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Fruit extracts of *Rosa canina* L. and *Rosa pimpinellifolia* L.: Phytochemical profiles, *in vitro* antioxidant, anti-inflammatory, xanthine oxidase inhibitory effects, and *in silico* molecular dynamics studies

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ABSTRACT

In this study, the phytochemical and biological activities of *Rosa canina* (RC) and *R. pimpinellifolia* (RP) fruit methanol extracts were investigated. HPLC analysis revealed that RP and RC extracts contained high amounts of ascorbic acid and gallic acid as the major components. In GC-MS/MS analysis, oleic acid, linoleic acid, and palmitic acid methyl ester were the most abundant compounds in both extracts. The total phenolic contents of RP and RC were 17.28±0.10 and 5.19±0,22 mg GAE/g extract, respectively. DPPH˙ scavenging activities of the extracts (13.45±0.21 µg/mL for RC and 3.41±0.05 µg/mL for RP) were observed to be higher than ascorbic acid (42.15±1.35 µg/mL). The reducing power capacities of RC and ascorbic acid were 55.57±3.23 and 87.24±2.44 µg/mL, respectively. According to the BSA denaturation assay, the anti-inflammatory effect of RP was found to be more effective at low doses with DFS and similar to the effect at high doses. RC extract showed high xanthine oxidase (XO) inhibition with an IC_{50} value of 2.28±0.25 µg/mL. The binding affinities of ascorbic and gallic acid with XO were determined as 6.20 and 6.60 kcal/mol, respectively. In addition, molecular dynamics simulations of the complexes were applied for 100 ns and observed to be stable. Binding energies were determined by performing MM/PBSA, and it was recorded that a high level of gallic acid was found at 21.50 kcal/mol. In this way, the phytochemical constituents and biological activities of two different rosehip species were compared, and ideas for their use in food, cosmetics, and medicine were presented.

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Rosa canina ve *Rosa pimpinellifolia* meyve özütleri: Fitokimyasal profilleri, *in vitro* antioksidan, anti-inflamatuar, ksantin oksidaz inhibitör etkileri ve *in silico* moleküler dinamik çalışmaları

ÖZET

Bu çalışmada, *Rosa canina* (RC) ve *R. pimpinellifolia* (RP) meyve metanol özütlerinin fitokimyasal ve biyolojik aktiviteleri araştırılmıştır. HPLC'de, RP ve RC özütleri ana bileşenler olarak büyük miktarlarda askorbik asit ve gallik asit içermektedir. GC-MS/MS'de, her özütte en yüksek miktarlarda oleik asit, linoleik asit ve palmitik asit metil esteri bulunmuştur. RP ve RC'nin toplam fenol içerikleri sırasıyla 17.28±0.10 ve 5.19±0,22 mg GAE/g özüt olarak bulunmuştur. Özütlerin DPPH˙ süpürücü aktivitesinin (RC için 13.45±0.21 µg/mL ve RP için 3.41±0.05 µg/mL), askorbik asitten (42.15±1.35 µg/mL) daha yüksek olduğu gözlenmiştir. RC ve askorbik asidin indirgeyici güç kapasiteleri sırasıyla 55,57±3,23 ve 87,24±2,44 µg/mL olarak bulundu. BSA denatürasyon deneyine göre, RP'nin anti-inflamatuar etkisinin DFS ile düşük dozlarda daha etkili olduğu ve yüksek dozlardaki etkiye benzer olduğu bulundu. RC özütü, 2,28±0,25 µg/mL'lik IC_{50} değeri ile yüksek ksantin oksidaz (XO) inhibisyonu

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gösterdi. Askorbik ve gallik asidin XO ile bağlanma afiniteleri sırasıyla -6,20 ve -6,60 kcal/mol olarak belirlendi. Ayrıca, komplekslerin moleküler dinamik simülasyonları 100 ns boyunca uygulandı ve kararlı oldukları gözlendi. Bağlanma enerjileri MM/PBSA yapılarak belirlendi ve gallik asidin yüksek değerinin -21,50 kcal/mol olarak bulunduğu kaydedildi. Bu şekilde iki farklı kuşburnu türünün fitokimyasal bileşenleri ve biyolojik aktiviteleri karşılaştırılmış ve gıda, kozmetik ve ilaç gibi alanlarda kullanımına yönelik fikirler sunulmuştur.

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INTRODUCTION

In traditional medicine, plants rich in antioxidants are used as medicines to treat many diseases. Antioxidant compounds found in plants are often used in the traditional or food industry as vitamins, colorants, sweeteners, and additives in foods and beverages to extend the shelf life of products or to influence consumer behavior (Sarkar et al., 2009; Schmidt et al., 2007). Food supplements and nutraceuticals enriched with antioxidants are also necessary for a healthy life (Gostner et al., 2015). The rose hip is an indispensable and healing plant in alternative medicine. The rose hip, which can be consumed fresh or dried as a tea or jam any time of the year, also has significant health benefits. Rosehip, which has a very high nutritional value, is also rich in vitamins and minerals (Doğan et al., 2006; Güneş, 2011). It is a natural food that acts as an antioxidant and protects the body from many diseases (Shakibaei et al., 2012). Rosehip contains phenolic chemicals that are vital for humans. Since the natural antioxidants, minerals, carotenoids, bioflavonoids, tocopherol, fruit acids, vitamin C, pectin, tannin, and amino acids contained in it are good for human health, this type of fruit (Rosa spp.) has recently gained value (Çınar & Çolakoğlu, 2004; Su et al., 2007).

The plant known as rosehip is a member of the Rosaceae family's genus Rosa and subfamily Rosaideae (Keleş & Kökosmanlı, 1996; Yılmaz, 1996). The Rosaceae family is mainly found in the Northern Hemisphere, although it is widely distributed worldwide. According to Tanker et al. (1993), this nation has 35 genera and 250 species, while there are 100–120 genera and 3000–4000 species worldwide. The genus Rosa is divided into four subgenera: *Eurosa, Hesperhodos, Platyrhodon,* and *Hulthemia* (Wissemann, 2017). The Eurosa subgenus has more variants than the rest of all of these. According to Atienza et al. (2005), the subgenus consists of *Rosa banksianae* R.Br.*, R. bracteatae* Wendl.*, R. carolinae* L.*, R. chinensis* Jacq.*, R. cassiorhodon* Dumort.*, R. gallicanae* L.*, R. pimpinellifolia* L.*,* and *R. laevigatae* Michx.

Black-fruited rosehip, or *R. pimpinellifolia* L., is a small tree that resembles a shrub and reaches a maximum height of one meter. The fruits are hairless, round, and flattened laterally. It's a purple-black color. At 1200-2750 meters, *R. pimpinellifolia* L. grows on rocky, arid slopes, volcanic rocks, or limestone soils (Kutbay & Kılınç, 1996). *R. canina* L. grows 1.5–3.5 meters tall. The fruits range in shape from spherical to egg-shaped. Fruits range in size from 3 cm to 5 cm, with colors ranging from dark pink to yellowish red. In general, fall is when fruits ripen. The species *R. canina* L. is widespread throughout this nation (Kutbay & Kılınç, 1996).

