

Determination of Phenolic, Flavonoid Content and Antioxidant Activity of Oil Rose Products

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Abstract

Rose and rose products are used as raw materials in many sectors including cosmetics, food and medicine. The *Rosa damascena* Mill. rose genotype, which is intensively cultivated in the Isparta region of Türkiye, is an important export product. The flower of the plant is main source of raw materials for rose oil, herbal teas and cosmetic products due to its high essential oil and polyphenols contents. In this study, *Rosa damascena*, *Rosa centifolia*, *Rosa alba* L., *Rosa alba* 'Semiplena' genotypes were grown in Yalova, which is an alternative region in terms of agro-climatic conditions from Isparta and its surroundings where rose oil cultivation is carried out in Türkiye. Within the scope of the study, total phenolic matter, total antioxidant activity (DPPH and CUPRAC) and total flavonoid contents of flowers (whole flowers), rose water and wastewater of the genotypes were determined. It is important to identify alternative rose genotypes to *R. damascena* in Türkiye and their potential for cultivation under different climatic conditions. The highest phenolic content (4115 mg gallic acid equivalent GAE 100 g⁻¹) and DPPH antioxidant activity (4893 mg trolox equivalent TE 100 g⁻¹) values were obtained in *R. damascena* genotype at the first harvest period. The highest CUPRAC antioxidant activity (34237 mg TE 100 g⁻¹) values were obtained in *R. centifolia* genotype at the first harvest period. In the second harvest period, the highest phenolic content, DPPH antioxidant activity and CUPRAC antioxidant activity values were determined in *R. centifolia* genotype. Antioxidant activity, phenolic and flavonoid contents of dried rose flowers were higher than in rose water and wastewater. Rose petals were found to have the potential to be used as an important antioxidant source, while total phenolic matter and antioxidant activity values were found to be low in rose water and wastewater. The harvest period was found to be major factor in oil rose flowers, which can be an important polyphenol source.

1. Introduction

Roses have been one of the most valuable flowers for centuries. In addition to its socio-cultural importance and use as an ornamental plant, roses are also a rich source of biologically active substances. Although the genus *Rosa* includes 200 species and more than 18000 varieties (Gudin, 2000), only a few (*R. damascena*, *R. alba*, *R gallica*

L. subsp. eriastila Kell. var. *Austriaca* Grantz f. *panonica* Br, *R. francofurtana* var. *Agatha*, *R. centifolia*, *R. rugose*, *R. moschata*) are used industrially in the production of rose oil and its products (Georgiev and Stoyanova, 2006; Kovacheva et al., 2010; Rusanov et al., 2012; Baydar, 2016). *R. damascena* is the most commonly used species in oil rose production in Türkiye and in the world.

The most important products of roses are essential oil, concretes, absolutes and rose water (Kumar et al., 2013). All these products are used in the food, cosmetics and perfumer industry. Due to the antioxidant and phenolic contents in phytochemicals, it has also effect on the prevention of many diseases. Recently, the production of products derived from natural plants has been increasing due to the disadvantages of synthetic antioxidants. For this reason, interest in phytochemicals is increasing. Rose species have been reported to have anti-human immunodeficiency virus, antidepressant and anti-inflammatory properties (Nowak and Gawlik-Dziki, 2007). Each of the oil rose genotypes can be a powerful source of antioxidants such as phenolics, flavonoids, carotenoids and anthocyanins. Today, natural plant sources such as oil rose antioxidants are used in the treatment of diseases, nutrition, health care and inhibition of collagenase enzyme (Liu et al., 2020; Mohsen et al., 2020). There are several industrial demands that need to identify new chemotypes with beneficial effects (Moein et al., 2016). Since artificial antioxidants are suspected of toxicity and risks to human health, the demand for the use of natural antioxidants is increasing (Weisburger, 1999). Traditionally, it has been used for chest pain, constipation, depression, gastrointestinal disorders, inflammation, respiratory problems and menstruation (Moein et al., 2016). Rose petals are a natural and powerful antioxidant. Moreover, natural compounds from leaf extracts, such as polyphenols and flavonoids, have anti-aging properties and antioxidant activity (Mohsen et al., 2020). Anthocyanins, as water-soluble vacuolar pigments, are responsible for the color in flowers and fruits of plants and have strong antioxidant

properties. The amount of anthocyanins, like other metabolites, depends on environmental factors (temperature, light intensity, nutrition, pH) (Shameh et al., 2019). Rose oil cultivation in Türkiye has focused on *R. damascena* in a single region (Isparta and its surroundings), and the current research results represent the products grown in this region.

