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RELATIONSHIP BETWEEN VITAMIN AND ANTIOXIDANT ACTIVITIES OF ROSEHIP SPECIES GROWN IN THE SAME ECOLOGICAL CONDITIONS

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ABSTRACT

The realisation of the high chemical potential of the fruits of Rosa species, which were initially used for seasonal consumption and in the treatment of a wide range of ailments, led to an increase in consumption and research into the possibilities for use. In order to determine the usage possibilities of these fruits and their various chemical properties, it is necessary to know about the antioxidant activity, the amount of vitamins, phenolic and flavonoid substances within rose species. In this study, naturally growing ecological friendly fruits of *Rosa villosa* L. subsp. *mollis* (R1), *Rosa villosa* L. subp. *villosa* L. (R2), *Rosa pimpinellifolia* (R3), *Rosa iberica* (R4), *Rosa pisiformis* (R5) and *Rosa canina* (R6) were investigated with respect to their vitamin values (A, E and C vitamins), total phenolic (TPC), flavonoid (TFC) content, and antioxidant potentials. The correlation ratios of these properties were also checked during this study. The recent study showed that the highest amount of TPC and TFC were 142.08±2.16 mg GAE/g, 8.04±0.47 mg QE/g, respectively in R1, and the highest vitamin values were determined which were vitamin A at 397.17±13.58 µg/mL in R5, Vitamin E at 19.52±0.82 µg/mL in R4 and vitamin C at 606.53±0.38 µg/mL in R1. DPPH, FRAP and CUPRAC methods, which are reliable methods, were used to determine the antioxidant potential. The highest antioxidant potential was measured in R1 by DPPH and FRAP methods. In R3, it was found that the Cu^{2+} reduction antioxidant activity was the highest with the CUPRAC method. In addition, it was understood that the correlation analysis among the determined characteristics of the species was statistically significant. There was a high positive correlation between TFC and vitamin E value in R1 while this postive relationship was also found between TPC and E in R2. As a result, although significant quantitative differences were detected between Roseship species in this study, it was determined that the antioxidant potentials and vitamin values of all species were high. The results showed that the important the fruits of these species would be used for human nutrition and health. In addition, the antioxidant potential and vitamin C value of *Rosa villosa* L. subsp. *mollis* (R1) were found to be very high and it was concluded that the fruits of this species would be more suitable for use in the pharmaceutical, functional food and cosmetic industries. It can be suggested that the further studies should be conducted in order to spread the consumption of fruits belonging to these species.

Keywords: Rosaceae, correlation, antioxidant activity, vitamin A, vitamin E, vitamin C

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INTRODUCTION

Understanding the important relationship between nutrient and health has increased interest amoung customers in natural food sources. Wild fruits are, also, affected by this interest. Thus, their consumption and recognition of natural fruits increase. In addition, the bioactive substances in these fruits have contributed to the intensification of research in this direction (Barros *et al*., 2011). As a result of these studies, it is understood that the majority of wild fruits are rich in phytochemical structure and antioxidants (Skender *et al*., 2020). Rosehip species has an important place among wild fruits. Rosehip is a plant in the form of a bush that sheds its leaves in winter, belonging to the genus Rosa from the Rosaceae family (Tabaszewska and Najgebauer-Lejko 2020). The fruits, which are rich in beneficial components and delicious, reach maturity in autumn. These fruits have high physiological activities due to their rich phytochemical contents. In addition, Rosehip berries are considered a valuable source of polyphenols and ascorbic acid (Chrubasik *et al*., 2008; Rovná *et al*., 2020).

Phenolic and flavonoid biochemicals are at the beginning of the valuable polyphenols found in Rosehip fruit. Fruits have a very high biochemical activity due to the abundant polyphenols in their structures (Ercisli, 2007). Another important nutrient in Rosehip fruit is vitamins. The most important of these are vitamin C, carotenoids and tocopherol (Gao *et al*., 2000; Valnet,

2015). Besides antioxidant and anti-inflammatory effects of these fruits, they have antibacterial, antimutagenic, osteoarthritis, rheumatoid arthritis and anticarcinogenic effects (Fan *et al*., 2014; Gruenwald *et al*., 2019; Gulbagca *et al*., 2019). Also, some researchers report that it has metabolism-regulating, modulating and treatment-enhancing effects (Khojasteh Banan *et al*., 2015).

Worldwide, there are 200 Rosehip species belonging to the "*Rosaceae*" family. More than 30 of these species are present in the flora of Turkey (Ercisli, 2005). Especially in the Eastern Black Sea Region of Türkiye, 17 species show natural distribution (Öz *et al*., 2018). The region where the plant samples use in the study, are supplied is the Olur region, located in the south of the Eastern Black Sea Region of Turkey. This region is one of the important regions where rosehip species are naturally spread (Fig. 1). In addition to the diversity of the species growing in the region, more than one species can be found in the same environment.

Rosa pimpinellifolia L. (Black Fruit Rosehip) is, also, called Black Fruit Rosehip. The height of this plant can reach up to 1 m. It blooms in June and July. It grows at an altitude of 1200-2750 m. Its fruits are purplish black, spherical, flattened from the side and hairless (Öz *et al*., 2018; Pashazadeh *et al.*, 2021). *Rosa canina L.* is 150-350 cm is in height. This species is present almost in every region of Türkiye. It is usually used for making tea and molasses (Öz *et al*., 2018; Pashazadeh *et al.*, 2021).

