



Evaluation of total phenolic and flavonoid contents and DPPH radical scavenging activity in *Cotoneaster coriaceus* Franch. extracts

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Cotoneaster coriaceus Franch. ekstraktlarında toplam fenolik ve flavonoid içeriklerinin ve DPPH radikal temizleme aktivitesinin değerlendirilmesi

Abstract: This study aimed to evaluate the total phenolic and flavonoid contents, as well as the antioxidant capacities, of *Cotoneaster coriaceus* Franch. fruit and leaf extracts obtained using different solvents (acetone, ethyl acetate, and water). The highest levels of total phenolics ($996.41 \pm 8.30 \mu\text{g GAE/mg extract}$) and flavonoids ($403.53 \pm 2.04 \mu\text{g QE/mg extract}$) were detected in the leaf acetone extract. The leaf water and fruit acetone extracts also exhibited substantial phenolic and flavonoid contents. Among fruit extracts, acetone extract showed the highest phenolic ($773.41 \pm 12.67 \mu\text{g GAE/mg extract}$) and flavonoid ($135.28 \pm 3.55 \mu\text{g QE/mg extract}$) concentrations. The leaf ethyl acetate extract had a notably high flavonoid content ($339.95 \pm 3.97 \mu\text{g QE/mg extract}$) despite its lower phenolic level. The antioxidant capacities of the extracts were assessed using the DPPH radical scavenging assay at various concentrations ($12.5\text{--}400 \mu\text{g/mL}$). All extracts exhibited a concentration-dependent increase in antioxidant activity ($p < 0.05$), with the acetone extracts showing the highest scavenging capacity. The leaf acetone extract displayed $>92\%$ activity at $100 \mu\text{g/mL}$. IC_{50} values further confirmed the antioxidant strength, with the lowest IC_{50} ($10.55 \mu\text{g/mL}$) observed in the leaf acetone extract, followed by the leaf water and fruit acetone extracts. Overall, these findings suggest that both plant part and solvent type significantly influence the extraction of bioactive compounds, with leaf acetone extracts offering the most promising antioxidant potential.

Key words: Bioactive compounds, natural extracts, phytochemicals, polyphenols

Özet: Bu çalışma, *Cotoneaster coriaceus* Franch. meyve ve yaprak ekstraktlarının toplam fenolik ve flavonoid içerikleri ile antioksidan kapasitelerini farklı çözücüler (asetonu, etil asetat ve su) kullanarak değerlendirmeyi amaçlamıştır. En yüksek toplam fenolik ($996.41 \pm 8.30 \mu\text{g GAE/mg ekstrakt}$) ve flavonoid ($403.53 \pm 2.04 \mu\text{g QE/mg ekstrakt}$) düzeyleri yaprak aseton ekstraktında tespit edilmiştir. Yaprak su ekstraktı ve meyve aseton ekstraktı da kayda değer fenolik ve flavonoid içeriklerine sahipti. Meyve ekstraktları arasında, aseton ekstraktı en yüksek fenolik ($773.41 \pm 12.67 \mu\text{g GAE/mg ekstrakt}$) ve flavonoid ($135.28 \pm 3.55 \mu\text{g QE/mg ekstrakt}$) konsantrasyonlarını göstermiştir. Yaprak etil asetat ekstraktı düşük fenolik içeriğe rağmen oldukça yüksek flavonoid içeriği ($339.95 \pm 3.97 \mu\text{g QE/mg ekstrakt}$) sergilemiştir. Ekstraktların antioksidan kapasiteleri, çeşitli konsantrasyonlarda ($12.5\text{--}400 \mu\text{g/mL}$) DPPH radikal giderme testi ile değerlendirilmiştir. Tüm ekstraktlar, konsantrasyona bağlı olarak artan antioksidan aktivite göstermiştir ($p < 0.05$) ve en yüksek radikal giderme kapasitesi aseton ekstraktlarında gözlemlenmiştir. Yaprak aseton ekstraktı, $100 \mu\text{g/mL}$ konsantrasyonda $\%92$ 'nin üzerinde aktivite göstermiştir. IC_{50} değerleri de antioksidan etkinliği desteklemiş, en düşük IC_{50} değeri ($10.55 \mu\text{g/mL}$) yaprak aseton ekstraktında, ardından yaprak su ve meyve aseton ekstraktlarında gözlemlenmiştir. Genel olarak, bu bulgular bitki kısmı ve çözücü tipinin biyolojik olarak aktif bileşiklerin ekstraksiyonunda önemli rol oynadığını, özellikle yaprak aseton ekstraktının en yüksek antioksidan potansiyele sahip olduğunu göstermektedir.