This study was conducted in Yalova, as an alternative region whose agro-climatic conditions such as altitude, temperature and precipitation are completely different from Isparta. Total phenolic matter, antioxidant activities and total flavonoid contents of rose petals and rose water of two different periods of *R. damascena* and other oil rose genotypes (*R. alba*, *R. alba* 'sempilena', *R. centifolia*) about which there is limited information were investigated. In addition, the phenolic contents and antioxidant activity potential of the wastewater, which is formed as a residue in the rose oil process, were determined.

2. Material and Methods

2.1. Material

In this study, *R. damascena*, *R. centifolia*, *R. alba*, *R. alba* 'Semiplena' oil rose genotypes grown in Atatürk Horticultural Central Research Institute (Yalova) were used. The flowers of the oil rose genotypes were collected in the early morning hours (between 07:00 and 08:00) in two different periods on the dates which were specified in Table 1. The performances of rose genotypes under Yalova conditions were investigated during two different periods. Meteorological data covering these two periods in Yalova conditions were given in Figure 1.

Table 1. Harvest periods of the samples used in the study.

| Genotypes | First period | Second period |
|------------------------------|--------------|---------------|
| <i>Rosa damascena</i> | 23.05.2022 | 08.06.2022 |
| <i>Rosa centifolia</i> | 23.05.2022 | 08.06.2022 |
| <i>Rosa alba</i> | 01.06.2022 | 15.06.2022 |
| <i>Rosa alba</i> 'sempilena' | 01.06.2022 | 15.06.2022 |

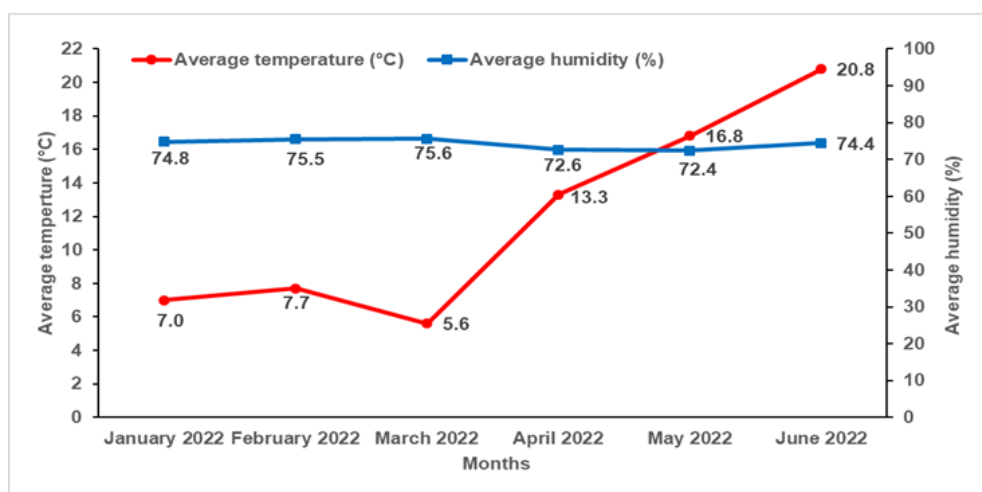


Figure 1. Meteorological data between January and June 2022 in Yalova.

2.2. Methods

In this study, phenolic content and antioxidant activity of the products (flowers, rose water and wastewater) of four different oil rose genotypes were investigated. After drying and powdering the whole rose flowers, phenolic content, antioxidant activity and total flavonoid content were determined by methanol extraction and spectroscopic methods. Rose water and wastewater were obtained by hydrodistillation method, phenolic contents were obtained by solvent extraction and total phenolic matter and antioxidant activity contents were analyzed (Figure 2).