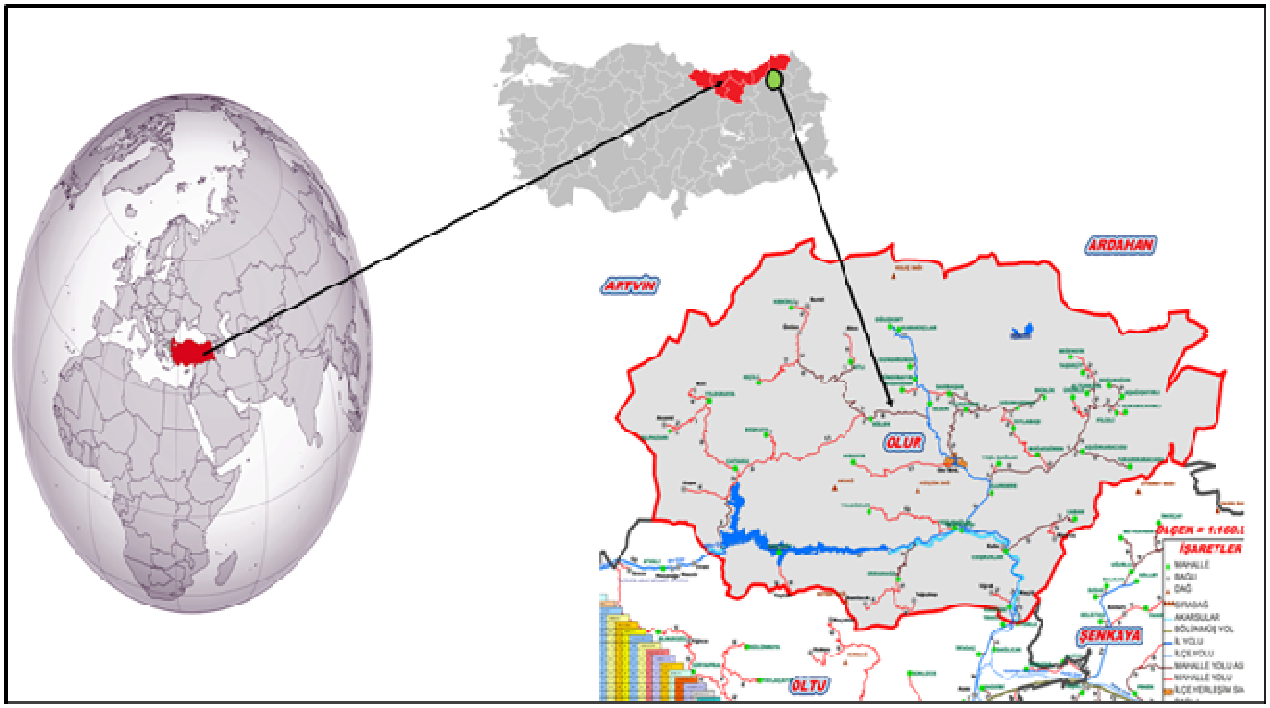

Figure 1. Erzurum Olur region of Türkiye

Rosa pisiformis (Christ) D. is, the only Rosa species, known endemic to Türkiye. They reach up to 2 meters in length. It grows at 1600–2000 m. It is used for decorative purpose (Ercisli, 2005; Hatipoglu *et al.,* 2021). *Rosa iberica* Stev. Bieb., can reach up to 200 cm in height. It grows at an altitude of 1200–2400 m (Nilsson, 1997; Ercisli, 2005). *Rosa villosa* L. subsp. *villosa* grows on rocky slopes, bushes and meadows at an altitude of 1300-2500 m. It blooms in June and July (Korkmaz *et al.,* 2013). *Rosa villosa* L. subsp. *mollis* (Sm.) Keller Gams grows on rocky slopes, bushes and meadows at an altitude of 1300-2500 m. It blooms in June and July. It has a unique aroma (Korkmaz *et al.,* 2013).

In this study; it was aimed to determine the comparative antioxidant capacities and vitamin status of the fruit extracts of rosehip species. In addition, it was requested to investigate whether there is a relationship between the antioxidant capacity and the amount of polyphenols specific to the species. In the literature review, no similar studies were found, except for a few species. This study is one of the rare studies using more than one rosehip species naturally distributed in the same region. In this study, fruits belonging to *Rosa villosa* L. subsp. *mollis*, *Rosa villosa* L. subsp. *villosa* L., *Rosa pimpinellifolia*, *Rosa iberica*, *Rosa pisiformis* and *Rosa canina* species were used. Antioxidant capacities, phenolic and flavonoid and vitamin A, E, C contents of these fruits were determined. In addition, Pearson analysis was applied to determine the correlation between the features detected in the study and to investigate the relationships between them.