Anahtar Kelimeler: Biyoaktif bileşikler, doğal ekstraktlar, fitokimyasallar, polifenoller

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1. Introduction

Antioxidants are compounds that play a crucial role in protecting cells by preventing oxidative damage caused by free radicals (Kıran et al., 2023; Ashraf et al., 2024). Oxidative stress is widely recognized as a key factor in the pathogenesis of various chronic diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions (Houldsworth, 2024; Muscolo et al., 2024; Selvaraj et al., 2025). Therefore, the investigation of antioxidants derived from natural sources is of great importance, both for the development of potential therapeutic agents and for their use as dietary supplements (Akbari et al., 2022; Mukherjee et al., 2024).

Naturally derived antioxidant compounds are generally considered safer and biologically more effective alternatives to synthetic antioxidants due to their structural diversity and bioavailability (Gutiérrez-del-Río et al., 2021; Stoia and Oancea, 2022). Plant-based materials, particularly those rich in phenolic compounds and flavonoids, offer a wide range of bioactive constituents with strong antioxidant capacity (Awad et al., 2021; Zhao et al., 2021; Sawicki et al., 2024). Phenolic compounds possess free radical scavenging activity due to the presence of hydroxyl groups, while flavonoids can mitigate oxidative damage through various mechanisms, such as metal ion chelation and inhibition of reactive oxygen species (ROS) formation (Hajam et al., 2023; Rao and Zheng, 2025). In

recent years, a growing number of scientific reports have focused on the antioxidant properties of medicinal plants (Asraoui et al., 2021; Ed-Dahmani et al., 2024; Doga Atıcı et al., 2025).

Cotoneaster coriaceus Franch., a member of the Rosaceae family (Kicel, 2020). Species belonging to the *Cotoneaster* genus are known to be rich in phenolic and flavonoid compounds and have been reported to exhibit a range of biological activities (Kicel et al., 2018a; Kicel et al., 2018b). However, data on the antioxidant potential of *C. coriaceus*, particularly regarding its leaf and fruit extracts, remain scarce in the scientific literature.

Therefore, a detailed investigation of the antioxidant potential of *C. coriaceus* is essential to reveal its pharmacological significance. In the present study, the antioxidant activity of *C. coriaceus* leaf and fruit extracts was evaluated, and their total phenolic and flavonoid contents were determined using spectrophotometric methods. The results obtained from this study will help assess the potential of this plant as a natural source of antioxidants.

2. Materials and Method

2.1. Collection and Extraction of Plant Material

Samples of *C. coriaceus* were collected in February, 2025 from Karaman, located in the Central Anatolia Region of Türkiye (37°10'N-33°15'E). The plant material was first air-dried at room temperature, then ground into a fine powder using an ultra-centrifugal mill. Powdered samples (10 g) were individually extracted with 250 mL of acetone, ethyl acetate, and water for 2 days employing a Soxhlet apparatus. The crude extracts were filtered through Whatman No. 1 filter paper. Following filtration, the solvents were removed under reduced pressure using a rotary evaporator. Finally, the dried extracts were subjected to lyophilization to produce ultra-dry powder forms.

2.2. Quantification of Total Phenolic Compounds

To assess the total phenolic content in the plant extracts, we employed a modified Folin-Ciocalteu assay with gallic acid as the reference standard. In a microplate, 20 µL of each extract (at a concentration of 400 µg/mL) and the gallic acid standard were placed into individual wells. To initiate the reaction, 20 µL of Folin-Ciocalteu reagent was added to

each well, followed by a 3-minute incubation in the dark. This step allows the phenolic compounds to reduce the Folin reagent. Afterward, 20 µL of sodium carbonate (Na_2CO_3) solution was added to each well to neutralize the reaction, and 140 µL of distilled water (dH_2O) was introduced to complete the mixture. This process facilitates the development of color, which is indicative of the phenolic content. The wells were then left in the dark for an additional 10 minutes to ensure color formation. After incubation, absorbance was measured at 725 nm using a spectrophotometer. The intensity of the resulting color correlates directly with the phenolic content in the samples. The phenolic content was calculated in terms of gallic acid equivalents (GAE) using a standard calibration curve (Fig. 1a) constructed from known concentrations of gallic acid (Kok et al., 2023).