2.2.1. Obtaining rose water and wastewater

200 g of fresh rose flowers were filled into the 5 L balloon of the Clevenger hydro-distillation unit and 2 L of distilled water was added into the mixture. The hydro-distillation process was continued for 3 hours after the water in the distillation flask started to boil with a jacketed heater. At the end of distillation, essential oil (rose oil) and aromatic water (hydrosol) accumulated under the oil were obtained as rose water. The wastewater was obtained by squeezing the remaining pulp in the hydro-distillation flask (Baydar and Baydar, 2017).

2.2.2. Extraction of rose flowers

Rose flowers were dried in a tray dryer (at 40°C) until the water content decreased below 5%, and

3 g were taken from the powdered samples, homogenized with 100 ml of pure methanol for 2 minutes and kept at room temperature for 24 hours. The solvent extract mixture was filtered through Toyo N. 2 filter paper, the solvent was removed in a rotary evaporator at 37°C until 50 ml of methanol remained. The resulting extract was stored at -18°C until analysis (Li et al., 2014).

2.2.3. Phenolic extraction of rose water and rose wastewater

60 mL of rose water and rose wastewater were mixed separately with 30 ml of 5% sodium bicarbonate and 60 mL of ethyl acetate in a vortex for 2 minutes. After separating the ethyl acetate phase, ethyl acetate was evaporated in a rotary evaporator and the remaining residue was dissolved in methanol and then filtered and used in the analysis (Baydar and Baydar, 2013).

2.2.4. Total phenolic analysis

It was measured spectrophotometrically by the Folin-Ciocalteu method 2400 µL of distilled water and 150 µL of 0.25 N Folin-Ciocalteu solution were added to 150 µL of extract and mixed in vortex for 3-4 minutes. 300 µL of sodium carbonate (Na₂CO₃) (1 N) was added to this mixture and left for 2 hours at room temperature, then the absorbance of the samples was measured at 725 nm wavelength in a spectrophotometer (Hitachi, model). Total phenolic content (mg GAE 100 g⁻¹) was calculated from the

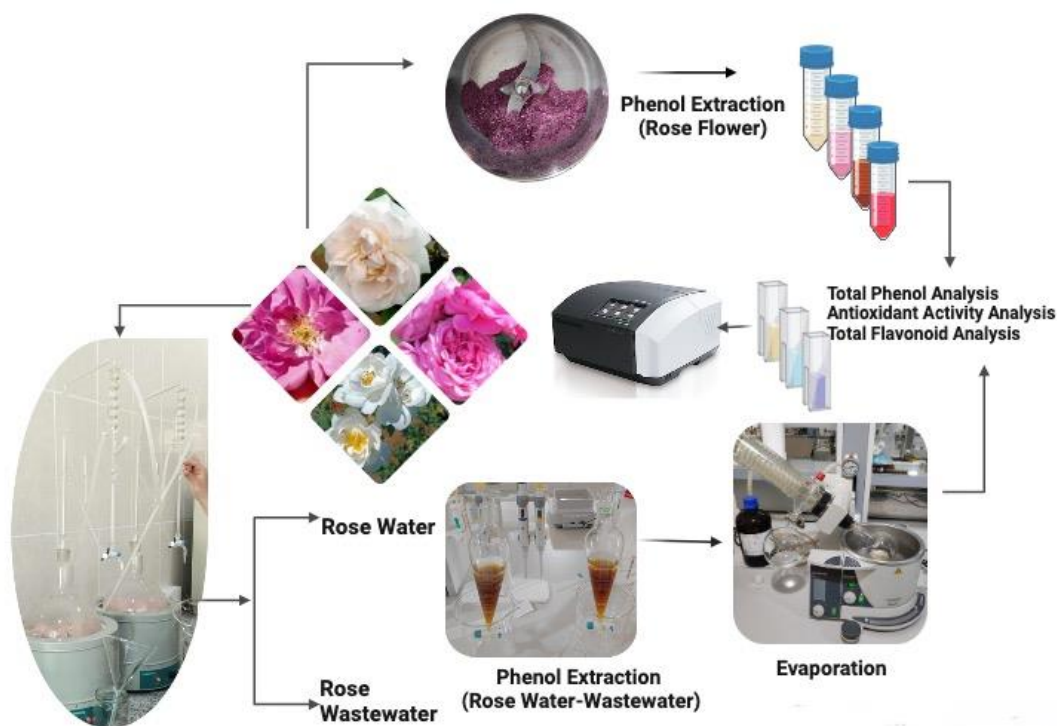


Figure 2. Flow chart examining the phenolic flavonoid and antioxidant properties of rose flowers rose water and rose wastewater.

calibration curve using a standard of gallic acid prepared at different concentrations (Thaipong et al., 2006).

