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ORIGINAL PAPER

# Investigation of antioxidant activity of *Rosa canina* L. fruit and gall extracts

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**Abstract**
Galls are abnormal plant growth structures formed as defense mechanisms against organisms like insects, nematodes, mites, bacteria, or fungi. This study investigated the antioxidant properties and chemical components of galls (G) formed on *Rosa canina* L. by *Diplolepis* sp., in comparison to adjacent normal mature fruit (R), through comprehensive laboratory analyses. The total phenolic content was found to be 338.34±23.10 mg GAE/g in G and 141.17±1.12 mg GAE/g in R, while the total flavonoid content was measured at 7.62±0.17 mg QE/g in G and 6.04±0.23 mg QE/g in R. It was determined that G exhibited higher ferric reducing power (FRAP), cupric reducing power (CUPRAC), and 1,1-diphenyl-2-picrylhydrazil (DPPH) free radical scavenging activity than R. The gas chromatography–mass spectrometry (GC/MS) analysis identified the primary constituents in the fruit and gall structures. The GC/MS analysis identified the key compounds in the fruit and gall structure. In the sample G, a high concentration of 2-methyloctacosane, which possesses antimicrobial properties and is absent in R, was determined. Additionally, compounds such as hexadecanoic acid, methyl ester, eicosane, and beta-sitosterol, which are not present in R and have pharmaceutical applications, were identified in G. In conclusion, it was established that the antioxidant capacity of *R. canina* galls is higher than that of mature fruits, and they contain distinct chemical components. Further research is required to elucidate the mechanisms of gall formation and to explore potential applications.

**Keywords** Gall · *Rosa canina* · Antioxidant capacity · Phenolic compounds · GC-MS

## Introduction

Plants are highly susceptible to harmful microbial and parasitic pests, and they are significantly affected by these organisms. The impact of these pests on plants varies depending on their species and environmental factors. Plants combat these pests through defense mechanisms, with the primary objective being the elimination of the pathogen and the reduction of potential damage. In some instances, plants exhibit various defense responses in addition to their existing defense mechanisms. One of the significant defense responses in plants is the formation of galls [1].

Gall formation: galls are abnormal growth forms that result from one of the plant's metabolic defense mechanisms against adverse effects caused by parasites, insects, mites, fungi, or bacteria living on plants [2]. Initially developed as a means to eliminate the pathogen, this defense strategy subsequently evolves towards isolating the causative agent. Galls, produced as a defensive product, possess a complex structure. Upon detection of the gall-inducing agent by the plant, the plant cells in the affected area rapidly undergo various modifications, reaching abnormal sizes. During this process, galls behave as if they were novel organs, utilizing photoassimilation products and various minerals inherent to the plant's structure. Consequently, these cells coalesce to form a structure that encapsulates, isolates, and harbors the gall-inducing agent [3]. Additionally, the plant's metabolism synthesizes various chemicals such as tannins and phenolic compounds in an attempt to eradicate the gall-inducing agent [4]. Plants recognize these structures on their surface

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as if they were distinct organs. Furthermore, to prevent the formation of new galls, plants synthesize various chemicals in their normal tissues outside the gall to inhibit the feeding of the pest larvae [5, 6].

*R. canina* is a shrub belonging to the genus *Rosa* within the Rosaceae family [7]. The fruits of this species are highly palatable. These fruits, which possess a rich composition, reach maturity in the autumn. The mature fruits exhibit high physiological activity and are rich in phytochemical constituents [8, 9]. In addition to their antioxidant and anti-inflammatory effects, these fruits also exhibit antibacterial, antimutagenic, anti-osteoarthritis, anti-rheumatoid arthritis, and anti-carcinogenic properties [10–12]. It has also been reported that they possess metabolic regulatory, modulatory, and therapeutic enhancement effects [13]. Among the primary agents causing gall formation in rosehip species are *Diplolepis* sp. Within this genus, *Diplolepis fructuum* and *D. rosae* (*Cynipidae*) are particularly significant [14]. These species are considered pests in production as they cause the destruction of a large portion of rosehip fruits. These species overwinter in the prepupal stage within the gall. In spring and early summer, the developed adults exit the gall through tunnels created using their mandibles. The lifespan of adults outside the gall is limited to a few days. After emerging, these agents mate and oviposit on suitable hosts, initiating the formation of new galls [15–17]. Galls can develop on fruits or leaves, causing curling in the leaves, while resulting in deformation and reduced quality in the fruits, which in turn leads to decreased yield in production [4, 18]. These galls are firm in structure, measuring 7–8 cm in diameter, and are covered with yellowish-brown and reddish hairs. These species are considered harmful in rosehip production.

The formation of galls in rosehips progresses through three distinct stages: the initial phase, the developmental stage, and the final maturation process [19, 20]. The initial phase begins when females deposit their eggs on the flower buds of the plant during the flowering period. It is believed that the fluids secreted by the adult female while laying her eggs, in conjunction with the eggs themselves, may influence the formation of galls during this period [4]. The growth phase commences with the onset of larval growth, followed by the maturation phase [21]. Mature gall structures accumulate different metabolites in their internal and external tissues. The metabolites collected in the external tissues have protective properties, while those in the internal tissues have nutritional properties [22]. (Fig. 1)

Previous studies conducted on *R. canina* have primarily focused on the chemical compositions and antioxidant properties of its fruits. Research related to fruit galls is limited and has mainly investigated gall formation physiology and the associated damage. This study aims to determine the antioxidant properties of fruits collected from the *R. canina* plant during the fruit maturation period and the galled fruits produced by *Diplolepis* sp. using various methods.