MATERIALS AND METHODS

Plant material: Fruit samples of *R. villosa* L. subsp. Mollis, *Rosa villosa* L. subp. villosa L, *Rosa pimpinellifolia*, *Rosa iberica*, *Rosa pisiformis* and *Rosa canina* species were collected from the location (40°49'49°N'42°07'54″E 1318 m / 40°49'47°N'42°07'54″E 1321 m / 40°49'48°N'42°07'56″E 1311 m/ 40°49'51°N'42°07'55″E 1318 m) in Erzurum Olur region of Türkiye in September 2022. The fact that the species we used in the study were found in the same environment and natural distribution influenced the preference for this region. By choosing the same region, the effect of environmental factors such as altitude, climatic conditions and soil properties, which had an impact on the phytochemicals of the plants, were tried to be equalized among the species. Collected fruit samples were washed with water and purified from physical dirt. It was, then, air dried. It was stored in the refrigerator at +4 ^{0}C.

Preparation of the plant extract: Samples were pulverized with the help of a grinder. Then, 10 g plant sample was weighed and methanol was added on it 200 mL (Ergün, 2022). It was mixed overnight under room conditions with the aid of a magnetic stirrer. The mixture was filtered to separate the extract. Methanol was added to the remaining precipitate and stirring was continued for a while. Afterwards, it was filtered again and the extract parts were combined. Concentrated extract was obtained by removing the solvent in the extracts in the evaporator at 45°C. A solution of 1000 ppm concentration was prepared from the obtained extracts and used in this trial (Ergün, 2021).

Determination of Total Phenolic Substance: The amount of phenolic substances in plant samples was determined using the Folin-Ciocaltaeu method (Slinkard and Singleton, 1977). The standard curve was obtained with gallic acid, and the result was expressed as mg gallic acid equivalent per gram extract (mg GAE/g extract). Briefly; 0.1 mL of extract was taken and the volume was made up to 1.840 mL with distilled water. 0.01 mL of Folin-Ciocaltaeu reagent was added to it. After 3 minutes of incubation at room temperature, 2% Na_2CO_3 solution was added. It was incubated for 2 hours at room temperature and absorbases were measured at 760 nm against a blank. Each extract was run in 3 replicates.

Determination of Total Flavonoids: Total flavonoid contents were determined by the aluminum nitrate method (Nieva Moreno *et al.,* 2000). Briefly, 0.1 mL of extract was taken and the volume was made up to 1.92 mL with methanol, and, then, 0.04 mL of KCH_3COO (1 M) was added. After 1 min, 0.04 mL $Al(NO_3)_3$ (10%) was added, and incubated for 40 min. Absorbances were measured at 415 nm. Measurements were made in 3 replicates. A standard graph was obtained with quercetin, and the results were expressed as mg quercetin equivalent per gram extract (mg QE/g extract).

Antioxidant Activity assessment

Free Radical Removal Activity (DPPH): DPPH radical removal activity was performed according to the method of Blois (1958). BHT (2,6-di-t-butyl-1-hydroxytoluene), trolox and α-tocopherol were used as standards. Different volumes (0.02, 0.04, 0.06 and 0.08 mL) were taken from the extracts prepared as 1000 ppm, and the total volume was completed to 0.4 mL with methanol. 1.6 mL of 0.1 mM DPPH solution was added, and incubated for 30 minutes in dark at room temperature. The absorbance changes of the samples were measured against methanol at 517 nm. DPPH radical removal activity was determined using the equation (%)=[(A_0 – A_1 / A_0) × 100]. In the formula, A_0 is the absorbance of control reaction, A_1 is the absorbance of the tested extract sample/standard solutions.

Ferric Reducing Power (FRAP): Ferric reducing capacity of plant samples was determined according to method of Oyaizu (1986). From the extract solutions (1000 ppm) to be measured, 20-100 µg/ml were taken into tubes, and volume was completed to 1 mL with distilled water. 2.5 mL of 0.2 M pH: 6.6 phosphate buffer and $K_3Fe(CN)_6$ (1%) solution were added to them. The tubes were kept in a water bath (50°C) for 20 min. 2.5 mL of TCA (10%) was added, and vortexed. From these tubes, 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%) were added onto the 2.5 mL mixture. Then, the absorbance against the blank was measured at 700 nm. BHT, Trolox and α-tocopherol were used as standard to compare the ferric reducing powers of the samples.

Copper (II) Reducing Capacity (CUPRAC): The Cu^{2+} reducing capacity of plant extracts was determined according to Apak *et al*. (2004). For this, 1 mL of $CuCl_2$, Neokuprine and NH_4CH_3COO solutions were each taken into 5 different tubes. 10-50 µL of the extract solution was added to them. Then, distilled water was added to give a total volume of 4 mL. After 30 min incubation, the absorbances against blank were measured at 450 nm. BHT, trolox and α-tocopherol were used as standards.

Determination of Vitamin A: Vitamin A was determined according to the method developed by Prasad *et al*. (1995). This method is based on the fact that vitamin A forms a colored compound with 4-hydroxy-3-methyl benzaldehyde and the absorbance of the resulting compound shows maximum absorbance at 610 nm. For this purpose, standard solutions of β-carotene were prepared in the concentration range 50-300 µg/mL. 100 µL of each of these standard solutions were taken and placed in separate tubes and the volume was made up to 1 mL with ethanol. 0.5 ml of 4-hydroxy-3-methoxy

benzatedehyde (0.5% w/v) and H_2SO_4 were added to the tubes and incubated for 5 minutes. Then, isopropanol was added to the mixture and its absorbance was measured at 610 nm against blank. A standard graph was obtained using absorbance values. 100 mL of plant extracts were taken, and the absorbance was determined at 610 nm by applying the same procedure. Using the standard graph, vitamin A values were determined as µg/mL extract.