2.2.5. DPPH antioxidant activity

The antioxidant activities of the samples were analyzed by applying the DPPH (2,2-diphenyl-1-picrylhydrazil) method. Stock solution; 0.12 mg of DPPH was weighed and dissolved in a 50 mL flask and stored at -20°C. Working solution; by adding 45 mL of methanol to 10 L of stock solution, an absorbance value of 1.1 ± 0.02 was obtained at 515 nm wavelength in the spectrophotometer. 2850 μ l of DPPH solution was added to 150 μ l of extract taken from the samples prepared before, stored at -20°C and kept in the dark for 24 hours. Measurements were made with a spectrophotometer at a wavelength of 515 nm. DPPH was calculated from the calibration curve obtained with the trolox standard (Thaipong et al., 2006).

2.2.6. Copper Reducing Antioxidant Capacity (CUPRAC)

Copper Reducing Antioxidant Capacity (CUPRAC) analysis was performed using the method of Apak et al. (2004). To 100 μ l of the extract, 1 mL 10 mM CuCl_2 , 1 mL 7.5 mM neocuproin and 1 mL 1M NH_4Ac (pH:7) were added respectively. 1 mL of distilled water was added immediately to the mixture to give a final volume of 4.1 mL. After incubation at room temperature for 60 min, absorbance values at 450 nm were determined. The CUPRAC antioxidant activity value was determined according to the calibration curve against a blank reagent with trolox standard prepared at different concentrations.

2.2.7. Total flavonoid analysis

4 mL of distilled water and 0.3 mL of 5% NaNO_2 were added to 1 ml of extract and then mixed. After

5 minutes, 0.6 mL of 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was added, after 5 minutes, 2 mL of 1 mol L^{-1} NaOH is added, the total volume was made up to 10 mL with distilled water. Readings were made at a wavelength of 510 nm. Total flavonoid contents were calculated using (+)-catechin standard calibration curve (Karadeniz et al., 2005).

2.2.8. Statistical analysis

All of the trials and analyses were repeated three times for each sample. Random plots design was used in the study. Analyzes were made using the JMP statistical package program. The significance value was taken as $p < 0.05$. And, results were given as mean \pm standard deviation.

3. Results and Discussion

The values of total phenolic matter and antioxidant activities (DPPH and CUPRAC) determined in rose flowers are given in Table 2. The highest total phenolic matter in first period harvested oil rose flowers was determined as 4115 mg GAE 100 g^{-1} in *R. damascena* genotype. In the other genotypes, it was 3529 mg GAE 100 g^{-1} in *R. centifolia*, while there was no statistical difference between *R. alba* 'Semiplena' (2064 mg GAE 100 g^{-1}) and *R. alba* (1888 mg GAE 100 g^{-1}). In the first harvest period, the highest DPPH antioxidant activity was found as 4893 mg TE 100 g^{-1} in *R. damascena* genotype, while there was no statistical difference between the other genotypes. As for CUPRAC antioxidant activity, the highest values were found in *R. centifolia* (34237 mg TE 100 g^{-1}) and *R. damascena* genotypes (27539 mg TE 100 g^{-1}), respectively. There was no statistical difference between *R. alba* 'Semiplena' (7422 mg TE 100 g^{-1}) and *R. alba* (6496 mg TE 100 g^{-1}). In the second harvest period, total phenolic content, DPPH and CUPRAC antioxidant activity was found in *R. centifolia* 3218 mg GAE 100 g^{-1} , 4860 mg GAE 100 g^{-1} , and

