpha tocopherol (2397.1±72.5 ppm) and gamma tocopherol (343.1±28.4 ppm) of rose absolute were found to be higher than that of essential oil and hydrosol. Their total phenolic contents were also evaluated. The total phenolic content of the tested extracts varied from 5.2 to 2134.3 GAE/mg L^{-1}. Rose absolute and essential oil contained high levels of phenolics and demonstrated strong antibacterial activity against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Chromobacterium violaceum* (ATCC 12472) and *Erwinia carotovora* (ATCC 39048) strains.

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Introduction

Rosa damascena Mill., commonly known as Damask rose, is a member of the Rosaceae family with pink flowers and a perennial shrub indigenous to Europe and Middle East countries, Iran and Turkey [1]. It is commercially cultivated for its essential oil. Besides rose essential oil, hydrosol and absolute are the abundant materials that can be obtained from *R. damascena* Mill. [2]. Rose essential oil is widely used in perfumery and cosmetic industry. In addition to its perfuming effects, it was reported to posses a wide range of biochemical activities, such as analgesic, hypnotic, antispasmodic, anti-inflammatory and anticonvulsant [3–5]. Rose extracts have some other benefits such as cooling, soothing, astringent and anti-inflammatory functions which allow them to be used in medicine, food and cosmetic industries [6–8].

In recent years, numerous studies have been also published on the antioxidant, antibacterial activities and chemical composition of rose essential oil [9–13]. However, to our knowledge, no report on the antioxidant and antibacterial activities, and total phenolic contents of *Rosa damascena* Mill. hydrosol and absolute is available to date. Rose essential oil has a high market value because of its use in perfumery and cosmetic industry. In comparison with rose oil, hydrosol and absolute are less expensive. Therefore, the purpose of the present study was to investigate and compare tocopherol, carotene, phenolic contents and

antibacterial properties of rose essential oil, hydrosol and absolute which are used as raw materials in various cosmetic and pharmaceutical applications.

Experimental

Rose Extracts

Rose essential oil, absolute and hydrosol (obtained from Sebat Ltd., Isparta commercial producers of rose oils and aromatic substances) were used in the present study.

Bacterial Strains

Gram-positive and gram-negative bacterial strains, representing a wide group of pathogenes, were used to test the antibacterial properties of rose extracts.

Microorganisms were obtained from the Department of Biology, Süleyman Demirel University, Isparta, Türkiye. Three strains of Gram-negative bacteria; *Chromobacterium violaceum* (ATCC 12472), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and two strains of Gram-positive bacteria; *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 6538) and a plant pathogen *Erwinia carotovora* (ATCC 39048) were used. Bacterial cultures were stored on appropriate agar slants at 4°C during whole study and used as stock cultures.

Antibacterial Assay

Antibacterial activities of rose essential oil, rose absolute and hydrosol were screened by well-diffusion assays in five different concentrations. The microorganisms were grown overnight at 37°C in 10 ml of Luria Broth (10 g/l tryptone, 5 g/l yeast extract, 5 g/l NaCl). Turbidity of the cultures were adjusted to McFarland no 0.5 standard with sterile saline solution. Five millilitre of molten LB agar (0.7% w/v) were inoculated with 1 ml of culture strain and immediately poured over the prewarmed plates. Up to 75 µl of the products (rose essential oils, absolute and hydrosol) were pipetted into the punched-wells in the solidified agar. The plates were incubated for 24–48 h at 37°C. Antibacterial activity was determined by the diameter of inhibition zones (mm) around the wells. Hexane was used as a negative control and gentamicin (200 µg/ml) was used as a positive control.

The rose essential oil and absolute were sterilised by filtration through a 0.45-µm membrane filter. All petri dishes were sealed with sterile laboratory parafilm to avoid the evaporation of test samples. The plates were left at room temperature for 30 min, and then they were incubated at 37°C for 24 h. After incubation, the zone of inhibition was determined with a calliper. Studies were performed in triplicate.

MIC Assay

Minimum inhibitory concentrations (MICs) were determined by broth dilution assay [14]. The assay was performed 0.5% (v/v) Tween-80 (Sigma) incorporation into broth to enhance oil solubility. A series of each rose products diluted for twice, ranging from 4 to 0.03% (v/v), was prepared in Luria Bertani broth. Inoculated tubes were incubated at 37°C for 24–48 h and the MIC was determined. Experiments were carried out in triplicate. The MICs were determined as the lowest concentration of oil inhibiting visible growth of each organism in the test tubes.

Gas Chromatography Mass Spectrometry (GC–MS)

The rose oil, hydrosol and rose absolute were analysed by Shimadzu GC-MS QP 5050 (Kyoto, Japan) gas chromatograph–mass spectrometer system. Separations were carried out by a CP WAX 52 CB capillary column (50 m × 0.32 mm ID, d_f :1.2 µm) purchased from Varian. Helium (99.999%), was used as carrier gas at a constant head pressure of 10 p.s.i (1 p.s.i = 6894.76 Pa). Injection volume was 1 µl. The GC oven was programmed as follows: the initial column temperature was 60°C, the column was heated to 220°C at a rate of 2°C min^{-1} and held at 220°C for 20 min. The GC–MS interface and injector were kept at 250 and 240°C, respectively. The mass spectrometer was run in the electron impact mode at 70 eV.