2.3. Quantification of Total Flavonoid Compounds

To measure the total flavonoid content in the extracts, a modified aluminum chloride colorimetric method was employed, using quercetin as the reference standard. In separate wells of a microplate, 50 µL of each extract (400 µg/mL) and the quercetin standard were added. To facilitate the reaction and dissolve the flavonoid compounds, 215 µL of 80% ethyl alcohol was introduced into each well. Subsequently, 5 µL of aluminum nitrate solution and 5 µL of potassium acetate were added to each well to complete the reaction mixture. The plates were incubated at room temperature for 40 minutes in the dark, allowing the formation of the aluminum-flavonoid complex, which is necessary for accurate detection. After the incubation period, the absorbance of the solutions was measured at 415 nm using a spectrophotometer. The absorbance values obtained were directly correlated with the flavonoid content in the extracts. The flavonoid content was expressed as quercetin equivalents (QE), which was calculated from a standard calibration curve (Fig. 1b) created using known concentrations of quercetin (Kok et al., 2023).

2.4. Free Radical Scavenging Activity

In the measurement of DPPH scavenging activity of acetone and water extracts obtained from the plants, applications were carried out with the final concentrations of the extracts in the plate wells of 12.5, 25, 50, 100, 200, and 400 µg/mL. According to the method, 20 µL of the

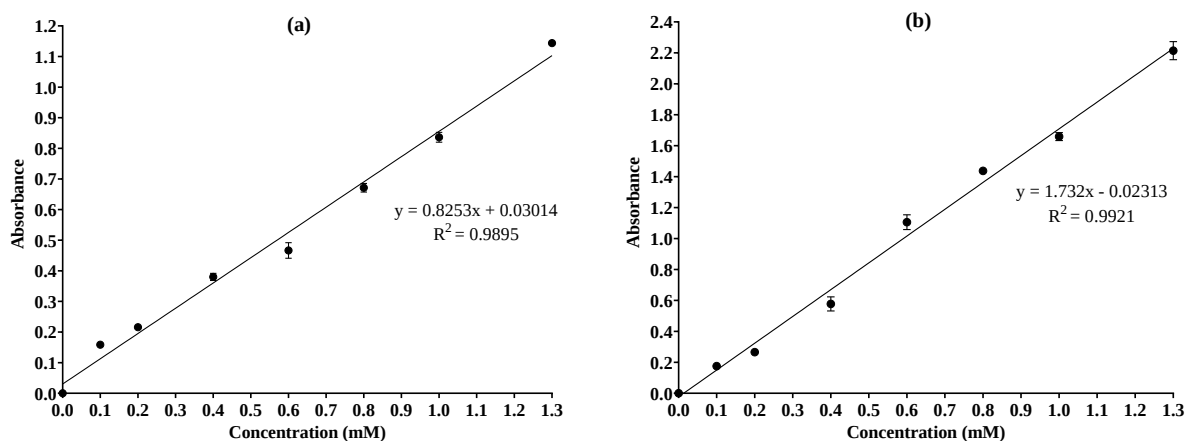


Figure 1. (a) Linear regression graphs for gallic acid, used as a standard in calculating total phenolic content, and (b) quercetin, used as a standard in calculating total flavonoid content ($n = 3$).

extracts were placed in each microplate well, and 180 μL of DPPH (0.06 mM in methanol) was added. The reduction of DPPH free radical was determined by measuring the absorbance values at 517 nm after 60 minutes in the dark. The free radical scavenging activities of the extracts were calculated as a percentage using the following formula (Kok et al., 2023).

$$\text{Radical scavenging activity (\%)} = \left[\frac{\text{Control absorbance} - \text{Extract absorbance}}{\text{Control absorbance}} \right] \times 100$$

2.5. Statistical Analyses

The activities of the extracts were evaluated through a one-way ANOVA followed by the Duncan test. Probit regression analysis was applied to calculate the median inhibitory concentration (IC_{50}) values. These statistical analyses were conducted using SPSS software (version 27.0, IBM Corporation, Armonk, NY, USA). Additionally, heatmap and hierarchical cluster analyses, employing Ward's minimum variance method, were used to identify similarities and differences among DPPH activities of the different extracts. These analyses were executed in the Rstudio console with R software (version 4.1.0).