Table 2. Total phenolic content and antioxidant activity of flowers of rose genotypes harvested at different periods.

| Periods | Genotypes | Total phenolic content (mg GAE 100 g^{-1}) | DPPH* Antioxidant activity (mg TE 100 g^{-1}) | CUPRAC Antioxidan capacity (mg TE 100 g^{-1}) |
|-----------------------------|----------------------------|--|---|---|
| Rose flower (First period) | <i>R. damascena</i> | 4115 \pm 33 a | 4893 \pm 56 a | 27539 \pm 1257 b |
| | <i>R. centifolia</i> | 3529 \pm 28 b | 4147 \pm 76 b | 34237 \pm 910 a |
| | <i>R. alba</i> | 1888 \pm 95 c | 4068 \pm 60 b | 6496 \pm 408 c |
| | <i>R. alba</i> 'Semiplena' | 2064 \pm 119 c | 4068 \pm 60 b | 7422 \pm 961 c |
| | CV | 2.75 | 1.49 | 4.95 |
| Rose flower (Second period) | <i>R. damascena</i> | 2196 \pm 22 b | 4169 \pm 18 b | 8576 \pm 881 b |
| | <i>R. centifolia</i> | 3218 \pm 47 a | 4860 \pm 19 a | 14863 \pm 414 a |
| | <i>R. alba</i> | 1991 \pm 53 c | 4077 \pm 13 b | 7288 \pm 370 b |
| | <i>R. alba</i> 'Semiplena' | 2094 \pm 150 bc | 3915 \pm 93 c | 8008 \pm 1137 b |
| | CV | 3.53 | 1.23 | 7.96 |

* DPPH: 2,2-diphenyl-1- picrylhydrazil CUPRAC: Copper Reducing Antioxidant Capacity.

Each value in the table was obtained by calculating the average of three analysis \pm standard deviation.

14863 mg GAE 100 g⁻¹, respectively. In the second period, phenolic contents were 2196 mg GAE 100 g⁻¹ in *R. damascena*, 2094 mg GAE 100 g⁻¹ GAE in *R. alba* 'Semiplena', 1991 mg GAE 100 g⁻¹ in *R. alba* for flower parts. There was no statistical difference between *R. damascena* (4169 mg GAE 100 g⁻¹) and *R. alba* (4077 mg GAE 100 g⁻¹) genotypes in terms of DPPH antioxidant activity. The lowest DPPH antioxidant activity (3915 mg GAE 100 g⁻¹) was detected for *R. alba* 'Semiplena' genotype. In terms of second period CUPRAC antioxidant activity, no statistical difference was detected between *R. damascena*, *R. alba*, *R. alba* 'Semiplena' genotypes. When the harvest period was compared, *R. damascena* genotype showed a significant decrease in total phenolic matter content and CUPRAC antioxidant activity content, while no significant changes were found in other genotypes.

Some research has focused on the antiradical and antioxidant activities of rose compared to other plants. Vinokur et al. (2006) analyzed hot water infusions (teas) of dried petals of twelve rose varieties for antioxidant activity. Phenolics, total anthocyanins and high antioxidant capacity of rose petal tea can be used as a caffeine-free beverage, consumed separately or in combination with other herbal ingredients. In a study investigating the total phenolic matter and antioxidants of fresh and dried flowers, flowers and green leaves of *R. damascena* genotype were dried and powdered, and then hot and cold extractions were carried out with methanol. While hot extractions yielded more extracts, cold extractions yielded higher total phenolic matter, flavanol and flavonol contents. The highest values for total phenolic matter were 478.34 mg GAE g⁻¹ and 530.40 mg GAE g⁻¹ in hot and cold extraction of leaves, respectively. The phenolic content of petals of 9 different rose genotypes were analyzed by Khurshid et al. (2018), and was found between 39.10-91.19 mg GAE g⁻¹. *Rosa moschata* was found to have the highest phenolic content, while *R. hybrida* (pink-yellow) had the lowest. In our study, total phenolic matter and antioxidant activities of rose flowers were found to be high by reported Khurshid et al. (2018) and Vinokur et al. (2006).