The ability of the rosehip plant to thrive in different types of soil, in high and low altitudes, and in harsh continental climates has led to its spread throughout Turkey and the creation of numerous varieties (İlisulu, 1992; Yamankaradeniz, 1983). It has diuretic, antimutagenic, and antibacterial properties and treats various diseases, including rheumatic diseases, gout, stomach ulcers, sciatica, gallstone formation, biliary tract diseases, and colds. It is also known to affect hemorrhoids and diabetes. Bronchitis is treated with leaves and root parts of the plant (Orhan et al., 2009). Rosehip is a traditional remedy for treating kidney and bladder stones, diarrhea, bleeding gums, side and chest pains, and other ailments. Rosehip seeds have been found to lower triglyceride and cholesterol levels (Güneş & Şen, 2001). Digestive disorders can be avoided by eating rose hip root, fruit, and blossom (Macit & Köse, 2015). Additionally, it is highly effective in preventing colds by bolstering the immune system (Chrubasik et al., 2008; Sen & Gunes, 1996).

In this study, we compared the phytochemical contents (total phenolic, total flavonoid, GC-MS/MS, HPLC) and biological activities (antioxidant, anti-inflammatory, and xanthine oxidase) of methanol extracts from two different

rosehip species, Rosa canina (RC) In and In Rosa In *pimpinellifolia* (RP), known as natural vitamin C. In addition, the inhibitory properties of the compounds that were most pronounced in the HPLC analysis of both species and their interactions with xanthine oxidase, which plays a role in uric acid metabolism, were determined using the AutoDock program and the binding energies corresponding to the most appropriate poses were calculated using GROMACS molecular dynamics simulations and MM/PBSA methods. In this way, the unknown aspects of black and red rosehip will be revealed, and their use in food and pharmacology will be improved.

MATERIAL and METHOD

Plant Collection and Extraction

Two rosehip species, RC and RP, were collected in September 2023. RC from Yenidoğan village (Ağrı Mountains) in the Aralık district of Iğdır province (INWM00000240, diagnosed by Prof. Dr. Ahmet Zafer Tel) and RP (ARTH3561, diagnosed by Prof. Dr. Emin Eminağaoğlu) from the Ardanuç district of Artvin province in Turkey

Dried RC and RP fruits were ground into powder using a grinder. 20 g of each sample was weighed and extracted with methanol in a 750 ml Erlenmeyer flask for 3 days. The solvent in the extract was evaporated using a rotary evaporator, and a methanol crude extract was obtained.

Analysis of Phenolic Contents of Extracts by HPLC

HPLC analysis determined the phenolic contents and amounts of RC and RP fruit methanol extracts (Başar et al., 2024b). It was analyzed using 15 phenolic standards on the HPLC device. The device had a DAD sensor (300/200 nm) and an 8-micron reversed-phase hi-plex analysis column (300x7.7). The column temperature was set to 30ºC for sensitive analysis. The solvents were eluent A, 83% water (0.1 formic acid), and eluent B, 17% acetonitrile (0.1 formic acid). The flow rate of the solvent was set to 0.8 mL/min, and the injection volume to 10 µL. Sample preparation: 20 mg of the extract was weighed on a precision balance and dissolved in methanol. 1 mL of the sample was taken with an automatic pipette and filtered through a 0.45-micron filter. It was then diluted 1:1 with pure water and injected into the device. The content analysis was carried out using 20 different standard phenolic compounds (Table 1).

Analysis of the fatty acid content by GC-MS/MS

RC and RP fruit *n*-hexane extracts were analyzed on a GC-MS/MS device, as this previously published article described. This analysis used an Agilent 7000 A GC/MS Triple Quad with 7890 GC, 7693 Autosampler, and 7697A Headspace Sampler (Başar et al., 2024c). The instrument was equipped with an Agilent HP-5 (5%-phenyl)-methylpolysiloxane) (30 m x 0.25 mm x 0.25 µm) GC column. According to the previously established procedure for the analysis, the initial temperature was set at 50°C and kept constant for two minutes. It was then gradually increased to 140, 220, and 270°C until it was fixed at 270°C, and the ion temperature of the MS detector was set to 280°C. 20 mg of the sample was taken, 1 mL of MeOH was dissolved, and 1 mL of *n*-hexane was added. Then, 1 mL of KOH solution (1 M) was added and mixed with a vortex device at 2500 rpm for 30 seconds to ensure phase formation. A 0.22 µm filtered sample was taken from the upper phase (*n*-hexane phase) containing fatty acid methyl ester and analyzed with a 1 mL He gas stream by injecting 1 µL volume at a ratio of 1:10.

Total Phenol and Flavonoid Contents

The total phenol (Folin-Ciocalteu method) and total flavonoid (aluminum chloride method) contents of *R. canina* and *R. pimpinellifolia* fruit methanol extracts were determined. Gallic acid (total phenol) and quercetin (total flavonoid) were used as standards (Golmakani et al., 2014).

In TPC, 100 µL of extract (or standard gallic acid) solution (1024 µg/mL) was mixed with 500 µL of Folin-Ciocalteu reagent. After 1 minute, 1.5 mL of 20% Na_2CO_3 was added, and the mixture was left in the dark at room temperature for 2 hours. The absorbance values of the mixture solutions were measured at 760 nm. The TPC values of the samples were expressed as mg gallic acid equivalent (GAE) using the equation $y = 1.9465x + 0.0262$ ($R^2 = 0.99$) in the calibration graph of gallic acid.

In TFC, 1.5 mL methanol, 100 µL 10% aluminum chloride, 100 µL 1 M potassium acetate, and 2.8 mL deionized water were added to 500 µL extract and standard quercetin (1024 µg/mL) solutions. The mixture was left at room temperature in the dark for 30 minutes, and absorbance values were measured at 415 nm. The TFC values of the samples were expressed as mg quercetin equivalent (QE) using the equation $y = 2,2406x + 0,0245$ ($R^2 = 0.99$) in the calibration graph of quercetin.

Antioxidant Activities

The antioxidant activities of RC and RP fruit methanol extracts were determined with the $DPPH^{\cdot}$ scavenging (Blois, 1958) and reducing power (Oyaizu, 1986) activities, and also compared with standard ascorbic acid. The results were recorded as $A_{0.5}$ (reducing power), IC_{50} ($DPPH^{\cdot}$ scavenging), and expressed µg/mL. The IC_{50} value is the concentration at which 50% absorbance is effective for $DPPH^{\cdot}$ scavenging activity. The $A_{0.5}$ value is the concentration at which half of the absorbance is effective for reducing power.

The reducing capacities of RC and RP fruit methanol extracts were observed spectroscopically with the Fe3+ to Fe2+ reduction assay.[24] Briefly, 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% $K_3Fe(CN)_6$ (potassium ferrisiyanür) were mixed with 1 mL of extract. The mixture was incubated in an aqueous bath at 50 °C for 20 minutes. After 2.5 mL of 10% TCA (trichloroacetic acid) was added to the mixture medium and centrifuged at 2500 x g for 10 minutes. 2.5 mL of the obtained supernatant was mixed with 0.5 mL of 0.1% $FeCl_3$ (iron (III) chloride) and 2.5 mL of ddH_2O (double deionized water). The reducing power capacities of the standards and extracts were measured at 700 nm, and changes in absorbance were followed. $A_{0.5}$ (µg/mL) values were calculated using the absorbance values.

This assay was evaluated by a 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$) radical scavenging assay.[26] 3 mL of the RC and RP fruit methanol extracts and solutions of standard antioxidant substances were mixed with 1 mL of 0.1 mM $DPPH^{\cdot}$ solution. Changes in absorbance at 517 nm were recorded. IC_{50} (µg/mL) values were calculated using the absorbance values.