3. Results

3.1. Analysis of Antioxidant Compounds of the Extracts

The total phenolic and flavonoid contents of *C. coriaceous* fruit and leaf extracts are presented in Table 1. Among all extracts, the leaf acetone extract exhibited the highest total phenolic content ($996.41 \pm 8.30 \mu\text{g GAE/mg extract}$) and total flavonoid content ($403.53 \pm 2.04 \mu\text{g QE/mg extract}$). This was followed by the leaf water extract and fruit acetone extract, which also showed considerable amounts of both phenolic and flavonoid compounds.

In fruit extracts, the acetone extract contained the highest levels of total phenols ($773.41 \pm 12.67 \mu\text{g GAE/mg extract}$) and flavonoids ($135.28 \pm 3.55 \mu\text{g QE/mg extract}$), compared to ethyl acetate and water extracts. Similarly, in leaf extracts, while the acetone extract was the most abundant in phenolic and flavonoid content, the ethyl acetate extract showed notably high flavonoid content ($339.95 \pm 3.97 \mu\text{g QE/mg extract}$) despite its relatively low phenolic content ($265.25 \pm 9.36 \mu\text{g GAE/mg extract}$).

These results indicate that both solvent type and plant part significantly influence the accumulation of antioxidant compounds, with acetone proving to be the most effective solvent for extracting phenolic and flavonoid compounds, particularly from leaves.

Table 1. Antioxidant compounds of *C. coriaceous* extracts ($\mu\text{g/mg}$)

Treatment	Total phenol (Gallic acid equivalent)	Total flavonoid (Quercetin equivalent)
F-AE	773.41 ± 12.67	135.28 ± 3.55
F-EAE	380.92 ± 4.07	75.12 ± 3.93
F-WE	325.82 ± 3.28	70.87 ± 2.03
L-AE	996.41 ± 8.30	403.53 ± 2.04
L-EAE	265.25 ± 9.36	339.95 ± 3.97
L-WE	568.12 ± 11.86	136.34 ± 5.14

F-AE: Acetone extract of the fruit of *C. coriaceous*; F-EAE: Ethyl acetate extract of the fruit of *C. coriaceous*; F-WE: Water extract of the fruit of *C. coriaceous*; L-AE: Acetone extract of the leaves of *C. coriaceous*; L-EAE: Ethyl acetate extract of the leaves of *C. coriaceous*; L-WE: Water extract of the leaves of *C. coriaceous*. Each value is expressed as mean $\pm$ standard deviation ($n = 3$)

3.2. Assessing DPPH Scavenging Abilities of the Extracts

The antioxidant capacities of fruit-derived extracts were evaluated using the DPPH radical scavenging assay. Extracts prepared with different solvents (acetone, ethyl acetate, and water) were tested at various concentrations ranging from 12.5 to 400 $\mu\text{g/mL}$.

In all extracts, a concentration-dependent increase in DPPH scavenging activity was observed ($p < 0.05$). The highest activity was recorded in the acetone extract at 400 $\mu\text{g/mL}$, showing over 95% radical scavenging. This was followed by the water and ethyl acetate extracts, respectively. At lower concentrations (12.5–50 $\mu\text{g/mL}$), all extracts showed relatively low scavenging activity.

When comparing different extracts at the same concentrations, the acetone extract consistently demonstrated the highest antioxidant activity, followed by the water extract, while the ethyl acetate extract exhibited the lowest activity. Statistical analysis indicated significant differences between groups, as denoted by different letters above the bars ($p < 0.05$). These results suggest that the fruit extracts, particularly those obtained with acetone, possess strong antioxidant potential (Fig. 2).

The antioxidant activities of leaf-derived extracts were assessed using the DPPH radical scavenging assay across a range of concentrations (12.5–400 $\mu\text{g/mL}$) and three solvent types: acetone, ethyl acetate, and water. As with the fruit extracts, a dose-dependent increase in radical scavenging activity was observed in all extracts ($p < 0.05$). The acetone extract displayed the highest scavenging activity, achieving over 90% inhibition at concentrations of 100 $\mu\text{g/mL}$ and above. This was followed by the water extract, while the ethyl acetate extract showed comparatively lower activity across all concentrations.