White rose petals (*Rosa* spp.) can be used in the treatment of allergic diseases due to their antioxidant effect. For this reason, the extraction of white rose petals was optimised for the independent variables of ethanol concentration, extraction

temperature, and extraction time. Predicted response values for the phenolic and flavonoid contents were 243.50 mg gallic acid equivalent/g dry mass and 19.93 mg catechin equivalent, CE g⁻¹ dry mass, respectively (Choi et al., 2015). Total phenolics values of white rose petals of *Rosa alba* and *R. alba* 'Semiplena' were found between 1888-2094 mg GAE 100 g⁻¹. Total flavonoid contents of *Rosa alba* and *R. alba* semiplena were 1401.0-1899.3 mg quercetin kg⁻¹. It was observed that the total phenolic substance contents agreed between similar values, but the difference between the total flavonoid contents is thought to be due to the fact that the same equivalent standard material was not used. In a similar study, they were collected early in the morning (from May to early July 2017, depending on the flowering time of each accession) from nine cities of Western and Eastern Azerbaijan. The percentage and composition of essential oil, total phenols, flavonoids, anthocyanins, anthocyanins, carotenoids and antioxidant capacity of 24 Damask rose accessions were investigated. The highest total phenolic content was 165 mg GAE g DW⁻¹ and flavonoid content was 81 mg quercetin g DW⁻¹). The antioxidant activities of the samples were determined using DPPH free radical scavenging activity and ferric reducing antioxidant power (FRAP) test. All the accessions (4-12 µg ml⁻¹) had lower IC₅₀ values than ascorbic acid (18 µg ml⁻¹). In the FRAP assay, they had high antioxidant activity in the range of 10-25 µmol Fe^{+2/g} DW. Based on the high and valuable bioactive compound source of Damask rose, elite accessions have potential to be used in cultivation and food applications (Alizadeh and Fattahi, 2021).

It was observed that rose flowers have the potential to be used in many fields as a natural antioxidant source. Table 3 shows the flavonoid values determined in the rose flowers of oil roses, flavonoids were not detected in the studies conducted in rose water. The highest flavonoid content was obtained in *R. damascena* genotype 3062.05 mg quercetin kg⁻¹ in the first harvest period, while *R. centifolia* 2805.45, *R. alba* 'Semiplena' 1899.30 and the lowest *R. alba* 1401 mg kg⁻¹. The second period flavonoid contents were highest in *R. centifolia* genotype, there was no statistical difference between the total flavonoid contents of other oil rose genotypes.

Khurshid et al. (2018) examined the total flavonoid content of nine different rose petals. They

Table 3. Total flavonoid content (mg quercetin equivalent kg⁻¹) of flowers of rose genotypes.

| Genotypes | Rose flower (First period) | Rose flower (Second period) |
|------------------------------|----------------------------|-----------------------------|
| <i>Rosa damascena</i> | 3062.05±36.27 a | 1247.50±35.23 b |
| <i>Rosa centifolia</i> | 2805.45±9.54 b | 1851.00±23.58 a |
| <i>Rosa alba</i> | 1401.00±63.07 d | 1413.13±88.07 b |
| <i>Rosa alba</i> 'Semiplena' | 1899.30±18.36 c | 1409.10±10.89 b |
| CV | 3.01 | 4.47 |

Each value in the table was obtained by calculating the average of three-analysis ± standard deviation.

reported that the total flavonoid content varied between 3.91-8.04 mg g⁻¹ quercetin equivalents. The highest total flavonoid content was determined in *R. moschata* genotype, while the lowest was determined in *R. hybrida* (pink yellow) genotype. In another study, the flavonoid content of methanolic rose extracts was found to vary between 3.6-23.7 mg g⁻¹ (Li et al., 2014). It was observed that the total flavonoid content of rose genotypes in the literature was compatible with the results of our study.