Anti-inflammatory Activity

The anti-inflammatory effect was evaluated *in vitro* by testing its effect on bovine serum albumin (BSA) denaturation (Kandikattu et al. (2013). Briefly, 500 µL of extract or standard (Diclofenac sodium, DFS) at different concentrations (512, 256, 128, 64 µg/mL) was added tubes to 500 µL of the solution of BSA (0.2% prepared in ddH_2O). A tube with 500 µL of BSA and 500 µL of methanol was also prepared as a control. Then, the mixture was incubated at 37 °C for 15 minutes and heated at 72°Cfor 5 min. After cooling, the absorbance was measured at 660 nm in a UV-visible spectrophotometer.

$$\% \text{ Inhibition} = 100 - \frac{OD_{test} - OD_{control}}{OD_{test}} \times 100$$

Xanthine Oxidase (XO) Inhibition

50 µL of sample or allopurinol solution, 100 µL of substrate solution (0.041 mM xanthine), and 50 µL of freshly prepared enzyme solution (0.1 U/mL xanthine oxidase in phosphate buffer (pH 7.5)) were added to the 96-well microplate. The mixture was incubated at 37°C for 5 min. The reaction was then stopped by adding 100 µL of 1 M HCl. The absorbance was measured using a 292 nm UV/VIS spectrophotometer (Li et al., 2025). IC_{50} (µg/mL) values were calculated using the absorbance values.

Statistical Analysis

The arithmetic mean ± standard deviation of the mean (std) was used to express the results; n = 3. An ANOVA was employed since the data acquired using IBM SPSS 20.0 software had a normal distribution. The antioxidant activities (DPPH and reducing power) and XO inhibition between the methanol extracts and standards were then compared using the Tukey $HSD^{a,b}$ test. Additionally, Cohen's d values between the samples were determined by performing an independent t-test, which showed a significant difference since d>2.0. For every test, the findings showed significant differences between the samples ($p < 0.05$).

Molecular Docking, Molecular Dynamics (MD) Simulation, and MM/PBSA Analysis

In the molecular docking studies, the molecular structures were drawn in ChemDraw ultra-18.0, the minimum energy was adjusted using Chem3D 18.0 programs, and the molecular structure was saved in mol2 format. Xanthine oxidase [3NRZ] was selected from RSCB (Protein Data Bank). The AutoDock Vina programs were used for the active site to determine a molecule's interaction with enzymes. All data were integrated to determine the 2D and 3D interaction of the molecules with the active sites of the enzymes using the Discovery Studio (Başar & Erenler, 2024; Çolak et al., 2025; Yenigun et al., 2024).

The stability of the complexes derived from the docking was investigated using MD modeling. The GROMACS package was used to perform the MD simulations (Abraham et al., 2015). The CHARMM force field was used for the MD simulations. The unbound enzyme and the complexes were placed in a tricyclic box and solvated with TIP3P water. Na^+ and Cl^- ions were added to the system to neutralize its overall charge. Next, 50,000 steps of the steepest descent method were used to minimize the energy. NVT/NPT then set the pressure and temperature of

the system to 100 kPa and 310 K, respectively. Finally, the MD simulation was run for 100 ns (Bjelkmar et al., 2010). RMSD (Root Mean Square Deviation), Rg (Radius of Gyration), RMSF (Root Mean Square Fluctuation), and ligand hydrogen bonding diagrams were plotted using qtgrace to investigate the MD simulation results (Akkoc et al., 2023; Başar et al., 2024a). The MM/PBSA method using the tool "gmx_mmpbsa" was preferred to determine the free energy of binding of the complex formed by protein and ligand" (Valdés-Tresanco et al., 2021; Yenigun et al., 2024).

RESULTS and DISCUSSION

As a result of the extraction, 1.2 grams (yield; 6%) of RP and 1.8 grams (9%) of RC extracts were obtained. The phytochemical contents, total phenolics, total flavonoids, antioxidant, anti-inflammatory and xanthine oxidase (XO) properties of the extracts were determined. In addition, the in silico properties of the main compounds and the XO inhibitor were investigated.

HPLC Analysis

The contents of phenolic components of the rose hips were determined by HPLC analysis, and it was found that both plant species contain 15 components. While the RP contained high amounts of ascorbic acid (107.162 ng/µl), gallic acid (473.077 ng/µl), protocatechuic acid (81.522 ng/µl) and *trans*-ferulic acid (49.163 ng/µl), the RC fruit contains high amounts of ascorbic acid (43.670 ng/µl) and gallic acid (281.070 ng/µl) (Figure 1 and Table 1). Catechin and rutin were found in the RC but not in the RP, and vanillic acid, gentisic acid, neohesperidin, and coumarin were found in the RP but not in the RC.

The RC species' biochemical characteristics, collected from six distinct regions in Van, Hakkari, and Şırnak, were investigated by Encü (2015). This species' chemical composition included ascorbic acid, ellagic acid, protocatechin, rutin, quercetin, catechin, gallic acid, chlorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, and phloretin. Gallic acid, chlorogenic acid, caffeic acid, ferulic acid, phloroglucinol, protocatechuic acid, *p*-coumaric acid, catechin, epicatechin, quercetin-3-glucoside, and resveratrol were detected in the HPLC analysis of the ethanol extract of RC (Fetni et al., 2020). In the HPLC analysis of the methanol extract of the root and fruit of RP, benzoic acid, caffeic acid, chlorogenic acid, protocatechualdehyde, and vanillic acid were determined as the main components (Guven et al., 2021). The content analyses vary depending on the standards used in HPLC analyses. They may also vary depending on the time of year, climate, and altitude at which the plant is harvested. However, this results are generally consistent with the literature.

Table 1. Analysis of the compounds in RP and RC methanol extracts using HPLC
Çizelge 1. RP ve RC metanol ekstraktındaki bileşiklerin HPLC ile analizi

No	Compound name	RT (min.)	LOD	LOQ	R^2	RP (ng/µL)	RC (ng/µL)
1	Ascorbic acid	3.307	1.972356	5.976836	0.99129	107.162	43.670
2	Gallic acid	4.161	2.15833	6.540393	0.99041	473.077	281.070
3	Protocatechuic acid	5.633	0.216179	0.655089	0.99989	81.522	17.079
4	Catechin	6.555	0.259225	0.785531	0.99986	ND	ND
5	Hydroxybenzoic acid	8.671	0.185641	0.562548	0.99992	ND	ND
6	Vanillic acid	9.628	0.202326	0.613109	0.99991	ND	ND
7	Gentisic acid	10.460	0.283662	0.859581	0.99983	16.511	ND
8	*p*-coumaric acid	17.214	0.136218	0.412782	0.99995	ND	ND
9	Rutin	19.288	0.237936	0.721018	0.99989	ND	ND
10	*trans*-ferulic acid	20.237	0.373309	1.131241	0.99983	49.163	9.237
11	Naringin	27.357	0.373309	1.131241	0.99972	17.684	11.986
12	*o*-Coumaric acid	28.565	0.260367	0.78899	0.99986	22.501	8.597
13	Neohesperidin	29.642	0.399215	1.209743	0.99968	ND	ND
14	Coumarin	30.696	0.048233	0.14616	0.99999	ND	ND
15	Resveratrol	32.573	0.153643	0.465586	0.99995	ND	ND
16	Quercetin	34.818	0.160099	0.485149	0.99995	9.753	6.673
17	*trans*-Cinnamic acid	35.616	0.23304	0.706183	0.99989	11.705	3.567
18	Hesperidin	36.763	0.354821	1.075215	0.99974	ND	ND
19	Alizarin	38.661	0.132739	0.402239	0.99995	ND	ND
20	Flavon	40.769	3.42617	10.38233	0.99995	ND	ND

RT: Retention time, RC: *R. canina* fruit methanol extract, RP: *R. pimpinellifolia* fruit methanol extract, ND: Not detected

GC-MS/MS Analysis

GC-MS/MS analysis determined both rosehip species' fatty acids and volatile oils content. The results showed that 21 compounds were detected in RP and seven compounds in RC (Figure 2 and Figure 3). Oleic acid methyl ester

(41.30%), linoleic acid methyl ester (32.43%), and palmitic acid methyl ester (16.47%) were detected in the highest amount in the *n*-hexane extract of RP. In comparison, oleic acid methyl ester (53.87%), palmitic acid methyl ester (22.72%), and linoleic acid methyl ester (15.46%) were detected in the *n*-hexane extract of RC (Table 2).