HPLC Determination of Tocopherol Isomers and Beta Carotene

In the tocopherol analysis, the HPLC method of Lampi et al. [15] was modified. Tocopherols (α-, β-, γ- and δ-tocopherol) were evaluated by HPLC with direct injection of samples in a mixture of heptane:tetrahydrofuran (THF) (95:5) solution. Detection and quantification were carried out with a Shimadzu LC-20AT prominence System controller (Kyoto, Japan), SIL-20AC prominence Autosampler, LC-20AT prominence pump and RF-10AXL Fluorescence Detector (Ex 295 nm, Em 330 nm) for tocopherols. The Luna Silica (250*4.6 mm) 5 μ (Supelco, Inc., Bellefonte, PA) column was used. The mobile phase was consisted of heptane/THF (95/5) (v/v) at a flow rate of 1.2 ml/min and the injection volume was 10 µl. For carotene analysis, detection and quantification were carried out with Shimadzu SCL-10Avp System controller (Kyoto, Japan), LC-10ADvp pump, CTO-10ACvp column oven and SPD-M10Avp (Diode Array Detector (450 nm) for beta carotene. The YMC-Pack ODS-AM (250*4.6 mm, 5 µm)

column was used. The mobile phase was consisted of Methanol/ACN/THF (73/20/7), (v/v/v) at a flow rate of 1 ml/min and the injection volume was 20 µl. Data were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software system. Tocopherol isomers (Sigma Chemical Co., St. Louis, MO, USA) were dissolved in mobile phase and used for identification and quantification of peaks. The amount of tocopherols and carotene in the rose essential oil, absolute and hydrosol were calculated as ppm using external calibration curves.

Determination of Total Phenolics

Total phenolic contents of the extracts were determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method [16]. Gallic acid (GA) was used to construct standard curve (0–70 mg/l). The results are expressed as milligrams of gallic acid equivalents (GAE) per litre of samples and calculated as mean value ± SD ($n = 3$).

Results and Discussion

GC–MS Analysis of Rose Products

Among the 15 constituents identified by GC–MS analysis of rose essential oil, citrenellol was found to be the major compound (35.23%) and followed by geraniol (22.19%), nonadecane (13.85%) and nerol (10.26%). Trace amounts of other chemical compounds were also identified. These results are in agreement with the previous studies [9, 12, 17–19]. Recently, Gochev et al. [13] reported citrenellol, geraniol and nonadecane as the major constituents in the Turkish, Bulgarian and Chinese rose oil.

GC–MS analyses of rose absolute showed that phenyl ethylalcohol (78.38%), citrenellol (9.91%), nonadecane (4.35%) and geraniol (3.71%) were the major compounds. In a similar study, the chemical composition of rose absolute was determined as phenyl ethylalcohol (72.73–73.80%), citrenellol (10.62–11.26%), nerol (2.42–2.47%) and geraniol (5.58–5.65%) [20]. Finally, hydrosol was found to be consisting of four constituents; geraniol was the major compound (30.74%) followed by citrenellol (29.44%), phenyl ethylalcohol (23.74%) and nerol (16.12%). To our knowledge, there is no published data on the chemical composition of rose hydrosol.

The major components and their retention times are summarised in Table 1.

Table 1 GC–MS analysis of rose extracts % major components and their retention times

Compound	rt (min)	Rose oil (%)	Rose absolute (%)	Hydrosol (%)
Alpha pinen	7.2	0.80	*	*
Linalool	35	0.53	*	*
Citrenellol acetate	42.3	0.70	*	*
Heptadecane	44.3	0.90	*	*
Germacrene-D	45	0.45	*	*
Geranyl acetate	47.9	2.00	*	*
Citrenellol	48.2	35.23	9.91	29.44
Nerol	50.4	10.26	1.43	16.12
Geraniol	53	22.19	3.71	30.74
Nonadecane	55.9	13.85	4.35	*
9-Nonadecane	56.8	2.79	*	*
Phenylethyl alcohol	57.1	2.30	78.38	23.70
Methyl eugenol	62.5	1.97	0.69	*
Heneicosane	66.7	4.85	*	*
Eugenol	70.5	1.18	1.52	*

* Not detected

The Total Phenolic Contents

The rose oil, absolute and hydrosol were examined for their total phenolic composition. Total phenolic contents varied from 5.2 to 2134.3 GAE/mg/L^{-1}. Total phenolic contents in rose oil, absolute and hydrosol were 839.5 ± 59.5, 2134.3 ± 91.4 and 5.2 ± 0.3 GAE/mg/L^{-1}, respectively. Total phenolic content of absolute was found remarkably high. Özkan et al. [12] found 276.02 mg gallic acid equivalent (GAE)/g in fresh rose flower extract and 248.97 mg GAE/g in spent rose flower extract. Several studies have reported on the relationships between phenolic content and antioxidant activity in some Rose species [21, 22].