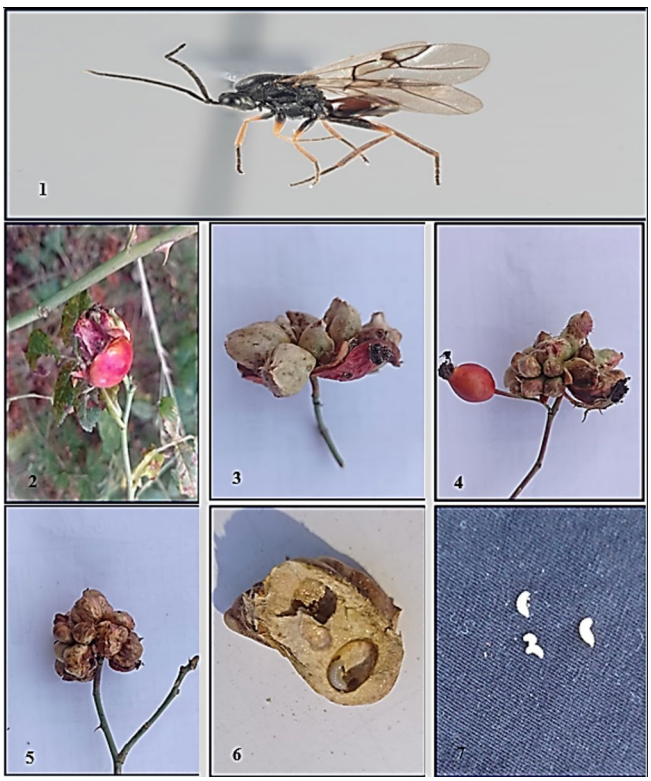


**Fig. 1** Gall formation (1: gall-inducing agent (*Diplolepis* sp.), 2: initial stage of gall formation, 3: growth stage of gall formation, 4: mature stage, 5: gall, 6: galleries inhabited by larvae, 7: larvae of the gall-inducing agent)

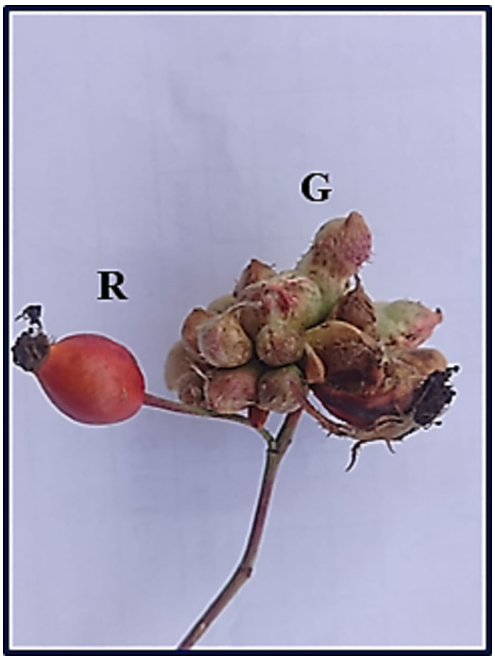


**Fig. 2** Samples of *R. canina* (dog rose). (R) Ripe fruit of *R. canina*, (G) Gall formation on *R. canina* plant

## Materials and methods

### Sample collection and preparation

Fruit and gall samples were obtained from *R. canina* populations located in the Olur district of Erzurum province of Türkiye (40°49'46"N 42°05'37"E, altitude 1,465 m) during the autumn season. It is easy to physically distinguish between ripe fruits and gall formations that have different biological developments. Ripe rosehip fruits are typically oval-shaped and bright red. The gall structure formed on the rosehip plant is abnormally large and hard compared to the fruits (Fig. 2). During sampling, normal fruits and gall structures were randomly selected to represent the whole from the same branch of the same bush to minimize environmental variability. The collected samples were first washed with tap water for 2 min to remove surface contaminants, then rinsed again with distilled water for 1 min and drained on sterile filter paper. Samples were dried in the shade for 3 weeks, without exposure to direct sunlight. Following the drying process, gall samples were opened, and the larvae inside were removed. Dried samples were ground into a homogeneous powder using a mechanical grinder. UV-visible spectroscopy (Shimadzu 2401PC model) was used to determine the total phenolic and flavonoid content, as well as the antiradical and reducing power activities of the samples. All spectroscopic measurements were performed in triplicate to ensure reliability.

### Extraction procedure

The air-dried and pulverized plant samples were extracted following a previously described method [23]. Briefly, 15 g of plant material was weighed and placed in bottles. A total of 300 mL of methanol was added, 20 mL per 1 g of the sample, and the mixture was stirred in a magnetic stirrer overnight. The extract was filtered through a Whatman No. 1 filter paper. Subsequently, 50 mL of methanol was added to the filtrate, stirred for 2 h, and filtered again. This process was repeated three times. The obtained methanol extracts were combined and concentrated using a rotary evaporator at 45 °C to remove the methanol. The resulting extracts were stored at 4 °C in darkness until further analysis.

### Assay for total phenolics

The total phenolic components of the methanol extracts of fruit and gall samples were determined using the Folin-Ciocalteu reagent and gallic acid standard, following the method described in the literature [24]. The method is based on the formation of a colored complex in alkaline medium with Folin reagent by phenolic compounds dissolved in water or organic solvents. The purple-violet complex formed gives maximum absorbance at 760 nm. 100 µL of a 1 mg/mL extract solution was taken, to which 100 µL of Folin-Ciocalteu reagent (FCR) and 4.5 mL of distilled water were added and mixed. After 3 min of incubation at room temperature, 300 µL of 2% $Na_2CO_3$ solution was added. The mixture was incubated at room temperature for 2 h. Spectrophotometric measurements of the samples were conducted at a wavelength of 760 nm. To determine the amount of phenolic compounds, a calibration curve was created using gallic acid as a standard. Using the slope equation of this curve ($y = 0.0153 \times$ gallic acid) the total phenolic substance amount of the extracts was calculated as mg GAE/mL.