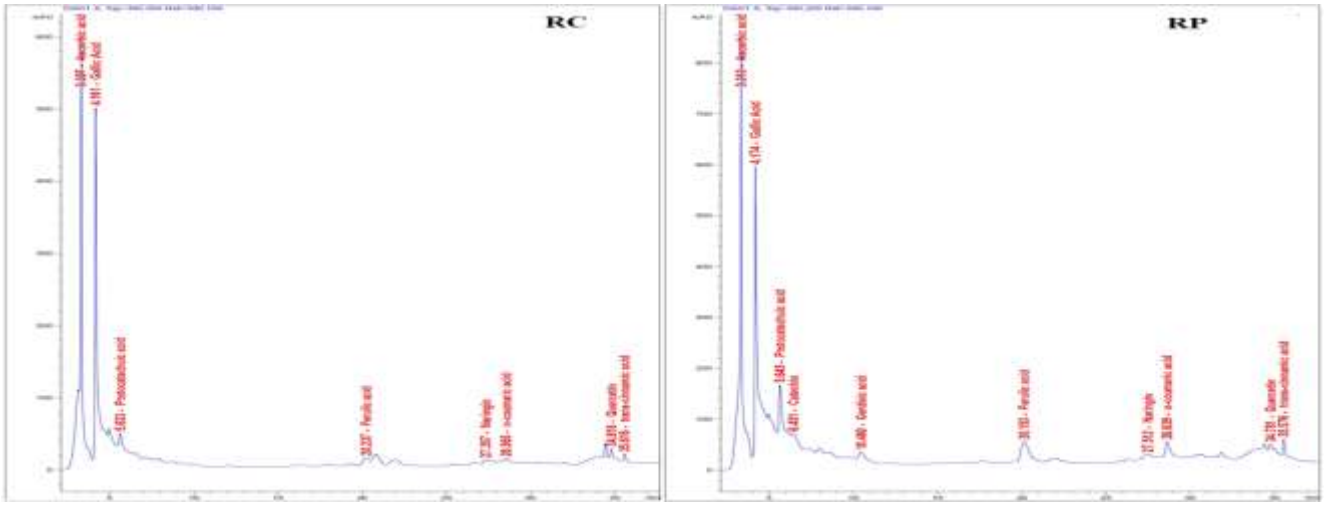

Figure 1. The HPLC chromatogram of the RC and RP
Şekil 1. RC ve RP'nin HPLC kromatogramı

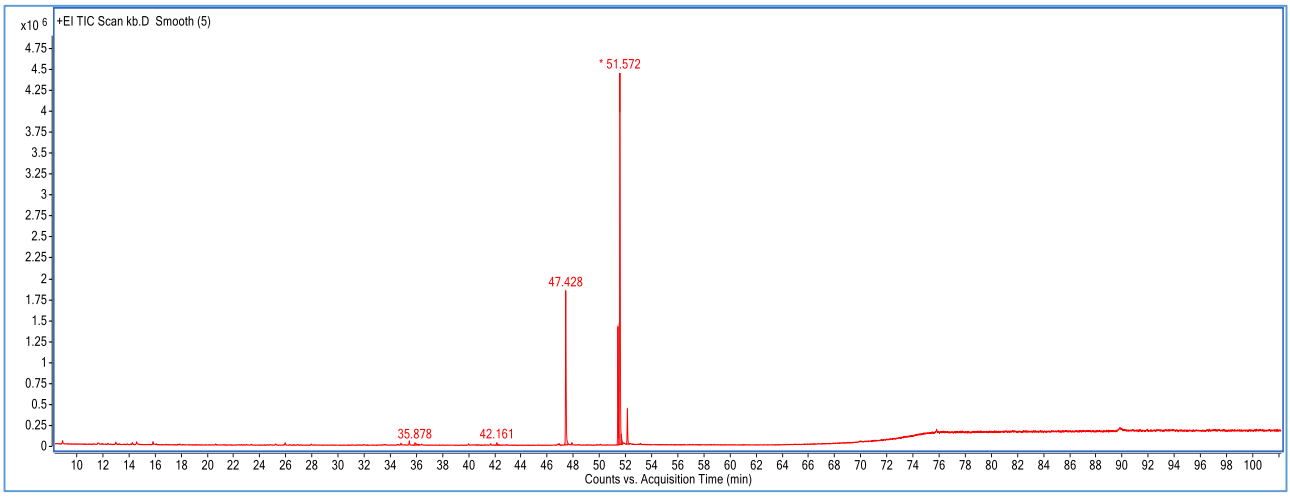

Figure 2. The GC-MS/MS chromatogram of the RP
Şekil 2. RP'nin GC-MS/MS kromatogramı

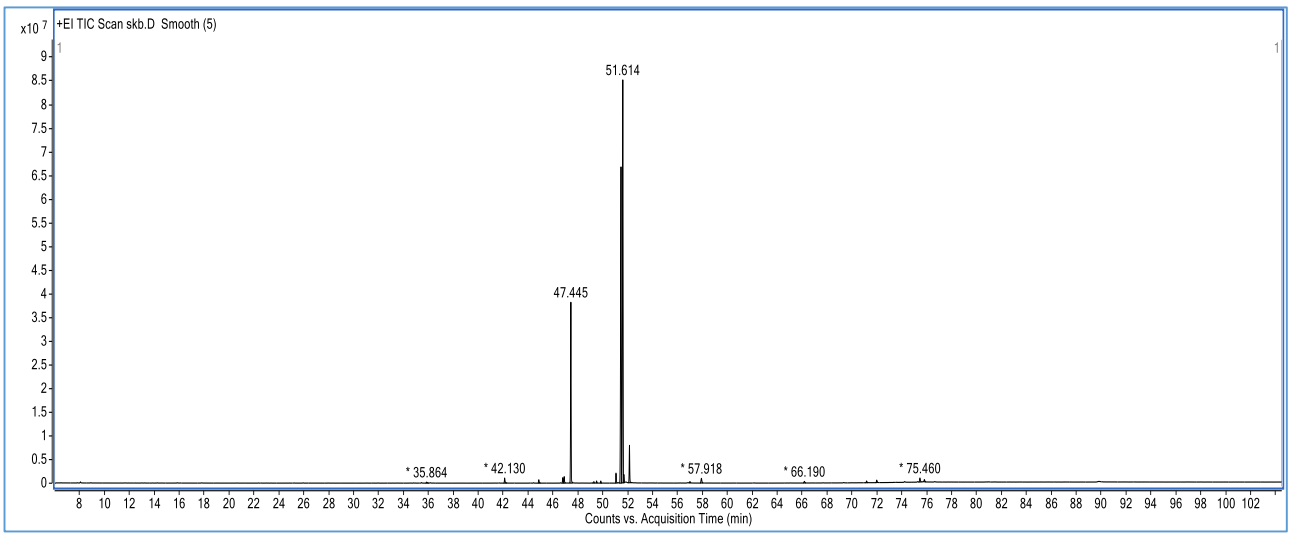

Figure 3. The GC-MS/MS chromatogram of the RC
Şekil 3. RC'nin GC-MS/MS kromatogramı