In our study, the total phenolic matter and antioxidant activity (DPPH and CUPRAC) values of rose water and wastewater, which were obtained as by-products of essential oil extraction from oil roses by hydrodistillation method, in two harvest periods are given in Table 4. In rose water, the highest total phenolic matter in *R. damascena* genotype was 4.92 mg GAE L⁻¹ in the 1st period, 4.82 mg GAE L⁻¹ in the second period, DPPH antioxidant activity was 3.18 mg TE L⁻¹ in the first period, 0.71 mg TE L⁻¹ in the second period, CUPRAC antioxidant activity was 24.56 mg TE L⁻¹ in the first period, 4.20 mg TE L⁻¹ in the second period. Total phenolic matter, DPPH antioxidant activity and CUPRAC antioxidant activity values were found to be very low in the first and second period rose waters of other oil rose genotypes and no statistical difference was found. By-products or residues of agricultural industries have attracted more attention in recent years as they are valuable sources of natural antioxidants. Taif rose water by-product obtained after hydrodistillation of Taif rose (*Rosa damascena* trigintipetala Dieck) was investigated for its biological and phytochemical properties. According to these results, it was found to have

SC50= 23.72±0.36 µg ml⁻¹ against DPPH radical. Direct infusion ESI (-and)-MS analysis of Taif rose water by-product showed the presence of the following substances: phenolic compounds belonging to hydrolyzable tannins and flavonoids. Acute, sub-chronic and chronic toxicity studies of taif rose water by-product on mice have shown that it is safe and non-toxic (Abdel-HameEd et al., 2015). From this study, it can be concluded that rose water has antioxidant activity and has the potential to be used safely due to its non-toxicity. In another study, the activities of cream formulations obtained by using different concentrations of rose water were examined. In this study, it was stated that rose water was helpful in reducing inflammation due to its polyphenolic substance content. It was determined that increasing the concentration of rose water increases antioxidant and anti-inflammatory activities (Safia et al., 2019).

In rose wastewater, the highest total phenolic content was 112.36 mg GAE 100 g⁻¹ in *R. alba* genotype and the lowest was 16.91 mg GAE 100 g⁻¹ in *R. centifolia*. The highest DPPH antioxidant activity value was determined in *R. alba* (87.73 mg TE 100 g⁻¹) and *R. alba* 'Semiplena' (89.41 mg TE 100 g⁻¹), *R. damascena* (85.76 mg TE 100 g⁻¹).

In CUPRAC antioxidant activity, the highest value of *R. damascena* was 726.69 mg TE 100 g⁻¹. *R. alba* 'Semiplena' 686.66 mg TE 100 g⁻¹, *R. alba* 364.31 mg TE 100 g⁻¹, while the lowest values were found in *R. centifolia* 184.26 mg TE 100 g⁻¹. In rose wastewater, *R. centifolia* showed the highest total phenolic matter (178.58 mg GAE 100 g⁻¹), DPPH antioxidant activity (58.02 mg TE 100 g⁻¹) and CUPRAC antioxidant activity (1933 mg TE 100 g⁻¹)

Table 4. Total phenolic and antioxidant content of the rose waters and wastewater of rose genotypes.

| Rose water and wastewater | Genotypes | Total phenolics (mg GAE L ⁻¹) | DPPH* Antioxidant activity (mg TE L ⁻¹) | CUPRAC Antioxidant activity (mg TE L ⁻¹) |
|------------------------------------|----------------------------|--|---|--|
| Rose water (First period) | <i>R. damascena</i> | 4.92±0.60 a | 3.18±0.78 a | 24.56±3.12 a |
| | <i>R. centifolia</i> | 0.09±0.04 b | 1.11±0.00 b | 2.07±0.98 b |
| | <i>R. alba</i> | 0.00±0.00 b | 0±0.00 b | 0.01±0.00 b |
| | <i>R. alba</i> 'Semiplena' | 0.00±0.00 b | 1.11±0.00 b | 0.00±0.01 b |
| | CV | 2.30 | 4.80 | 2.40 |
| Rose water (Second period) | <i>R. damascena</i> | 4.82±0.49 a | 0.71±0.53 a | 4.20±0.25 a |
| | <i>R. centifolia</i> | 0.13±0.00 b | 0.00±0.00 | 1.50±0.76 b |
| | <i>R. alba</i> | 0.086±0.00 b | 0.00±0.00 | 1.01±0.43 b |
| | <i>R. alba</i> 'Semiplena' | 0.00±0.05 b | 0.00±0.00 | 0.84±1.31 |
| | CV | 9.04 | 10.04 | 1.75 |
| Rose wastewater (First period) | <i>R. damascena</i> | 86.71±0.92 b | 85.76±6.80 a | 726.69±0.00 a |
| | <i>R. centifolia</i> | 16.91±0.04 c | 3.71±0.62 b | 184.26±4.06 d |
| | <i>R. alba</i> | 112.36±11.82 a | 87.73±10.15 a | 364.31±22.36 c |
| | <i>R. alba</i> 'Semiplena' | 68.45±15.97 b | 89.41±1.24 a | 686.66±13.20 b |
| | CV | 9.97 | 9.21 | 3.17 |
| Rose wastewater (Second period) | <i>R. damascena</i> | 87.80±0.06 b | 0±0.00 | 1542±33.49 b |
| | <i>R. centifolia</i> | 178.58±0.13 a | 58.02±2.76 | 1933±4.92 a |
| | <i>R. alba</i> | 68.76±1.48 d | 0±0.00 | 366±7.69 d |
| | <i>R. alba</i> 'Semiplena' | 81.15±1.48 c | 0±0.00 | 1265±14.19 c |
| | CV | 0.91 | 9.80 | 1.50 |