### Assay for total flavonoids

The total flavonoid components of the methanol extract of the samples were determined according to the aluminum nitrate method described by Moreno et al. (2000) [25]. In the method, aluminum chloride and flavonoids form stable complexes in acidic medium. The colored complex formed gives maximum absorbance at 415 nm. For measurements, firstly, 0.5 mL of 1 mg/mL prepared extract solution and 0.1 mL of 1 M potassium acetate were put into a tube. After one minute of incubation, 0.1 mL of aluminum nitrate (10% w/v) was added, and the mixture was shaken. The total volume was brought to 5 mL with methanol. After an incubation period of 40 min, it was read spectrophotometrically at a wavelength of 415 nm. Quercetin was used as the standard.

The total flavonoid substance amounts were calculated as mg QE/mL according to the equation obtained from the standard quercetin graph (y = 0.0319 × quercetin).

## Evaluation of reducing capacity (FRAP assay)

The reducing capacity was assessed using a modified version of the method described by Oyaizu (1986) [26]. The procedure involved combining 1 mL of methanolic extract (concentration range: 20–100 µg/mL) with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide solution (1% w/v). This mixture was then subjected to incubation at 50 °C for a duration of 20 min. Following incubation, 2.5 mL of trichloroacetic acid (10% w/v) was introduced, and the resulting solution was centrifuged. From the supernatant, a 2.5 mL aliquot was withdrawn and mixed with an equal volume of distilled water and 0.5 mL of ferric chloride solution (0.1% w/v). The absorbance of this final mixture was determined spectrophotometrically at 700 nm, using a blank for reference. Butylated hydroxytoluene (BHT) served as the positive control in this assay.

## DPPH free radical scavenging assay

The antioxidant capacity of the extracts and reference compounds was evaluated using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method adapted by Blois (1958) [27]. This method is widely used to measure the antioxidant capacity of natural extracts because it is simple, fast, and inexpensive. The purple DPPH radical used is easy to use, has high sensitivity, and allows rapid measurement of antioxidant activity of many samples. First, methanol solutions of the standards and extracts used for the test were prepared at concentrations ranging from 20 to 80 µg/mL. 0.5 mL of the solutions prepared in different solutions were taken and added to a tube. Then, 1.5 mL of DPPH solution (0.1 mM in methanol) was added. After vigorous shaking, the mixtures were incubated at ambient temperature in the dark for 30 min. Subsequently, the absorbance was measured spectrophotometrically at 517 nm, using methanol as a blank. BHT served as the positive control. The radical scavenging activity was expressed as the percentage inhibition of DPPH radicals, calculated using the equation:

$$\text{DPPH } (\%) = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

where $A_{\text{control}}$ represents the absorbance of the reaction mixture without the test compound, and $A_{\text{sample}}$ denotes the absorbance in the presence of extract or standard.

The half-maximal inhibitory concentration ($IC_{50}$), representing the concentration required to neutralize 50% of DPPH radicals, was determined from the linear regression of the %I-concentration curves plotting inhibition percentage against sample concentration.

## Cupric reducing antioxidant capacity (CUPRAC)

The cupric reducing antioxidant capacity was determined according to the method of Apak et al.. (2004) [28]. The CUPRAC method is among the widely used methods. The reason for choosing this method is that it is fast and easy to apply. In addition, the reagents used in the method are cheap and do not require much equipment. Before the test measurements, solutions of extracts and standards were prepared at different concentrations (10–50 µg/mL). 1 mL of Cu (II) chloride (10 mM), 1 mL of Neocuproine (7.5 mM), and 1 mL of $NH_4CH_3COO$ (1 M) solution were added to a tube. 0.5 mL of the solution at different concentrations was added, and the total volume was brought to 4 mL with distilled water. After thorough mixing, it was incubated at room temperature for 30 min in a closed container, and the absorbance was read at 450 nm against a blank. Results are presented as absorbance values compared to the absorbance of the BHT standard.

## Gas chromatography/mass spectrometry (GC/MS) analysis

The GC-MS analysis of the plant samples was performed using the Shimadzu GCMS-QP2010 ultra device. The GC was equipped with a fused silica capillary Rtx-5MS column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The ion source temperature was set to 300 °C. The interface temperature was determined to be 280 °C. The solvent exit was closed for 0.5 min. The total analysis time was set to 121 min. The gas chromatography (GC) analysis started at an initial temperature of 40.0 °C, held for 2 min, and then heated to 270 °C at a rate of 10 °C/min. Finally, it was held isothermally for 20 min. Helium was used as the carrier gas, with a flow rate set to 2 mL/min. For GC-MS detection, an electon an ionization system with ionization energy of 70 eV was used. One microliter of diluted samples (1/100 v/v in methanol) was injected into the device's heated section in splitless mode [29].