Table 2. GC-MS/MS analysis of the compounds in the RP and RC hexane extract
Çizelge 2. RP ve RC hekzan ekstraktındaki bileşiklerin GC-MS/MS analizi

No	Compound name	RT (min.)	RI	RP (%)	RC (%)
1	Lauric acid, methyl ester	35.86	1526	0.13	0.99
2	Myristic acid, methyl ester	42.13	1725	0.54	0.59
3	Pentadecanoic acid, methyl ester	44.87	1820	0.34	ND
4	7-Hexadecenoic acid, methyl ester	46.79	1900	0.53	ND
5	Palmitoleic acid, methyl ester	46.90	1899	0.62	ND
6	Palmitic acid, methyl ester	47.45	1926	16.47	22.72
7	Methyl 8-heptadecenoate	49.29	1986	0.18	ND
8	Methyl 9-heptadecenoate or 9-17:1	49.51	1989	0.18	ND
9	Heptadecanoic acid, methyl ester	49.85	2028	0.18	ND
10	γ-Linolenic acid, methyl ester	51.07	2092	0.84	ND
11	Linoleic acid, methyl ester	51.47	2092	32.43	15.46
12	Oleic acid, methyl ester	51.61	2091	41.30	53.87
13	Oleic acid, methyl ester-isomer	51.70	2091	0.62	1.43
14	Stearic acid, methyl ester	52.15	2128	3.38	4.94
15	11-Eicosenoic acid, methyl ester	57.00	2306	0.16	ND
16	Eicosanoic acid, methyl ester	57.92	2329	0.66	ND
17	Behenic acid, methyl ester	66.19	2528	0.21	ND
18	Heptacosane	71.18	2700	0.20	ND
19	Lignoceric acid methyl ester	71.99	2728	0.28	ND
20	Hexacosanol	75.46	2852	0.51	ND
21	Nonacosane	75.82	2900	0.27	ND

RT: Retention time, RI: Retention Index, RC: *R. canina*, RP: *R. pimpinellifolia,* ND: Not detected

When GC-MS analyzed the *n*-hexane extract of RP to determine the fatty acid content, linoleic acid and homo-γ-linolenic acid were determined as the main components (Guven et al., 2021). The fatty acid content of RC was reported to consist of linoleic acid, palmitic acid, and stearic acid as the main components (Ercisli et al., 2007). Therefore, this data are generally consistent with the literature.

Phytochemical Contents, Antioxidant and XO Inhibition Activities

The total phenolic content was higher in the RP than in the RC (Table 3). It is hypothesized that the reason why the total phenolic content of the RP extract is higher than that of the RC extract is due to the higher amounts of 11 compounds determined in the HPLC analysis, the presence of gentisic acid and vanillic acid only in the RP extract, and the absence of catechin and rutin in the RP extract. The $DPPH^{\cdot}$ scavenging activity was strongly influenced by ascorbic acid in both rosehip fruit extracts. However, RP also showed higher activity than other rosehip species and ascorbic acid. Although RC had a higher effect than ascorbic acid in reducing power capacity, RP had no effect (Table 3). The antioxidant activity of the extract and the standards differed significantly ($p < 0.05$) according to statistical analysis using the Tukey test. The test verified that the RC extract's activity was noticeably higher than expected ($p < 0.05$, Table 3). The methanol extracts of RC and RP had a total phenolic content of 176.48 and 225.65 mg GAE/100 g, a total flavonoid content of 0.41 and 2.02 mg QE/100 g, and a $DPPH^{\cdot}$ scavenger content of 79.16% and 87.78%, respectively (Fattahi et al., 2012).

XO is an important enzyme that catalyzes the conversion of hypoxanthine to xanthine and then to uric acid. XO also releases superoxide and hydrogen peroxide anions, which are required to catalyze the primary steps of purine metabolism. Hyperuricemia is the result of excessive uric acid synthesis, which can lead to gout. XO is an important and specific target for treating gout and hyperuricemia-related diseases, such as metabolic syndrome, diabetes, and cardiovascular disease (Singh et al., 2020). The discovery of natural substances with xanthine oxidase-inhibiting properties has increased recently. The activity of this enzyme is mediated by mechanisms that have been shown to lower uric acid levels in many natural flavonoids, phenylpropanoids, alkaloids, saponins, and polysaccharides. Flavonoids have attracted much attention due to their efficacy and safety (Xue et al., 2023).

Table 3 shows the xanthine oxidase inhibition values of the extracts and allopurinol. According to these results, no inhibitory effect of the RP extract was observed. However, it was noted that the RC extract had higher XO inhibition than the drug. This situation is thought to be because the amount of palmitic acid and oleic acid among the fatty acids it contains is higher than in the RP extract, and the catechin and rutin compounds are in the RC extract but not in the RP extract. The XO inhibition of the extract and the standards differed significantly ($p < 0.05$) according to statistical analysis using the Tukey test. The test verified that the RC extract's inhibition was noticeably higher than expected ($p < 0.05$, Table 3).

Table 3. The total phenol and flavonoid, antioxidant and enzyme inhibition activities of the RP and RC
Çizelge 3. RP ve RC'nin toplam fenol ve flavonoid, antioksidan ve enzim inhibisyon aktiviteleri

Name	Total phenol (mg GAE/g)	Total flavonoid (mg QE/g)	DPPH˙ scavenging (IC_{50} µg/mL)	Reducing power ($A_{0.5}$ µg/mL)	XO inhibition (IC_{50} µg/mL)
RP	17.28±0.10	0.03±0.00	3.41±0.05[a]	NA	NA
RC	5.19±0.22	0.13±0.05	13.54±0.53[b]	55.57±3.23[a]	2.28±0.25[a]
Ascorbic acid	NT	NT	42.15±1.35[c]	87.24±2.44[b]	NA
Allopurinol	NT	NT	NT	NT	19.95±1.441[b]
Cohen's d					
Between RP and RC	NT	NT	26.78	24.32	13.06
Between RP and standard	NT	NT	40.50	50.58	20.05
Between RC and standard	NT	NT	20.40	9.47	12.46
P value	NT	NT	0.001	0.000	0.002

GAE: Gallic acid equivalent, QE: Quercetin equivalent, RC: *R. canina* fruit methanol extract, RP: *R. pimpinellifolia* fruit methanol extract, XO: xanthine oxidase. NT: Not tested, NA: Not activity
The average values ± standard deviation of three separate samples are represented in the data. A Tukey test indicates that significant differences are shown by different letters [a-d]. ($p < 0.05$).

Anti-inflammatory Activity

Numerous studies indicate that the denaturation of proteins is one of the causes of rheumatoid arthritis. In many rheumatic diseases, the denaturation of proteins can lead to the formation of autoantigens *in vivo*. Disulfide, hydrophobic, hydrogen, and electrostatic bond changes will likely be part of the denaturation process. Several anti-inflammatory drugs have been shown to stop heat-induced protein denaturation dose-dependently (Rahman et al., 2015).