Notably, the acetone extract reached near-maximal activity ($>92\%$) at concentrations as low as 100 $\mu\text{g/mL}$. The water extract also exhibited strong activity, with over 90% scavenging at 400 $\mu\text{g/mL}$, whereas the ethyl acetate extract required higher concentrations to achieve moderate levels of activity. At the lowest concentrations (12.5 and 25 $\mu\text{g/mL}$), all extracts showed limited antioxidant potential.

Statistical analysis revealed significant differences among treatments, as indicated by the lettering above the bars ($p <$

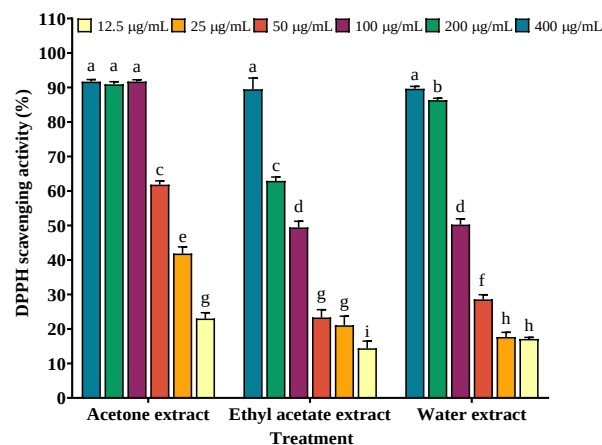


Figure 2. DPPH radical scavenging activity of different fruit extracts is presented as mean $\pm$ standard deviation ($n = 3$). Distinct letters indicate statistically significant differences at $p < 0.05$.

0.05). The acetone extract consistently outperformed the others at comparable concentrations. These findings suggest that leaf extracts, particularly when obtained using acetone, possess potent antioxidant capacity (Fig. 3).

The heatmap based on DPPH scavenging activity (%) provided a detailed comparison of the antioxidant capacities of *C. coriaceus* fruit and leaf extracts across different concentrations. A clear clustering pattern was observed, where acetone extracts of both fruit and leaves exhibited consistently high scavenging activity, particularly at higher concentrations (100–400 µg/mL), as indicated by the intense red color blocks. Leaf acetone extract showed the most prominent red intensity across nearly all concentrations, reflecting its superior radical scavenging potential. In contrast, ethyl acetate extracts displayed weaker DPPH activity, with white and purple color tones especially dominant at lower concentrations (12.5–50 µg/mL), indicating minimal scavenging effect. Water extracts demonstrated moderate activity, with leaf water extract showing stronger responses than its fruit counterpart in higher concentrations. In the analysis consisting of three clusters, the leaf acetone extract was grouped in a separate cluster among all extracts. This visualization reinforces the concentration-dependent nature of the antioxidant response and supports earlier quantitative findings, confirming that acetone extracts—particularly from leaves—are the most potent in neutralizing free radicals via the DPPH assay (Fig. 4).

The IC₅₀ values obtained from the DPPH scavenging activities of *C. coriaceus* extracts are presented in Table 2. Among all tested samples, the leaf acetone extract demonstrated the strongest antioxidant activity, with the lowest IC₅₀ value of 10.55 µg/mL (95% CI: 8.21–12.94), indicating high free radical scavenging potential. This was followed by the leaf water extract and fruit acetone extract, with IC₅₀ values of 34.03 µg/mL and 31.15 µg/mL, respectively. In contrast, the fruit ethyl acetate extract exhibited the weakest activity, with an IC₅₀ value of 97.27 µg/mL, followed by the leaf ethyl acetate extract and fruit water extract, with IC₅₀ values of 69.85 µg/mL and 75.51 µg/mL, respectively. The slope values obtained from the probit analysis of DPPH scavenging activities of *C. coriaceus* extracts ranged from 1.22 to 1.90. Among the extracts, the lowest slope value was observed in L-AE (1.22