## Data analysis

The experimental results underwent statistical evaluation using version 22 of the SPSS software package. To assess the significance of differences among treatments, a one-way analysis of variance (ANOVA) was performed, following the approach outlined by Genç and Soysal (2018) [30]. In cases where the ANOVA indicated significant variations, Duncan's multiple range test was applied to

**Table 1** Total phenolic content, flavonoid content, and $IC_{50}$ values

| Groups | TPC (mg GAE/g) | TFC (mg QE/g) | $IC_{50}$ (µg/mL) | TEAC-FRAP |
|---|---|---|---|---|
| *R* | 141.17 ± 1.12$^{b}$ | 6.04 ± 0.23$^{b}$ | 56.32 ± 0.94$^{a}$ | 0.41 |
| *G* | 338.34 ± 23.10$^{a}$ | 7.62 ± 0.17$^{a}$ | 14.00 ± 3.01$^{c}$ | 1.82 |
| *BHT* | - | - | 46.14 ± 1.10$^{b}$ | 1.48 |
| *Trolox* | - | - | - | 1 |

*:Differences between means indicated by the same letter are not significant at the $P<0.05$ level

*:R; *R. canina* fruit, G; *R. canina gall*, and BHT (butylated hydroxytoluene)

pinpoint specific differences between treatment groups [31]. Throughout all statistical analyses, a significance level of $P<0.05$ was employed as the criterion for determining statistical significance.

## Results

Phenolic compounds and flavonoids are compounds with antimicrobial, antioxidant, antibacterial, and many other beneficial properties. Phenolic acids are secondary metabolites found widely in plants. Flavonoids are particularly interesting compounds because they not only inhibit the production of radicals but also facilitate their removal [32]. In the study, the total phenolic content of *R. canina* fruits was determined to be 141.17 ± 1.12 mg GAE/g (Table 1).

Yılmaz and Ercisli reported a total phenolic content of 102 mg GAE/g for ripe fruits in their study [33]. In another different study conducted on the fruits, this value was reported as 70.58 ± 6.23 mg GAE/g [23]. It is believed that geographical, climatic, and harvest time differences may contribute to these discrepancies. Additionally, the total phenolic content of the galls was found to be 338.34 ± 23.10 mg GAE/g (Table 1). Tataroğlu et al. (2023) reported that the total phenolic content of galls formed by *Diplolepis* sp. on *R. canina* was 243.93 ± 21.00 mg GAE/g in methanol extract, 279.98 ± 22.72 mg GAE/g in acetone extract, and 286.22 ± 11.27 mg GAE/g in ethanol extract [34]. Çoruh and Ercişli (2010), in their study with gal formed by *Diplolepis* sp. in *R. canina* leaves, found that the total phenolic matter content was 75.91 GAE/g and 82.04 GAE/g in leaves without gal [21]. In the study, the difference in total phenolic content between fruit and gall extracts was significant ($P<0.05$). The results we obtained support the findings of Hall et al. (2017), which indicate that gall-inducing insects significantly cause higher amounts of phenolic compounds in their hosts [4]. It has been reported that the increase in phenolic compound content in gall structures may be influenced by the plant's defensive responses [35]. Furthermore, it is known that this situation varies according to the plant species, the gall-inducing agent, the density, and the plant organ in which the gall forms [36].

In the study, the difference in total flavonoid content between fruit and gall extracts was significant ($P<0.05$) (Table 1). The total flavonoid content was calculated as 6.04 ± 0.23 mg QE/g in R and 7.62 ± 0.17 mg QE/g in G. In studies conducted on *R. canina* fruits, the total flavonoid content has been reported as 1.80 ± 0.50 mg QE /100 g [37], 1.01 to 1.63 mg QE/g [38], and 0.52 mg QE/g [39]. The values we found for the fruit and gall structures in this study are higher than those reported in the literature. This difference may be associated with the plant's defensive responses to eliminate the pest.

The antioxidant properties of plant extracts are evaluated by spectrophotometric methods based on radical scavenging. In the presence of an antioxidant, the purple color of the DPPH solution disappears or lightens. A decrease in the maximum absorbance of the DPPH solution at 517 nm is observed [40]. If *R. canina* fruit and gall formation extracts

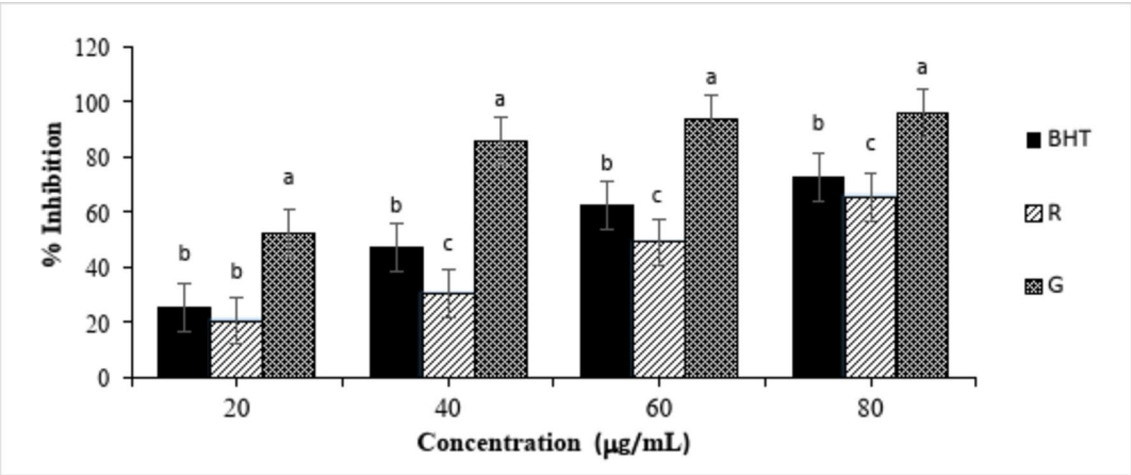


**Fig. 3** DPPH radical removal activities of *R. canina* fruit and its gall. [a-c]: The same letters within the same coloumn show no statistical difference between rose species the $P<0.05$ level. **R**; *R. canina* fruit, **G**; *R. canina* gall, **BHT**; 2,6-di-t-butyl-1-hydroxytoluene.