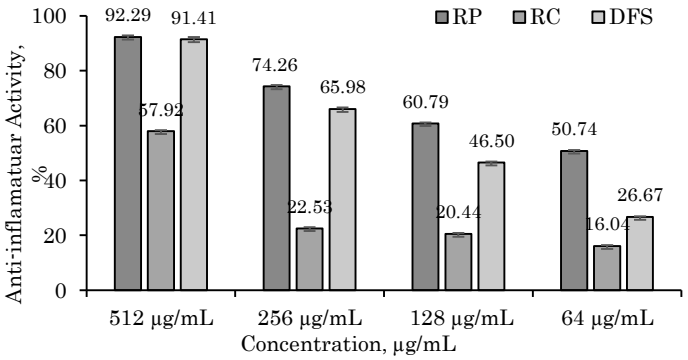

Figure 4. Anti-inflammatory activity of RP, RC, and DFS
Şekil 4. RP, RC ve DFS'nin anti-inflamatuar aktivitesi

The anti-inflammatory effect of diclofenac sodium, which was used as a standard, decreased with decreasing concentration. On the other hand, RP and RC extracts were found to denature BSA. Of these two extracts, it was observed that the RP extract denatured more than the standard, while the RC extract denatured less than the standard (Figure 4). According to this result, it is clear that RP can be used as an anti-inflammatory agent.

Molecular Docking Studies

In this study, we investigated the interactions of gallic acid and ascorbic acid, present in large amounts in both rosehip species, with xanthine oxidase using the AutoDock Vina program in a computer environment. Ascorbic acid was found to form ten hydrogen bonds that interact with XO. Four hydrogen bonds are conventional (VAL259, GLY260, SER347), while the other six are carbon-hydrogen bonds (VAL258, GLY260, ASN261, ALA346, THR262, SER347) (Figure 5 and Table 4). Gallic acid was shown to interact hydrophobically with xanthine oxidase once, forming five hydrogen bonds. Two hydrogen bonds are carbon-hydrogen bonds (GLY260, ASN351), and the other three are conventional (GLY260, SER347). The hydrophobic interaction is an amide-pi stacked interaction (GLY350) (Figure 5 and Table 4). The binding affinities of ascorbic acid and gallic acid were determined to be -6.20 kcal/mol and -6.60 kcal/mol, respectively (Table 4).

MD Simulation Studies

The investigation of the dynamic behavior of molecules and the complex systems with which they interact is of crucial importance for drug development methods. MD simulation is used as a computational method for this

purpose. Molecular docking simulations take into account the flexibility of targets, which is not the case with conventional docking techniques. Binding energy estimates can more accurately identify putative inhibitors (Liu et al., 2018).

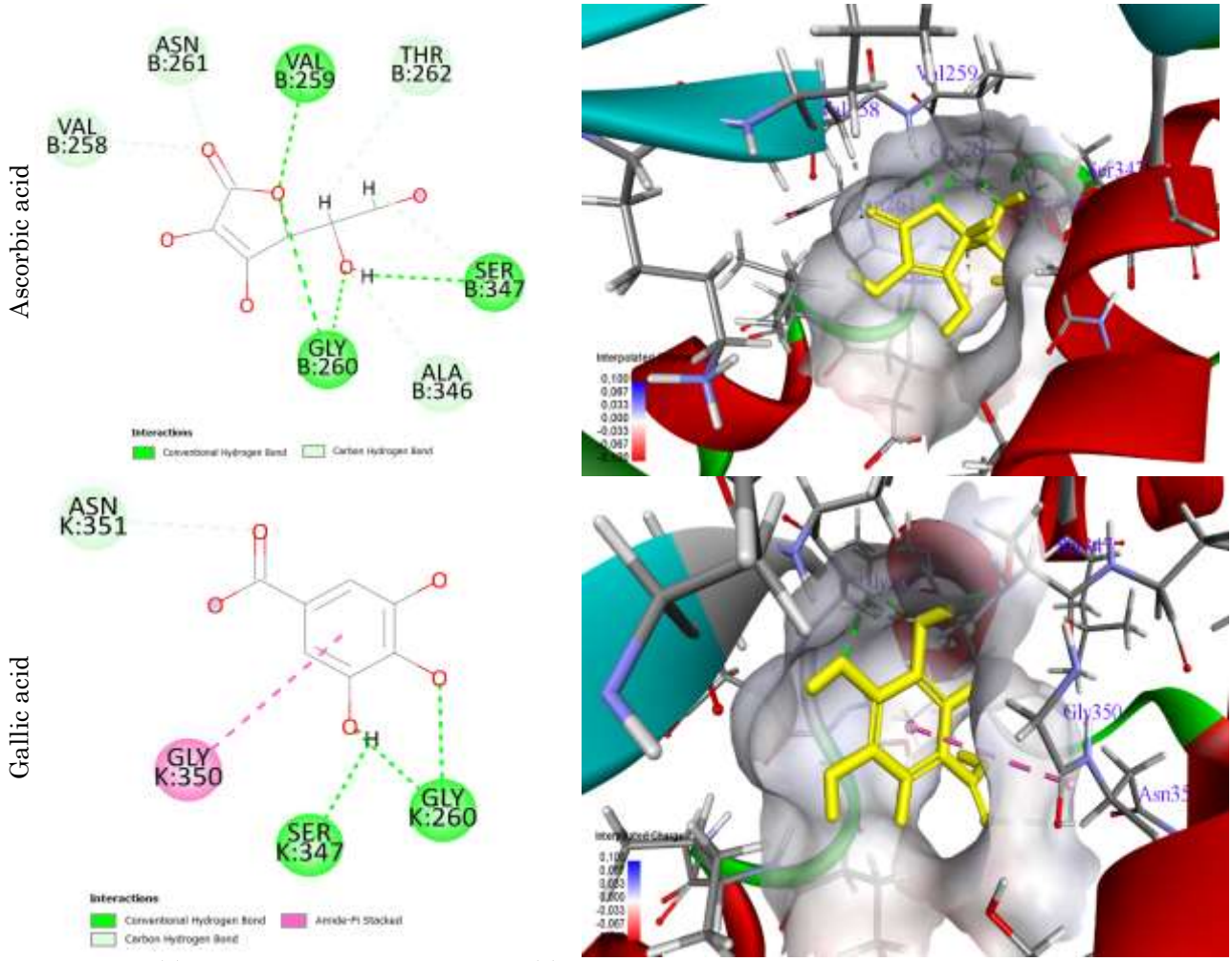

Figure 5. 2D (a), and 3D interpolated charge (b) of the interaction of compounds with XO
Şekil 5. Bileşiklerin XO ile etkileşiminin 2D (a) ve 3D interpole edilmiş yükü (b)

Table 4. XO-compounds interaction categories, species and molecular docking distance
Çizelge 4. XO-bileşiklerin etkileşim kategorileri, türleri ve moleküler yerleştirme mesafesi