* DPPH: 2,2-diphenyl-1-picrylhydrazil CUPRAC: Copper Reducing Antioxidant Capacity.

Each value in the table was obtained by calculating the average of three analysis ± standard deviation.

values in the second harvest period in contrast to the first harvest period. In the second harvest period, the total phenolic matter contents of the wastewater were 87.80 mg GAE 100 g⁻¹ in *R. damascena*, 178.58 mg GAE 100 g⁻¹ in *R. centifolia*, 81.15 mg GAE 100 g⁻¹ in *R. alba* 'Semiplena', and 68.76 mg GAE 100 g⁻¹ in *R. alba*, respectively. DPPH antioxidant activity values of second period wastewater were not detected in genotypes other than *R. centifolia*. CUPRAC antioxidant activity values were determined as *R. centifolia* 1933 mg TE 100 g⁻¹, *R. damascena* 1542 mg TE 100 g⁻¹, *R. alba* 'Semiplena' 1265 mg TE 100 g⁻¹ and *R. alba* 366 mg TE 100 g⁻¹, respectively.

4. Conclusion

In our study, total phenolic matter, total antioxidant activity (DPPH and CUPRAC) and total flavonoid contents of whole flowers, rose water and wastewater of *R. damascena*, *R. centifolia*, *R. alba*, *R. alba* 'Semiplena' oil rose genotypes at two different harvest periods were determined in the climatic conditions in Yalova. There were differences in phenolic content and antioxidant activity content of oil rose genotypes according to harvest period. *R. damascena* showed higher total phenolic matter and antioxidant activity in the first harvest period, while *R. centifolia* showed an increase in total phenolic matter and antioxidant activity in the second harvest period.

In our study, total phenolic content and antioxidant activity values of dried flowers of rose genotypes were found to be higher than rose water and wastewater. While it was determined that rose flower has the potential to be used as an important antioxidant source, total phenolic matter and antioxidant activity values of rose water and wastewater were found to be low. Despite the low total phenolic matter and antioxidant activity values, the fact that rose water and wastewater can be supplied at lower costs compared to rose flower. Lower cost can still make them an important resource for the cosmetic industry. Total phenolic matter and antioxidant activity values of rose water were very low in both periods. Only in *R. damascena* genotype, a small amount of total phenolic matter and antioxidant activity values were detected. In wastewater, harvest period had a significant effect on phenolic content. Phenolic contents of the wastewater were found to be the highest in the second period of *R. centifolia*, while the first period phenolic contents of *R. alba* genotype were found to be high. Rose wastewater has much higher phenolic content and antioxidant activity than rose water. The rose genotype with the highest phenolic content as an alternative to *R. damascena* was determined as *R. centifolia* genotype. Similar to this study, it is important to determine the contents of by-products such as rose

water and wastewater, along with antioxidants in rose, with future studies. In this way, by-products of rose industry will be evaluated more and resource waste will be reduced.

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