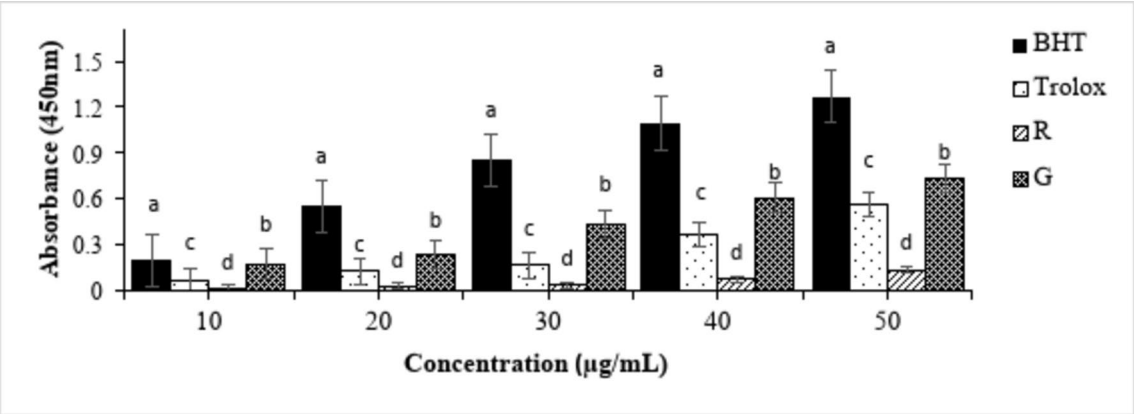


**Fig. 4** Copper(II) reducing capacity (CUPRAC) of *R. canina* fruit and its gall. [a-d]: The same letters within the same coloumn shows no statistical difference between rose species the $P<0.05$ level. **R**; *R. canina* fruit, **G**; *R. canina* gall, **BHT**; 2,6-di-t-butyl-1-hydroxytoluene

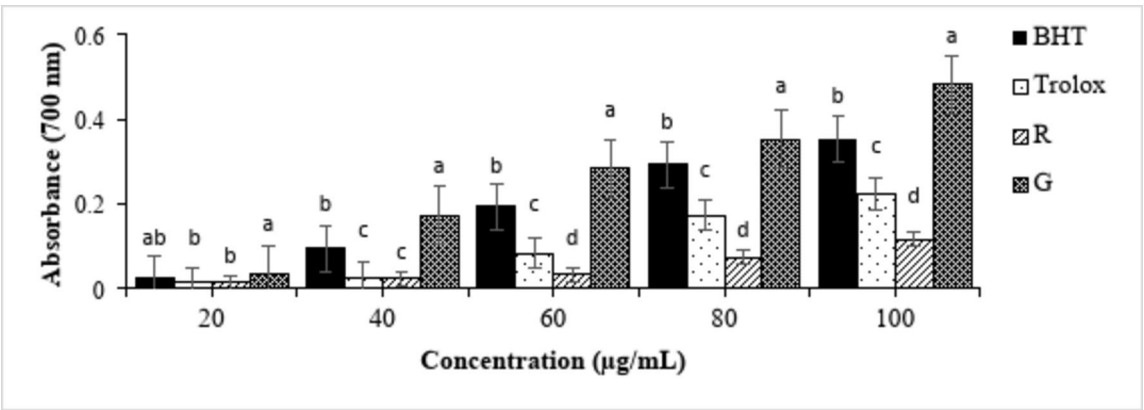


**Fig. 5** Ferric reducing capacity of *R. canina* plant and its gall. [a-d]: The same letters within the same column shows no statistical difference between rose species the $P<0.05$ level. **R**; *R. canina* fruit, **G**; *R. canina* gall, **BHT**; 2,6-di-t-butyl-1-hydroxytoluene.

show DPPH scavenging effect, it can be said that they have natural antioxidant capacity. R and G samples decolorized the DPPH solution. The free radical scavenging activity (DPPH) of the study samples was determined at various concentrations (Fig. 3). The gall extract exhibited notably high DPPH radical scavenging activities. When examining the inhibition percentages at a concentration of 80 µg/mL, the order was as follows: G (95.31%)>BHT (72.76%)>R (65.45%).

The $IC_{50}$ values for the plant fruit and gall formation were calculated (Table 1). For this purpose, concentration-% inhibition graphs were plotted, and calculations were made using the slope equations of these graphs. Significant differences were found among the samples ($P<0.05$). The $IC_{50}$ value was calculated as 56.32±0.94 µg/mL for R and 14.00±3.01 µg/mL for G. In the DPPH radical scavenging activity measurements conducted at different concentrations (20–80 µg/mL) of the study samples, the percent inhibition value calculated as 95.02% in G is considerably higher than the value of BHT (72.77%), which is a standard antioxidant. The methanol extracts of *R. canina* fruit and gall showed very high antiradical activities ($IC_{50}$ for R: 56.32±0.94 and $IC_{50}$ for G: 14.00±3.01 µg/mL) compared to BHT ($IC_{50}$: 46.14±1.10 µg/mL), which was used as a reference and has strong antiradical activity. Especially gal extract has a better antioxidant potential than standard antioxidant. The smaller the $IC_{50}$ value, the higher the antioxidant activity. In a similar study, the $IC_{50}$ value was reported as 47.74±1.10 for BHT and 208.58±2.26 for rose hip fruits [23]. The values we found are lower than these reported values. In this case, it can be said that gall formation affects the antioxidant activity of the neighboring fruit.