Determination of Vitamin E: First, 50-300 µL of the standard solution prepared from α-tocopherol was taken into tubes and 0.5 mL of tetrazolium blue (0.01 M) was added. 5 mL of NaOH (0.2 M) and 5 mL of methanol were added and incubated at 90°C for 10 minutes. Then, the absorbance was determined at 526, and a standard graph was created (Amin, 2001). The same procedure was applied by taking 0.1 mL of plant extracts, and their absorbance was determined at 526 nm. The vitamin E content of the plant extracts was calculated as µg/mL extract using the standard graph.

Determination of Vitamin C: The determination of vitamin C in plant extracts was carried out spectrophotometrically (Optima SP-3000, Tokyo/Japan). The method is based on the reduction and decolorization of 2,6 dichloroindophenol dyestuffs with vitamin C (ascorbic acid) (Güzel and Akpınar, 2019). The absorbance of the dye remaining in the medium at the end of the reaction is determine at 518 nm. The amount of remaining dye and the amount of vitamin C are inversely proportional. Briefly, 0.9 mL of oxalic acid solution (0.4%) was added to the 0.1 mL sample taken from the extract solutions. Then, 9 ml of 2.6 dichloroindophenol solution was added to it. Absorbance was measured at 518 nm against the blank, in which water was used instead of dye solution. By applying the same procedure at different (1-5 mg/100 mL) concentrations of ascorbic acid, absorbance measurements were made and a standard graph was obtained. With the help of the graph obtained, the vitamin C content in the extracts was calculated as mg/100 mL extract.

Statistical Analysis: SPSS 22 V ® statistical package program was used to evaluate the analyis made on 6 Rosehip species (Aşcı and Durmus, 2015). First of all, the data regarding the total phenolic, flavonoid content, antioxidant capacity and vitamin contents of the plant species were evaluated using one-way analysis of variance (ANOVA). Duncan's test was used to determine which species or species caused the difference in the study (Duggan *et al.,* 2017). In addition, Pearson analysis was applied to determine the correlation between 8 biochemical properties of the species. In the study, the level of significance was determined at ($P < 0.05$).

RESULTS AND DISCUSSION

Determination of Total Phenolic Substance: The differences between species were significant in terms of total phenolic content of the rosehip fruit extracts tested in this study ($P<0.05$) (Table1). The amounts of total phenolic content were determined as 142.08±2.16 mg GAE/g in R1, 125.92±4.49 mg GAE/g in R2, 116.77±2.22 mg GAE/g in R3, 96.50±5.89 mg GAE/g in R4, 94.98±2.47 mg GAE/g in R5, and 70.58±6.23 mg GAE/g in R6 group, respectively.

Table 1. Total phenolic and flavonoid contents of Rose species.

Sample	TPC (mg GAE/g)	TFC (mg QE/g)
R1	142.08±2.16^{a}	8.04±0.47^{a}
R2	125.92±4.49^{b}	6.99±0.47^{b}
R3	116.77±2.22^{c}	4.17±0.17^{c}
R4	96.50±5.89^{d}	3.33±0.17^{d}
R5	94.98±2.47^{d}	3.23±0.17^{d}
R6	70.58±6.23^{e}	2.92±0.17^{d}

$^{a-e}$: The same letters within the same coloumn shows no statistical difference between rose species at $P<0.05$ level.
R1 (*Rosa villosa* L. subsp. *mollis*), **R2** (*Rosa villosa* L. subp. *villosa* L.), **R3** (*Rosa pimpinellifolia*), **R4 (***Rosa iberica*), **R5** (*Rosa pisiformis*) and **R6** (*Rosa canina*), **GAE** (Gallic acid equivalent), **QE** (Quercetin equivalent), **TPC** (Total phenolic content), **TFC** (Total flavonoid content).

In the studies conducted on fruits of similar species, the total amount of phenolic compounds were 16.4±0.4 mg GAE/g in *R. pimpinellifolia* (Demir *et al.,* 2021), 186.84±4.11 mg GAE/g in *R. iberica* (Gidik *et al.,* 2019), *R. pisiformis* to 83 mg GAE/g (Yılmaz and Ercisli, 2011), and *R. canina* to 92.19±0.43 mg GAE/g (Çömlekçioğlu *et al*., 2022). When these values were compared with our values, similar amounts were found in *R. pisiformis,* high in *R. pimpinellifolia*, and low in *R. iberica* and *R. canina*. It is thought that this situation may be caused by the difference between species and the difference in growth such as geography, climate and light. It is known that the process of biosynthesis of phenolic structures in plants is affected by drought stress, heavy metals intoxication, light intensity and various environmental factors (Martín Lara *et al.,* 2018; Bistgani *et al.,* 2017; Sharma *et al.,* 2019).