Compound Name	Amino acid Names	Distance	Bond Types	Binding Affinities (kcal/mol)
Ascorbic acid	VAL259	2.28	Hydrogen Bond (Conventional)	-6.20
	GLY260	2.25	Hydrogen Bond (Conventional)	
	GLY260	2.41	Hydrogen Bond (Conventional)	
	SER347	2.14	Hydrogen Bond (Conventional)	
	VAL258	2.39	Hydrogen Bond (Carbon)	
	GLY260	2.75	Hydrogen Bond (Carbon)	
	ASN261	2.42	Hydrogen Bond (Carbon)	
	ALA346	2.73	Hydrogen Bond (Carbon)	
	THR262	2.87	Hydrogen Bond (Carbon)	
	SER347	2.63	Hydrogen Bond (Carbon)	
Gallic acid	GLY260	1.71	Hydrogen Bond (Conventional)	-6.60
	GLY260	2.31	Hydrogen Bond (Conventional)	
	SER347	1.85	Hydrogen Bond (Conventional)	
	GLY260	2.82	Hydrogen Bond (Carbon)	
	ASN351	2.89	Hydrogen Bond (Carbon)	
	GLY350	4.74	Hydrophobic (Amide-Pi Stacked)	

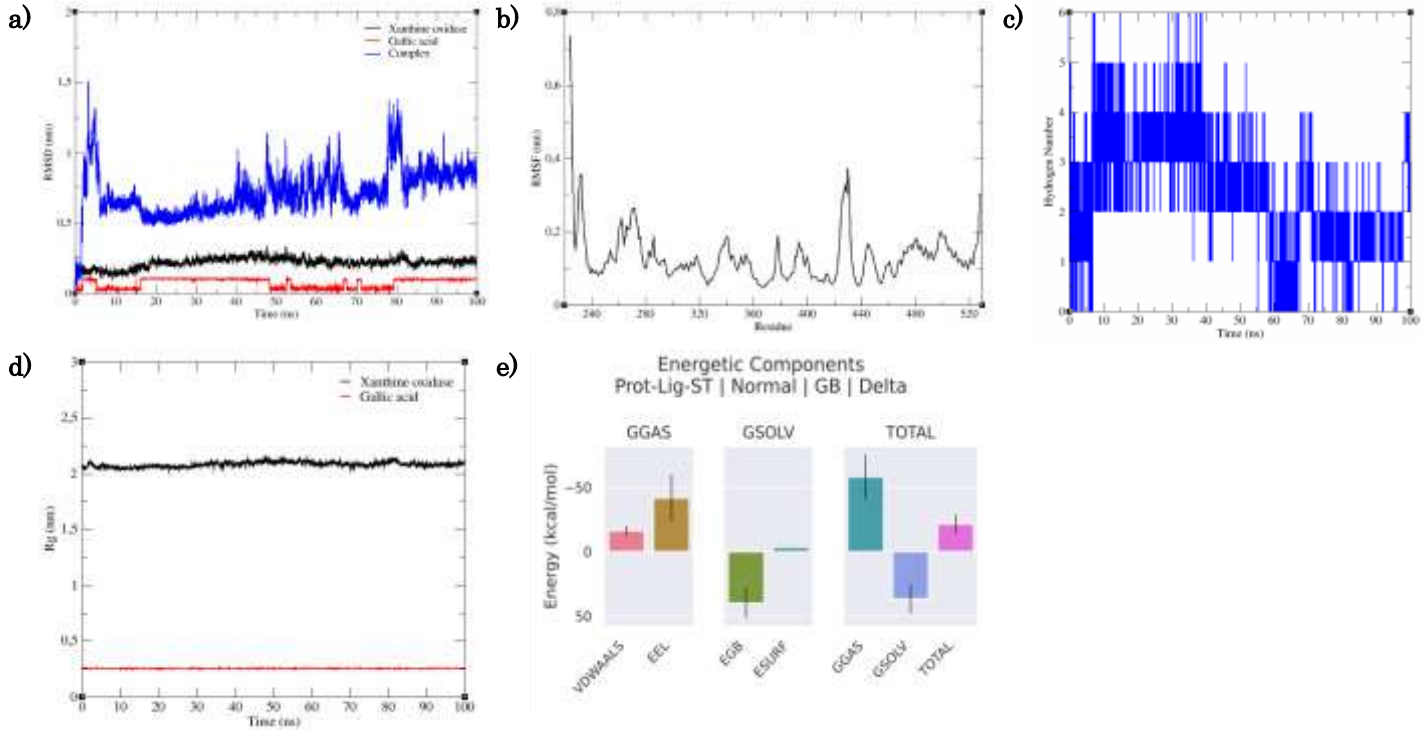

Figure 6. RMSD (a) and RMSF (b) backbone variations within MD trajectories for peptides and the complex, time-dependent H-bond interactions (c), Rg plotting (d), MM-PBSA energy (e) with gallic acid XO

Şekil 6. Peptitler için MD yörüngeleri içindeki RMSD (a) ve RMSF (b) omurga varyasyonları ve karmaşık, zamana bağlı H-bağ etkileşimleri (c), Rg çizimi (d), gallik asit XO ile MM-PBSA enerjisi (e)

The protein remained stable during the entire simulation period, as can be seen from the reduced RMSD values. RMSD mapping showed that the ascorbic acid and gallic acid complexes changed over a period of 100 ns in 9.0-9.5 nm and 0.5-0.8 nm, respectively. It was found that the complexes formed became more stable (Figures 6a and 7a). Using the Cα atoms of XO, the RMSF was calculated for all complex systems and showed that the fluctuation intensity persisted within 0.05-0.45 nm (Figures 6b and 7b). Thus, the stability of the protein structure and the presence of flexible regions required to achieve the optimal conformations were demonstrated by the RMSF plots. To determine the stability of a ligand-receptor complex, it was crucial to investigate the binding connections between proteins and ligands during MD simulations (Majewski et al., 2019). The H-bond that the ligands formed with xanthine oxidase during the 100-ns MD simulations is shown in Figures 6c and 7c. Throughout the MD simulation period, a continuous interaction of H-bonds between one and six and one and ten occurred for the complexes of gallic acid and ascorbic acid, respectively. The degree of compactness of a protein is indicated by its Rg value. The ability of a drug to alter the structure of proteins can be accurately and usefully measured using Rg. The loose molecular packing of a protein is indicated by its Rg value. The dynamics calculations for ascorbic acid, gallic acid, and XO for 100 ns were consistently around 2.10–2.20, 0.25, and 0.25 nm, respectively, as shown in Figures 6d and 7d.

MM/PBSA Analysis

MM-PBSA is a frequently used technique for determining the free energy of binding. It assumes that a ligand-protein combination with a lower predicted binding free energy is more stable and has higher ligand activity and potency. $\Delta G_{binding}$, the free energy of binding of protein-ligand complexes, was determined using MM-PBSA. The energy contributions are G_{gas} and G_{solv}, and MM-PBSA is ranked according to binding energy criteria. The GB estimates in Table 5 show that EGB and ESURF represent the polar and non-polar contributions, respectively. Although all mutants have a strong electrostatic contribution, this is balanced by a sizable positive polar contribution (EGB), which is why the van der Waals term contributes the most to the total binding free energy. As a result, the total polar contribution (EEL + EGB) is positive (Gautam et al., 2021). The $\Delta G_{binding}$ values for ascorbic acid and gallic acid are -17.37 kcal/mol and -21.50 kcal/mol for xanthine oxidase (Figures 6e and 7e). Further analysis of the MM-PBSA data revealed that the van der Waals interaction force, as opposed to the electrostatic interaction force, is important for protein-ligand binding. The output parameters of the MD simulation show a strong correlation with the docking results, indicating that the docked protein-ligand complexes remain stable during the simulation.