The reducing ability of a chemical can indicate the potential antioxidant capacity of the compound. Chemicals that exhibit antioxidant properties can convert radicals into stable inert structures [32]. The reducing power of *R. canina* fruit and bile extracts was determined using CUPRAC and ferric reduction methods. Their antiradical abilities were investigated using the DPPH analysis method. The copper-reducing antioxidant capacity of the samples at different concentrations was measured following the appropriate procedure, and the results were presented

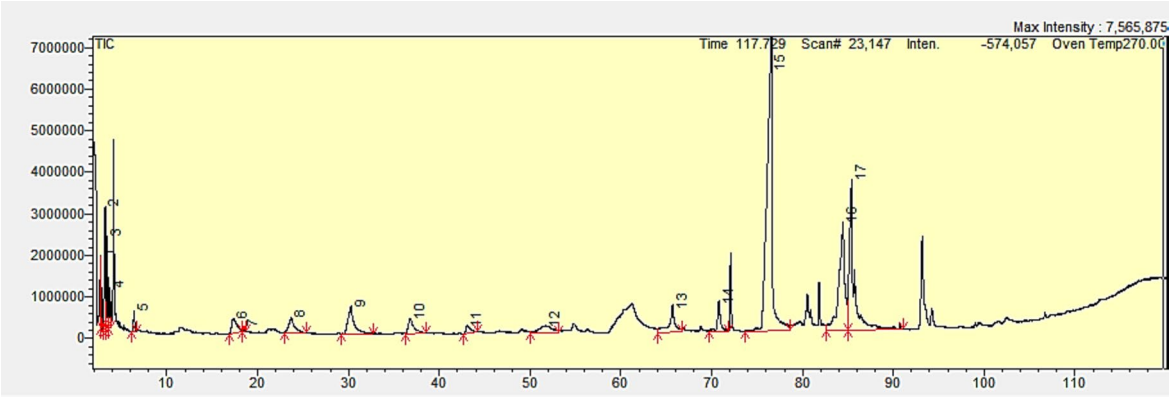


**Fig. 6** GC-MS profile of *R. canina* plant extract

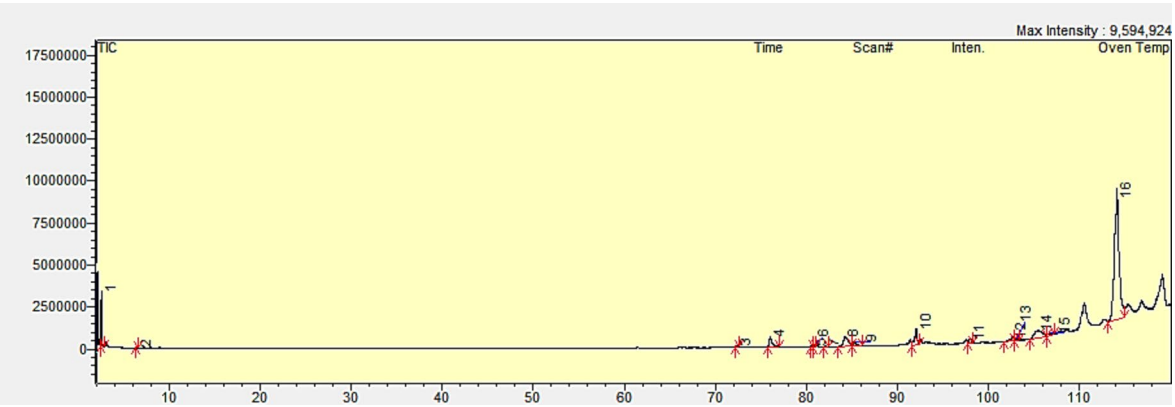


**Fig. 7** GC-MS profile of *R. canina* gall extract

as absorbance-concentration graphs (Fig. 4). It was found that there was a relationship between the Cu $^{2+}$ reducing effects in R and G extracts and the amount of phenolic compounds. On the other hand, phenolic compounds, especially in G extract, showed a significant absorption of reducing ability at 50 µg/mL. In the study, where BHT and Trolox were used as standards, the CUPRAC capacity at 50 µg/mL concentration was in the order of BHT (1.266 ± 0.005 > G (0.733 ± 0.012) > Trolox (0.563 ± 0.021) > R (0.133 ± 0.003). An increase in absorbance indicates an increase in reducing capacity. Here, the highest activity after BHT was observed in G.

FRAP analysis is a colorimetric analytical method applied to assess how well extracts reduce ferric ions. Depending on the reducing power of a sample, the reduction of $Fe^{+3}$ to $Fe^{+2}$ results in the reaction mixture's yellow color gradually shifting to a blue-green tone [32]. Oyaizu's ferric reduction method was used to determine the reducing capacity of the extracts. The addition of $Fe^{+3}$ to the mixture containing the extracts increases the amount of $Fe^{+2}$ formed, resulting in maximum absorption at 700 nm. The ferric reducing antioxidant power (FRAP) of the samples was determined in the study (Fig. 5). The $Fe^{3+}$ reducing power of the samples increased with increasing concentration. According to the results obtained, G showed a higher iron-reducing capacity than BHT, Trolox, and R. The order of iron-reducing effectiveness was G > BHT > Trolox > R. In addition, FRAP activity has been expressed in terms of Trolox equivalent antioxidant capacity (TEAC) (Table 1). This value was obtained by calculating the ratio between the slope of the Trolox calibration curve and the slopes of the sample extract curves. Upon examination of the TEAC values derived from the FRAP method, it was determined that BHT, a synthetic antioxidant widely used as a food preservative additive, exhibited an antioxidant activity approximately 1.48 times higher than an equivalent amount of Trolox. In contrast, the G sample demonstrated an antioxidant activity 1.82 times higher than the reference compound Trolox. These findings indicate that the G sample possesses a stronger antioxidant potential than both the synthetic antioxidant BHT and the standard reference Trolox.