Table 2 HPLC analysis results of rose extracts

Sample	α-Tocopherol (ppm)	β-Tocopherol (ppm)	γ-Tocopherol (ppm)	δ-Tocopherol (ppm)	β-Carotene (ppm)
Essential oil	*	*	9.6 ± 0.56	*	*
Absolute	2397.1 ± 72.5	*	343.1 ± 28.4	33.6 ± 2.1	422.3 ± 35.6
Hydrosol	*	*	*	*	*

* Not detected

HPLC Analysis of Tocopherol and Carotene Levels

Antioxidant effects are very important due to the destructive role of free radicals in biological systems and foods. Vitamin E active compounds are part of the antioxidant system inactivating free radicals and take part in the oxidative stress [23]. It was reported that carotenoid or β-carotene-rich diets may prevent cardiovascular disease [24]. Therefore, we examined tocopherol and carotene levels of rose extracts. HPLC analysis of rose oil, absolute and hydrosol showed that tocopherol and carotene levels of rose absolute were significantly higher than that of essential oil and hydrosol (Table 2). Because of solvent extraction yields about 5–10 times that obtained by steam distillation [2], the composition of the absolute depends on method. High tocopherol and carotene levels of rose absolute suggest that it can be used a potent natural antioxidant for commercial exploration.

Antimicrobial activity

Antimicrobial activities of rose products were determined by agar diffusion and MIC tests against six pathogenic bacteria. Hexan (control) had no inhibitory effect on the strains while rose oil and absolute had showed high antimicrobial performance against microorganisms (Tables 3, 4). *C. violaceum* 12472 was the most sensitive microorganism against rose oil and absolute with the highest inhibition zone (>25 mm) and lowest MIC values 0.25 and 0.5%, respectively. *E. coli* 25922 was also sensitive against rose essential oil and rose absolute with MIC values of 0.25 and 0.5%, respectively. According to MIC values rose essential oil showed higher efficiency compared to absolute. However, hydrosol had no antimicrobial activity against any of the microorganisms. Antimicrobial activities and MIC levels of rose essential oil, absolute and hydrosol were given Tables 3 and 4. These results suggested that the antibacterial activity of the tested extracts were mainly due to their phenolic contents.

Antibacterial effect of major components of rose oil (citrenellol, geraniol and nerol) was reported previously [9, 13]. To date, the bacterial activity of *R. damascena* Mill. absolute and hydrosol has not been evaluated. Our results indicated that rose absolute also possess antibacterial activity against both gram-negative and gram-positive bacteria. Antibacterial properties of rose absolute can be attributed to its high phenylethyl alcohol content (PEA). The antimicrobial properties of alcohols have been known for a long time [25]. Because of rose-like aroma of PEA, it is used as a fragrance ingredient in a wide variety of cosmetic products and foods such as beer, wine, olive oil, grapes, tea, apple juice and coffee [26].

Table 3 Antimicrobial activities of rose essential oil, hydrosol and absolute using agar diffusion method

	P. aeruginosa 27853					*C. violaceum* 12472					*E. coli* 25922				
Concentration	1:1	1:5	1:10	1:20	1:100	1:1	1:5	1:10	1:20	1:100	1:1	1:5	1:10	1:20	1:100
Rose oil	*	*	*	*	*	>25	>25	>25	>25	>25	>20	>20	*	*	*
Rose absolute	18 ± 0.5	14 ± 0.3	*	*	*	25 ± 0.5	23 ± 0.5	20 ± 0.5	16 ± 0.6	11 ± 0.5	14 ± 0.7	13 ± 0.5	11 ± 0.8	*	*
Hydrosol	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Gentamicin 200 µg/ml	25 ± 0.8					18 ± 0.3					20 ± 0.8				
	E. carotovora 39048					*B. subtilis* 6633					*S. aureus* 6538				
Rose oil	>20	>20	20 ± 0.5	*	*	>25	>25	>25	>25	14 ± 0.5	*	>25	>25	>25	*	*
Rose absolute	>20	17 ± 0.5	12 ± 0.5	*	*	14 ± 0.5	13 ± 0.8	*	*	*	15 ± 0.5	14 ± 0.5	*	*	*
Hydrosol	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Gentamicin 200 µg/ml	20 ± 0.6					25 ± 0.5					20 ± 0.6				

Gentamicin (200 µg/ml) as a positive reference standard; values are mean inhibition zone (mm), diameter of the well, 5 mm, included

* Not detected

Table 4 Minimum inhibitory concentration (MIC) of rose extracts (% v/v)

Sample	*S. aureus*	*B. subtilis*	*E. carotovora*	*C. violaceum*	*P. aeruginosa*	*E. coli*
Rose oil	0.5	0.25	0.5	0.25	>4	0.25
Rose absolute	2	1	0.5	0.5	2	0.5
Hydrosol	*	*	*	*	*	*
Gentamicin (ppm)	12	3	12	12	6	12

Gentamicin as a positive reference standard

* Not detected

In conclusion, our study showed that because of strong antibacterial effects of rose oil and rose absolute, and high tocopherol, carotene content of rose absolute, they could be used as natural preservative additives in food industry and medicine production and antibacterial agent for disinfection of various surfaces.

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