Total Flavonoid substance determination: There were significant differences between species in terms of total flavonoid amounts ($P<0.05$) (Table 1). The highest value was determined as 8.04±0.47 mg QE/g in R1, and the lowest value was determined as 2.92±0.17 mg QE/g in R6. In similar studies conducted on rosehip fruits, flavonoid amounts were 5.2±0.2 mg QE/g in *R. pimpinellifolia* (Demir *et al*., 2021) 5.97 ± 0.12 mg GAE/g in *R. iberica* (Tepe *et al.,* 2011), in *R. canina*. It

was reported to be between 0.08 and 2.03 mg QE/g (Rovná *et al.,* 2020). The total flovonoid activity values of the species identified in this study were higher than the reported values. It was concluded that this situation may have resulted from differences in growing conditions and species-specific differences.

Free Radical Removal Activity (DPPH): The % DPPH removal activities of the samples were calculated, and given in Table 2. The standard substances BHT, trolox and α-tocopherol were used to compare their radical removal activities. In the measurements of Rosehip extracts in the concentration range of 20-80 µg/mL, radical removal activities increased in parallel with the increase in concentration. Although there was an increase in all species used in the study, the highest increase in activity was observed in R1 species. At the highest concentration tested, BHT was decreased by 73%, trolox by 96% and α-tocopherol by 95%, while the R1 species showed high radical removal activity by reducing it by 66%. In the R6 species, the antiradical activity was the lowest with 18.62% reduction of DPPH radical at the highest concentration. When examined at lower concentrations, the most active extract always belonged to the R1 species among other species. The concentration at which half of the DPPH radical was reduced was calculated as IC_{50}. There was an inverse relationship between the IC_{50} and DPPH radical removal activity. A low IC_{50} value indicated a strong radical removal activity (Table 2).

Table 2: DDPH radical removal activities and IC_{50} values of Rose species and standards.

Sample	DPPH (% inhibition)				IC_{50} (µg/mL)
	20 µg/mL	**40 µg/mL**	**60 µg/mL**	**80 µg/mL**	
R1	19.88±0.32^{d}	31.15±1.54^{c}	52.24±1.12^{c}	66.09±5.03^{c}	60,69±0,94^{d}
R2	3.95±0.27^{f}	14.4±1.05^{e}	15.66±0.50^{f}	31.25±1.50^{e}	113,01±9.74^{c}
R3	7.79±0,57^{e}	17.71±0.51^{d}	30,25±2.69^{d}	36.20±0.64^{d}	127,51±6.72^{bc}
R4	7.69±0.15^{e}	11.16±0.53^{fg}	13.89±2.06^{fg}	22.33±0.84^{f}	133,06±19.25^{b}
R5	0.16±0.07^{h}	13.68±0.50^{ef}	20.85±0.62^{e}	29.27±0.66^{e}	194,21±19.17^{a}
R6	2.51±0.14^{g}	9.69±1.06^{g}	10.40±0.50^{g}	18.62±0.21^{g}	208,58±2.26^{a}
BHT	22.74±0.51^{c}	46.18±1.10^{b}	62.91±0.60^{b}	73.64±2.03^{b}	47,74±1.10^{d}
Trolox	57.53±0.62^{a}	95.74±2.00^{a}	96.34±3.09a	96.42±0.91^{a}	14,24±0.21^{e}
α-tocopherol	52.46±0.57^{b}	95.33±2.03^{a}	95.84±2,05^{a}	95.60±1.02^{a}	15,27±0.24^{e}

$^{a-h}$: The same letters within the same coloumn shows no statistical difference between rose species at P<0.05 level
R1 (*Rosa villosa* L. subsp. *mollis*), **R2** (*Rosa villosa* L. subp. *villosa* L.), **R3** (*Rosa pimpinellifolia*), **R4 (***Rosa iberica*), **R5** (*Rosa pisiformis*) and **R6** (*Rosa canina*), **BHT**; 2,6-di-t-butyl-1-hydroxytoluene, **DPPH**; Free radical removal activity, **IC_{50}**; The concentration of the extract that inhibits 50% of the DPPH radical.

The lowest IC_{50} value was 60.69±0.94 in R1, and the highest IC_{50} value was 208.58±2.26 in R6. According to the calculated IC_{50} values, the radical scavenging activity is trolox> α tocopherol> BHT> R1> R2> R3> R4> R5> R6. In similar studies, DPPH Antioxidant activity was reported to be 9434.09 mg gallic acid/100 g in *R. canina* (Kayahan *et al.,* 2022), while it was reported between 6.99 and 7.73 mg TEAC/g in another study (Rovná *et al.,* 2020).

Ferric reducing Power (FRAP): The reducing capacity of the extracts was determined by monitoring the colour change at 700 nm caused by the reduction of Fe^{3+} to Fe^{2+}. The results are given in Table 3. The increase in absorbance in the graph is an indication of an increase in the reducing capacity. As with the radical removal activity, the reducing power increases as the concentration of the extracts increases. It has reducing power activity in all species, although it is low compare to standard compounds. When the species were compared among themselves, except for the R1 species, the others species showed similar activity. The reducing power of the extracts and standards at 100 µg/mL concentration were in order as BHT>α-tocopherol>trolox>R1>R3>R2≥R5>R4>R6. As with the radical scavenging activity, the species with the highest reducing power was the R1 species. In different studies on fruits of Rosehip species, the FRAP value was reported as 38.55 mmol TE/g FW in *Rosa iberica* (Abacı *et al.,* 2016), and 39 ± 0.03 µg/mL (IC_{50}), in *Rosa canina* (Sabahi *et al.,* 2022).