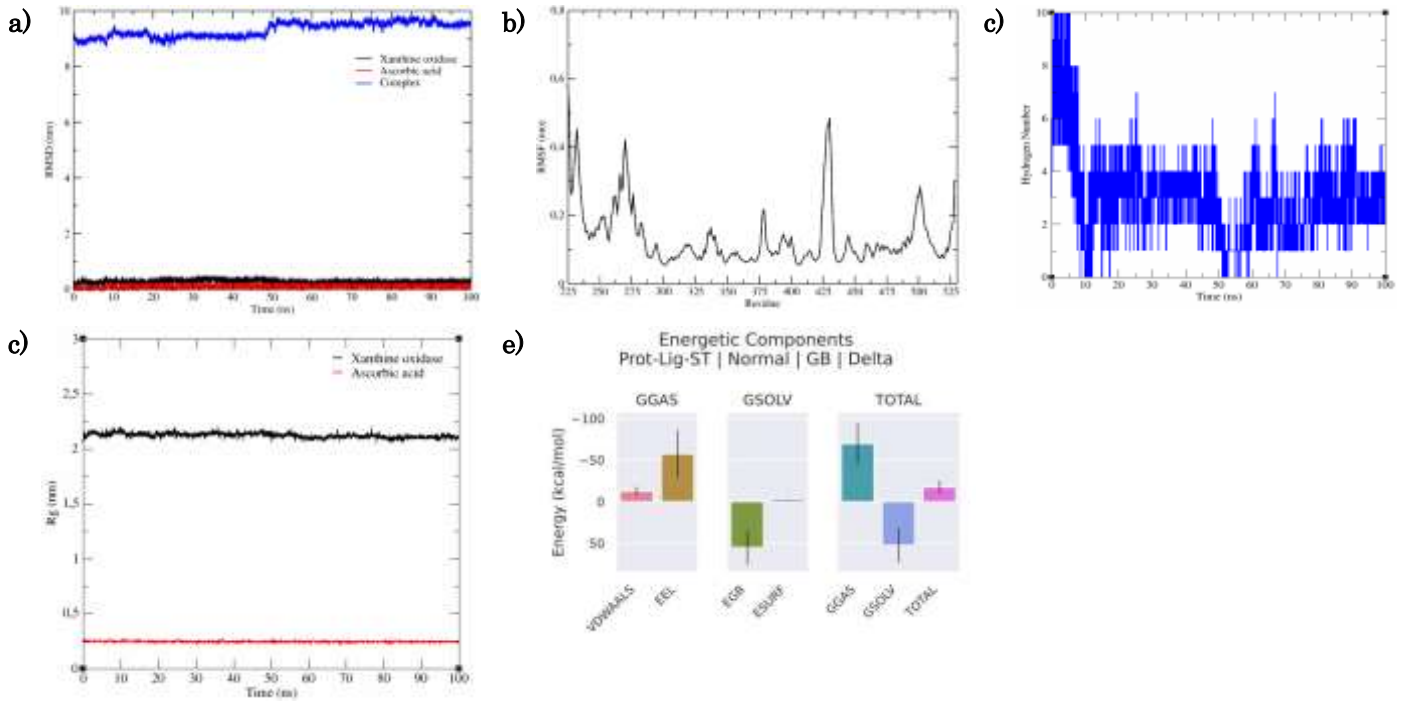

Figure 7. RMSD (a) and RMSF (b) backbone variations within MD trajectories for peptides and the complex, time-dependent H-bond interactions (c), Rg plotting (d), MM-PBSA energy (e) with ascorbic acid XO

Şekil 7. Peptitler için MD yörüngeleri içindeki RMSD (a) ve RMSF (b) omurga varyasyonları ve karmaşık, zamana bağlı H-bağ etkileşimleri (c), Rg çizimi (d), askorbik asit XO ile MM-PBSA enerjisi (e)

Table 5. Results of the energy calculation of the compound-XO complex with MM/PBSA

Çizelge 5. MM/PBSA ile bileşik-XO kompleksinin enerji hesaplamasının sonuçları

Compound-Enzyme	VDW (kcal/mol)	EEL (kcal/mol)	EGB (kcal/mol)	E_{SURF} (kcal/mol)	ΔG_{GAS} (kcal/mol)	ΔG_{SOLV} (kcal/mol)	$\Delta G_{Binding}$ (kcal/mol)
Ascorbic acid	-12.46±5.25	-57.28±28.55	55.23±20.72	-2.86±0.45	-69.74±25.31	52.38±20.79	-17.37±7.76
Gallic acid	-16.39±3.48	-41.72±18.40	39.95±11.54	-3.35±0.35	-58.11±17.29	36.61±11.38	**-21.50±7.21**

VDW: van der Waals contribution from MM, **EEL:** electrostatic energy as calculated by the MM force field, **EGB:** the electrostatic contribution to the solvation-free energy calculated by GB, **E_{surf}:** hydrophobic contribution to solvation-free energy for GB calculations, **ΔG_{gas}:** total gas phase energy (ELE + VDW + INT), **ΔG_{solv}:** sum of nonpolar and polar contributions to solvation, **$\Delta G_{Binding}$:** final estimated binding free energy calculated from the terms above (kcal/mol)

CONCLUSION

Rosehips are used to make tea, juice, jam, and marmalade. It has the highest concentration of vitamin C in all cultivated and natural plants. In addition to its diuretic, antimutagenic, and antibacterial properties, it is used to treat various diseases (rheumatic diseases, gout, stomach ulcers, sciatica, gallstone formation, biliary tract diseases, and colds). For this reason, the phytochemical analyses (total phenol, total flavonoid, GC-MS/MS, and HPLC) and the bioactivities (antioxidant activities) of the methanol extracts of the fruits of RC and RP were investigated in this study. According to the GC-MS/MS analysis results, oleic acid methyl ester, linoleic acid methyl ester, and palmitic acid methyl ester were obtained in high amounts from RP and RC. The HPLC analysis revealed that ascorbic acid, gallic acid, protocatechuic acid, and trans-ferulic acid were present in high amounts in RP, whereas RC contained high levels of ascorbic and gallic acid. The total phenolic content was higher in the fruit extract of RP, likely due to its greater phenolic compound content. The total phenolic content was higher in the fruit extract of RP. This could be due to the fact that it contains more phenolic compounds. $DPPH^{\cdot}$ scavenging activity was strongly affected by ascorbic acid in both rosehip fruit extracts. The fruit extract of RC had a higher effect than ascorbic acid in terms of reducing power. It was observed that RP and RC extracts denatured BSA in their anti-inflammatory effect. Of these two extracts, the RP extract was more denatured than the standard, while the RC extract was less denatured than the standard. RC extract also showed a higher inhibition effect than the standard in XO inhibition. In addition, the interaction of ascorbic acid and gallic acid with XO was investigated by molecular docking, and it was found that the complex formed by molecular dynamics simulation was stable at 100 ns. In MM/PBSA analysis, the binding energy of gallic acid was found to be higher than that of ascorbic acid. This result is directly proportional to the docking results. In other words, while the binding affinity of gallic acid is high in docking, the binding energy of gallic acid is high in MM/PBSA analysis. It was found that RP from both rose hips can be used as a dietary supplement and medicine due to their high phenolic compound content and high

antioxidant effect. According to these results, both types of rosehips showed a high antioxidant effect. It is assumed that this high effect is due to the phenolic compounds and fatty acid esters they contain. It is, therefore, important to use these plants in areas such as nutrition and pharmacology.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors declare that no financial conflicts or interpersonal relationships known to them could have influenced the work published in this publication.

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