In this measurements related to reducing activity, absorbance measurements increased with increasing concentration. Görmez (2022) reported that CUPRAC activity increased in parallel with concentration increase in their study with *R. canina* fruits [41]. Our result is consistent with

**Table 2** GC-MS analysis results of *R. canina* fruit and gall extracts

| No | Name | | *R* | G | Areas of use |
|---|---|---|---|---|---|
| 1 | 1,2,3-Propanetriol (CAS) | *RT* | 2.707 | 2.504 | In the field of food and industry |
| | | A | 1.18 | 2.17 | |
| | | *H* | 7.13 | 22.24 | |
| 2 | Acetic acid (CAS) | *RT.* | 3.203 | - | |
| | | *A* | 2.67 | - | nf |
| | | *H* | 12.07 | - | |
| 3 | 1-propylmethyl ether | *RT* | 3.374 | - | |
| | | *A* | 2.15 | - | nf |
| | | *H* | 9.10 | - | |
| 4 | Acetic acid, hydroxy-, methyl ester (CAS) | *RT* | 3.378 | - | |
| | | *A* | 1.32 | - | nf |
| | | *H* | 3.91 | - | |
| 5 | 2-Cyclopenten-1-one | *RT* | 6.351 | - | |
| | | *A* | 0.61 | - | nf |
| | | *H* | 2.03 | - | |
| 6 | Nonanoic acid | *RT* | 17.304 | - | |
| | | *A* | 1.98 | - | nf |
| | | *H* | 1.40 | - | |
| 7 | (E)−3-(Dimethylamino)−2-pentene | *RT* | 18.372 | - | |
| | | *A* | 0.22 | - | nf |
| | | *H* | 0.54 | - | |
| 8 | Heptanoic acid | *RT* | 23.733 | - | |
| | | *A* | 2.23 | - | nf |
| | | *H* | 1.48 | - | |
| 9 | Octanoic acid (CAS) | *RT* | 30.332 | - | |
| | | *A* | 4.94 | - | nf |
| | | *H* | 2.75 | - | |
| 10 | Nonanoic acid (CAS) | *RT* | 36.836 | - | |
| | | *A* | 2.06 | - | nf |
| | | *H* | 1.44 | - | |
| 11 | n-Decanoic acid | *RT* | 43.122 | - | |
| | | *A* | 0.92 | - | nf |
| | | *H* | 0.74 | - | |
| 12 | 2-Amino-9-(3,4-Dihydroxy-5-Hydroxymethyl-Tetrahydro- | *RT* | 51.752 | - | |
| | | *A* | 2.28 | - | nf |
| | | *H* | 0.67 | - | |
| 13 | Tetradecanoic acid (CAS) | *RT* | 65.738 | - | Antifungal, antiviral, anticancer, antiparasitic, immunomodulating activities |
| | | *A* | 3.71 | - | |
| | | *H* | 2.51 | - | |
| 14 | Pentadecanoic acid | *RT* | 70.834 | - | |
| | | *A* | 2.54 | - | In the fields of industry |
| | | *H* | 2.66 | - | |
| 15 | n-Hexadecanoic acid | *RT* | 76.551 | 76.051 | Antioxidant, anticancer activity |
| | | *A* | 39.14 | 3.05 | |
| | | *H* | 28.14 | 4.34 | |
| 16 | 9-Octadecanoic acid, (E)- | *RT* | 84.476 | - | |
| | | *A* | 15.50 | - | nf |
| | | *H* | 9.79 | - | |
| 17 | Octadecanoic acid (CAS) | *RT* | 85.380 | 85.289 | In the fields of pharmaceuticals, cosmetics, and industry |
| | | *A* | 17.25 | 1.57 | |
| | | *H* | 13.64 | 1.83 | |
| 18 | 3,3-Dimethoxy-2-butanone | *RT* | - | 6.408 | |
| | | *A* | - | 0.02 | nf |

**Table 2** (continued)

| No | Name | | *R* | G | Areas of use |
|---|---|---|---|---|---|
| | | *H* | | 0.20 | |
| 19 | Hexadecanoic acid, methyl ester | *RT* | - | 72.350 | Pharmacology, bioactivity |
| | | *A* | - | 0.28 | |
| | | *H* | - | 0.77 | |
| 20 | 9,12-Octadecadienoic acid, methyl ester, (E, E)- | *RT* | - | 80.558 | nf |
| | | *A* | - | 0.23 | |
| | | *H* | - | 0.47 | |
| 21 | 9-Octadecenoic acid, (Z)-, methyl ester | *RT* | - | 80.831 | Bioactivity |
| | | *A* | - | 0.49 | |
| | | *H* | - | 1.01 | |
| 22 | Heptadecanoic acid, 16-methyl-, methyl ester (CAS) | *RT* | - | 82.100 | nf |
| | | *A* | - | 0.10 | |
| | | *H* | - | 0.26 | |
| 23 | Oleic acid | *RT* | - | 85.251 | |
| | | *A* | - | 5.62 | Bioactivity |
| | | *H* | - | 3.96 | |
| 24 | Vitamin E | *RT* | - | 92.103 | |
| | | *A* | - | 4.94 | Bioactivity |
| | | *H* | - | 6.46 | |
| 25 | 2-methyloctacosane | *RT* | - | 98.018 | |
| | | *A* | - | 1.51 | Antimicrobial |
| | | *H* | - | 1.95 | |
| 26 | Eicosane | *RT* | - | 102.622 | Antifungal, skin regenerating, antibacterial, anti-inflammatory, antioxidant |
| | | *A* | - | 1.80 | |
| | | *H* | - | 1.37 | |
| 27 | Nanodecane (CAS) | *RT* | - | 103.095 | |
| | | *A* | - | 0.74 | nf |
| | | *H* | - | 1.10 | |
| 28 | Beta-Sitosterol | *RT* | - | 105.482 | Anti-inflammatory, antimicrobial, anti-diabetic, antioxidant, hepatoprotective |
| | | *A* | - | 6.99 | |
| | | *H* | - | 2.97 | |
| 29 | Docosane (CAS) | *RT* | - | 106.885 | |
| | | *A* | - | 1.08 | nf |
| | | *H* | - | 0.83 | |
| 30 | 2-methyloctacosane | *RT* | - | 114.214 | |
| | | *A* | - | 69.41 | Antimicrobial |
| | | *H* | - | 50.24 | |