Copper (II) reducing capacity (CUPRAC): CUPRAC is one of the methods used to determine the reduction capacity. The darkness of yellow color is directly proportional to the amount of antioxidants. Copper (II) reducing capacity (CUPRAC) of the species was determined and the results were given in table 4. In the study in which measurements were made in different concentration range (20-100 µg/mL), the increase in absorbance values parallel to the increase in concentration shows that all species have Cu^{2+} reduction ability. The highest Cu^{2+} reduction capacity at 100 µg/mL

was in order as BHT>α-tocopherol>Trolox>R3>R4> R5>R6>R1>R2. In a study with *R. canina*, it was reported that the water extract to have CUPRAC activity (Mihaylova *et al*., 2015). In addition, in another study conducted with Rosehip berries, the antioxidant capacity value of CUPRAC was reported to be 1600.75 mg trolox/100 g (Kayahan *et al.,* 2022).

Table 3: Ferric reducing capacity of Rose species and standards

Sample	FRAP (Absorbance at 700 nm)				
	20 µg/mL	**40 µg/mL**	**60 µg/mL**	**80 µg/mL**	**100 µg/mL**
R1	0.017±0.001^{a}	0.025±0.002^{c}	0.035±0.002^{d}	0.071±0.007^{c}	0.121±0.005^{c}
R2	0.005±0.005^{c}	0.009±0.005^{d}	0.015±0.002^{ef}	0.020±0.005^{e}	0.025±0.002^{e}
R3	0.005±0.001^{c}	0.012±0.001^{d}	0.019±0.001^{e}	0.030±0.002^{d}	0.040±0.005^{d}
R4	0.007±0.005^{c}	0.013±0.005^{d}	0.017±0.001^{e}	0.020±0.001^{e}	0.023±0.001^{e}
R5	0.004±0.001^{c}	0.006±0.001^{d}	0.012±0.005^{fg}	0.017±0.001^{e}	0.025±0.005^{e}
R6	0.004±0.001^{c}	0.007±0.005^{d}	0.009±0.001^{g}	0.011±0.005^{e}	0.013±0.005^{f}
BHT	0.020±0,004^{a}	0.090±0.011^{a}	0.198±0.005^{a}	0.290±0.007^{a}	0.353±0.010^{a}
Trolox	0.010±0.004^{b}	0.0027±0.005^{c}	0.070±0.005^{c}	0.170±0.006^{b}	0.228±0.006^{b}
α-tocopherol	0.016±0.006^{a}	0.041±0.003^{b}	0.078±0.004^{b}	0.171±0.009^{b}	0.238±0.006^{b}

$^{a-f}$: The same letters within the same coloumn shows no statistical difference between rose species at P<0.05 level
R1 (*Rosa villosa* L. subsp. *mollis*), **R2** (*Rosa villosa* L. subp. *villosa* L.), **R3** (*Rosa pimpinellifolia*), **R4 (***Rosa iberica*), **R5** (*Rosa pisiformis*) and **R6** (*Rosa canina*), **BHT**; 2,6-di-t-butyl-1-hydroxytoluene**, FRAP**; Ferric reducing power.

Table 4: Copper (II) reducing capacity of Rose species and standards.

Sample	**CUPRAC (Absorbance at 450 nm)**				
	10 µg/mL	**20 µg/mL**	**30 µg/mL**	**40 µg/mL**	**50 µg/mL**
R1	0.010±0.004^{g}	0.020±0.004^{d}	0.035±0.004^{g}	0.043±0.001^{h}	0.065±0.005^{g}
R2	0.005±0.001^{h}	0.010±0.001^{d}	0.012±0.001^{h}	0.013±0.001^{ı}	0.015±0.001^{h}
R3	0.086±0.005^{b}	0.115±0.004^{b}	0.126±0.004^{d}	0.203±0.005^{d}	0.212±0.004^{d}
R4	0.060±0.001^{d}	0.070±0.001^{c}	0.100±0.001^{ef}	0.137±0.001^{e}	0.200±0.032^{d}
R5	0.040±0.003^{f}	0.058±0.001^{c}	0.089±0.010^{f}	0.099±0.002^{g}	0.165±0.007^{e}
R6	0.050±0.003^{e}	0.065±0.002^{c}	0.096±0.002^{ef}	0.116±0.002^{f}	0.122±0.005^{f}
BHT	0.192±0.009^{a}	0.550±0.040^{a}	0.850±0.028^{a}	1.093±0.009^{a}	1.269±0.022^{a}
Trolox	0.068±0.001^{c}	0.121±0.008^{b}	0.162±0.003^{c}	0.364±0.006^{c}	0.568±0.012^{c}
α-tocopherol	0.018±0.003^{g}	0.120±0.005^{b}	0.350±0.015^{b}	0.613±0.003^{b}	0.734±0.008^{b}

$^{a-ı}$: The same letters within the same coloumn shows no statistical difference between rose species at P<0.05 level
R1 (*Rosa villosa* L. subsp. *mollis*), **R2** (*Rosa villosa* L. subp. *villosa* L.), **R3** (*Rosa pimpinellifolia*), **R4 (***Rosa iberica*), **R5** (*Rosa pisiformis*) and **R6** (*Rosa canina*), **BHT**; 2,6-di-t-butyl-1-hydroxytoluene**, CUPRAC**; Copper (II) reducing capacity.

Determination of Vitamin A: In this study, the amount of vitamin A of the fruit extracts of Rosehip species was determined. Differences between species were found tobe significant (P<0.05) (Table 5). Total vitamin A values were determined as 397.17±13.58 µg/mL in R5, 319.40±5.43 µg/mL in R1, 313.80±12.47 µg/mL in R6, 291.64±5.42 µg/mL in R3, 241.52±4.66 µg/mL in R4, and 149.97±8.36 µg/mL in R2 group, respectively.

Kazaz *et al*. (2009) reported that the vitamin A content of the fruits of *R. canina* rosehip species was 3.25 µg/g. In a similar study, the vitamin A value was reported in the range of 102.72-236.23 µg/g (Anderson *et al.,* 2011). The value we found for the species is greater than the value reported by Kazaz *et al*. (2009), but it is similar to the value found by Andersson *et al*. (2011). In addition, it is thought that differences may occur due to the fact that vitamin A is very sensitive to light, pH, temperature and oxygen before and during the analysis.

Determination of Vitamin E: Vitamin E is an important radical scavenger. Vitamin E reduces the damage that can be caused by radicals by capturing free electron from hydroxyl radicals in lipophilic environments. In addition, membranes sensitive to lipid peroxidation are protected by this feature of vitamin E (Gutteridge, 1995). In this study, it was determined that the differences between the species were significant in terms of vitamin E amount (P<0.05) (Table 5). The highest value was calculated as 19.52±0.82 µg/mL in R4 and the lowest value as 4.52±0.41 µg/mL in R2. In the similar studies, the vitamin E value of different species was reported as 10.36-1.725 µmol/100 g in *R. canina* species (Kayahan *et al.,* 2022), 3.57 µg/g in *R. villosa* species (Yörük *et al.,*

2008) and 17.60 µg/g in R. *pisiformis* species (Yörük *et al.,* 2008). These results are agree with our values.

Determination of Vitamin C: Water-soluble radical scavengers include ascorbic acid (Özcan *et al.,* 2015). In this study, the vitamin C values of Rosehip fruits were found statistically significant ($p<0.05$) (Table 5). Total vitamin C values were determined as 606.53±0.38 mg/100 mL in R1, 417.06±0.39 mg/100 mL in R6, 415.52±0.31 mg/100 mL in R3, 412.03±069 mg/100 mL in R2, 401.85±0.08 mg/100 mL in R5 and 398.41±0.28 mg/100 mL in R4 group, respectively. In different studies on *Rosa canina* species, the vitamin C values of this species were 354.50 ± 128.21 mg AA/100 g (Maloupa *et al.,* 2021), 112.20 ± 2.82/ 360.22 ± 2.87 mg AA/100 g (Roman *et al.,* 2013) and 347.12 - 621.31 mg/100 g (Ropciuc *et al*.,2011). In the studies conducted on different species, it was reported as 199.90 ± 2.11-305.92 ± 2.45 mg AA/ 100 g in *Rosa pimpinellifolia* L. species (Öz *et al.,* 2018), 119.83 ± 3.3 mg AA/ 100 g in *R. villosa* species (Murathan *et al.,* 2016) and 503.26 ±18.8 mg/100g in *Rosa iberica* species (Abacı *et al.,* 2016). Similarly, although there are small quantitative differences between the values, our values were different due to species factor.

Correlation shows the relationship between variables. Correlations between each of the phytochemical properties in plants are very important in determining their possibilities of use. Pearson correlation coefficients were calculated between the TPC, TFC, DPPH, CUPRAK, FRAP, vitamin A, vitamin E and vitamin C values of the species used in the study (Table 6). As a result of the evaluation, it was found that there was a high correlation between TFC, FRAP and vitamin E values in R1. A negative correlation was found between TFC and FRAP while a positive correlation was seen in between TFC and vitamin E. In addition, a high negative correlation was found between FRAP and vitamin E in R1. It was determined that there was a negative correlation between TPC and FRAP, while a positive correlation was also found in between TPC and vitamin E. A negative correlation was also seen in between FRAP and vitamin E in R2. It was also found that there was a high negative correlation between TPC and TFC values in R3, between DPPH and vitamin A values in R5 and between DPPH and FRAP and vitamin A values in R6.

Table 5. Vitamin A, E and C values of Roseship species.