*:RT: Retention time, A: Area%, H: Height%

*:R: *R. canina* fruit, G: *R. canina* gall, nf: not found

the literature. Furthermore, the fact that the G group was higher than R at all concentrations can be explained by the possibility that the enzymes and chemicals secreted by the plant during gall formation and accumulated in galled fruits may have high reducing properties.

GC/MS analyses were performed on the gall and rosehip samples in this study (Figs. 6 and 7, and Table 2). The analysis revealed 17 compounds in R. In R, the compounds with the highest area percentages were n-hexadecanoic acid (39.14%), octadecanoic acid (17.25%), and 9-octadecanoic acid (15.50%). Cömlekcioglu et al. (2022) identified 15 fatty acids in their study on dried fruits of *R. canina*, reporting the most abundant as oleic acid (24.13 ± 0.71%), linoleic acid (60.37 ± 1.85%), palmitic acid (4.32 ± 0.6%), and gamma-linolenic acid (7.36 ± 0.54%) [42]. The primary fatty acids in wild rosehip fruits have been reported as linoleic (37.07%), oleic (17.58%), linolenic (9.78%), and palmitic (7.21%) acids [43]. Furthermore, Babalau Fuss et al. (2021) reported the major fatty acids in rosehip fruits as linoleic acid (62.3%), oleic acid (28.6%), and palmitic acid (6.22%)

[44]. The major components found in our study differ from these previous findings.

GC-MS analysis revealed 16 compounds in G. The compounds with the highest area percentages in the bile extract were identified as 2-methyloctacosane (50.24%), 1,2,3-propanetriol (22.24%), and oleic acid (3.96%). Haghparasti et al. (2023) reported that *R. canina* fruit galls contained oleic acid (36.66%), palmitic acid (14.40%), octadecenoic acid (13.21%), benzenetriol (2.46%), valeric acid (2.21%), methyl palmitate (2.09%), pyrogallol (1.92%), and stearic acid (0.59%). The major components found in our study differ from those reported in the literature. Similar components show proportional differences. Examining the fatty acid profiles obtained from plants is important as it provides information about the specific roles of fatty acid types in health and diseases. The difference in fatty acid compositions found in fruit and gall extracts may be attributed to different biosynthesis and accumulation stages of these compounds [45].

The compounds identified through GC-MS analysis in the gall extract have various biological applications. 2-methyloctacosane, which was found in the highest proportion in the gall extract, has been reported to possess antimicrobial properties [46]. Research conducted by Pelo et al. (2021) identified 2-methyloctacosane as a compound with antimicrobial properties. Their findings demonstrated its effectiveness against a diverse range of microorganisms, including both fungal and bacterial species [47]. 1,2,3-propanetriol (glycerol), found at 22.24% in the extract, is used as a humectant, solvent, and sweetener in foods and beverages. It also aids in food preservation [48]. Glycerol and its derivatives are also used as plasticizing materials in the edible film and coating industry [49]. Hexadecanoic acid, identified in the GC-MS analysis of the gall extract, is used as a surfactant, emulsifier, and softener in cosmetic products. Bharath et al. reported that hexadecanoic acid isolated from *T. ornata* exhibited potential antioxidant and anticancer activities [50]. Additionally, octadecanoic acid is known to play a crucial role in defense against ultraviolet wavelengths [51]. Eicosane, detected at 1.37% in the extract, has been reported to possess antifungal, skin regenerative, antibacterial, anti-inflammatory, and antioxidant properties [47, 52, 53]. Beta-sitosterol was identified at 2.97%. It is the most common phytosterol used in herbal supplements for prostate health maintenance. Herbal supplements containing beta-sitosterol are among the best-selling herbal supplements in the USA [54]. Furthermore, beta-sitosterol has been reported to show positive effects on breast, colon, prostate, and kidney cancer types and to possess anti-inflammatory, antimicrobial, antidiabetic, antioxidant, and hepatoprotective effects [55].

In this study, total phenolic and flavonoid content and antioxidant activities were evaluated in *R. canina* ripe fruit and gal formation. Our results have contributed to our knowledge of the compounds present in gal and ripe fruit extracts. It was determined that both had antioxidant activity and contained high levels of phenolic and flavonoid substances. However, all values in gal formation were found to be higher than the values in ripe fruit. The results found in the study support the hypothesis that plants secrete various bioactive compounds as a defense mechanism during gall formation. As a result, new studies are needed to investigate the gal structures formed on plants and to determine the possibilities of their use.

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**Data availability** Data will be made available on reasonable request.

## Declarations

**Conflict of interest** The authors declare no competing interests.

**Consent for publication** All authors have agreed to publish the paper.



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