Sample	A (µg/mL)	E (µg/mL)	C (mg/ 100 mL)
R1	319.40±5.43^{b}	8.50±0.07^{c}	606.53±0.38^{a}
R2	149.97±8.36^{c}	4.52±0.41^{d}	412.03±0.69^{b}
R3	291.64±5.42^{c}	8.57±0.017^{c}	415.52±0.31^{b}
R4	241.52±4.66^{d}	19.52±0.82^{a}	398.41±0.28^{c}
R5	397.17±13.58^{a}	8.33±0.41^{c}	401.85±0.08^{c}
R6	313.80±12.47^{b}	14.05±0.40^{b}	417.06±0.39^{b}

$^{a-d}$: The same letters within the same coloumn shows no statistical difference between rose species at $P<0.05$ level.
R1 (*Rosa villosa* L. subsp. *mollis*), **R2** (*Rosa villosa* L. subp. *villosa* L.), **R3** (*Rosa pimpinellifolia*), **R4 (***Rosa iberica*), **R5** (*Rosa pisiformis*) and **R6** (*Rosa canina*), **A (**vitamin A), **E (**vitamin E), **C (**vitamin C**)**.

Table 6. Pearson correlation coefficients between 8 main traits examined in rosehip species.

		TPC	TFC	DPPH	CUPRAK	FRAP	A	E	C
R1	**1**	1							
	2	-0.996	1						
	3	0.182	-0.089	1					
	4	-0.303	0.213	-0.992	1				
	5	0.996	**-1.000****	0.093	-0.217	1			
	6	0.748	-0.683	0.789	-0.859	0.685	1		
	7	-0.996	**1.000****	-0.093	0.217	**-1.000****	-0.685	1	
	8	0.104	-0.197	-0.959	0.916	0.193	-0.582	-0.193	1
R2	**1**	1							
	2	-0.127	1						
	3	0.017	0.990	1					
	4	-0.441	0.946	0.890	1				
	5	**-0.998***	0.193	0.050	0.500	1			

	6	0.829	-0.660	-0.545	-0.868	-0.864	1		
	7	**0.998***	-0.193	-0.050	-0.500	**-1.000****	0.864	1	
	8	0.441	-0.946	-0.890	-1.000**	-0.500	0.868	0.500	1
	1	1							
	2	**-1.000****	1						
	3	-0.989	0.989	1					
R3	**4**	0.984	-0.984	-0.948	1				
	5	0.577	-0.577	-0.452	0.712	1			
	6	-0.868	0.868	0.931	-0.766	-0.094	1		
	7	0.189	-0.189	-0.330	0.013	-0.693	-0.652	1	
	8	-0.077	0.077	0.221	0.100	0.770	0.562	-0.994	1
	1	1							
	2	0.064	1						
	3	0.988	-0.090	1					
R4	**4**	-0.896	-0.500	-0.817	1				
	5	0.126	-0.982	0.277	0.327	1			
	6	-0.601	0.759	-0.717	0.184	-0.869	1		
	7	0.832	-0.500	0.908	-0.500	0.655	-0.943	1	
	8	0.481	-0.844	0.610	-0.042	0.930	-0.990	0.886	1
	1	1							
	2	0.380	1						
	3	0.480	0.994	1					
R5	**4**	0.893	0.756	0.824	1				
	5	0.056	0.945	0.903	0.500	1			
	6	-0.490	-0.993	**-1.000****	-0.830	-0.898	1		
	7	-0.611	0.500	0.401	-0.189	0.756	-0.391	1	
	8	0.803	0.856	0.908	0.985	0.640	-0.913	-0.019	1
	1	1							
	2	-0.817	1						
	3	-0.618	0.958	1					
R6	**4**	0.932	-0.971	-0.861	1				
	5	0.655	-0.971	**-0.999***	0.885	1			
	6	0.581	-0.944	**-0.999***	0.837	0.996	1		
	7	0.091	0.500	0.727	-0.277	-0.693	-0.758	1	
	8	-0.880	0.446	0.171	-0.648	-0.218	-0.126	-0.552	1

R1 (*Rosa villosa* L. subsp. *mollis*), **R2** (*Rosa villosa* L. subp. *villosa* L.), **R3** (*Rosa pimpinellifolia*), **R4 (***Rosa iberica*), **R5** (*Rosa pisiformis*) and **R6** (*Rosa canina*). **TPC** (Total phenolic content), **TFC** (Total flavonoid content), **DPPH** (Free radical scavenging activity), **CUPRAC (**Copper (II) reducing capacity), **FRAP (**Ferric reducing power), **A (**Vitamin A), **E (**Vitamin E), **C (**Vitamin C)

*. Correlation is significant at the 0.05 level

**. Correlation is significant at the 0.01 level

Conclusion: In this study, it was concluded that species diversity may be one of the most determining factors on the main compounds and antioxidant potential in plants and the relationship between them. In addition, it should be focused that all species have high antioxidant potential and are a source of vitamins, even if there are differences between the species in the study. The results of this study showed that five other species, especially Rosa *villosa* L. subsp. *mollis* (R1), had the potential to be natural sources of antioxidants. As a result, it is thought that the studied species can be used as an alternative to butylated hydroxyanisole and butylated hydroxytoluene in the nutrient industry, as well as their use in food, medicine and cosmetics. It was also concluded that it could be used as a natural source of antioxidants in the development of bio-functional food products in vitro. Similar studies on different species are needed to get the detailed information on them with respect to the present parameters.

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Contribution Statement: F.E., designed the study and conducted the experiments; M.Y., did the analysis and reporting of the data. All authors contributed to the writing and revisions.

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