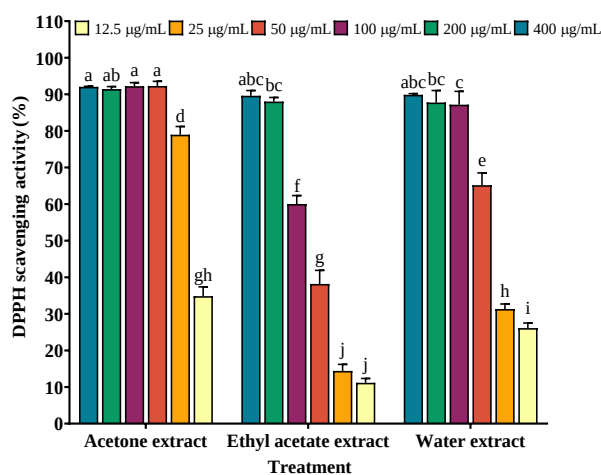


Figure 3. DPPH radical scavenging activity of different leaf extracts is presented as mean ± standard deviation ($n = 3$). Distinct letters indicate statistically significant differences at $p < 0.05$.

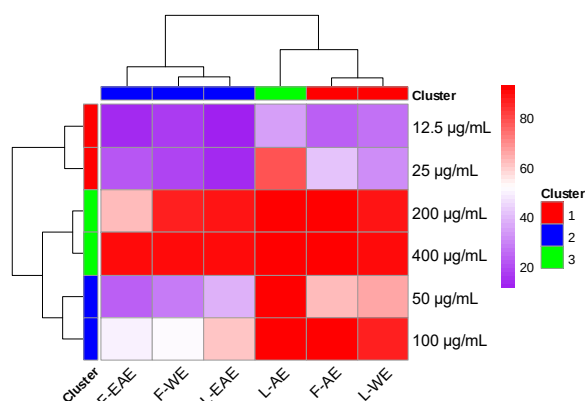


Figure 4. Heatmap and hierarchical clustering analyses of DPPH scavenging activities (%) of different concentrations of plant-derived extracts. Red represents high activity levels, whereas purple corresponds to low activity levels. F-AE: Acetone extract of the fruit of *C. coriaceus*; F-EAE: Ethyl acetate extract of the fruit of *C. coriaceus*; F-WE: Water extract of the fruit of *C. coriaceus*; L-AE: Acetone extract of the leaves of *C. coriaceus*; L-EAE: Ethyl acetate extract of the leaves of *C. coriaceus*; L-WE: Water extract of the leaves of *C. coriaceus*.

± 0.07), indicating a more gradual dose–response curve, while the highest slope value was recorded for L-EAE (1.90 ± 0.07), reflecting a steeper concentration-dependent scavenging effect. Fruit extracts exhibited slope values between 1.47 and 1.69, suggesting moderate response curves with relatively consistent activity across the tested concentrations. In contrast, leaf extracts showed a wider variation in slope values, implying differences in the homogeneity and sensitivity of antioxidant responses depending on the solvent used. Overall, the slope values confirmed that the IC₅₀ estimates were obtained from well-fitted dose–response curves with acceptable levels of precision.

Overall, these findings reveal that both plant part and extraction solvent significantly affect the antioxidant capacity, with leaf extracts—especially those obtained using acetone—demonstrating superior free radical scavenging efficiency.

4. Discussions

In this study, the antioxidant potential of *C. coriaceus* fruit and leaf extracts was systematically evaluated through DPPH radical scavenging activity, IC₅₀ values, and total phenolic and flavonoid content.

The DPPH assay results revealed that all extracts exhibited

Table 2. IC₅₀ values (µg/mL) resulting from DPPH scavenging activities of *C. coriaceus* extracts

Treatment	IC ₅₀ (Limits)	Slope ± Standard error of the mean (Limits)
F-AE	31.15 (27.93–34.48)	1.64 ± 0.07 (1.49–1.79)
F-EAE	97.27 (87.94–108.02)	1.47 ± 0.06 (1.34–1.61)
F-WE	75.51 (69.04–82.67)	1.69 ± 0.07 (1.55–1.83)
L-AE	10.55 (8.21–12.94)	1.22 ± 0.07 (1.07–1.37)
L-EAE	69.85 (64.33–75.84)	1.90 ± 0.07 (1.75–2.05)
L-WE	34.03 (30.38–37.82)	1.52 ± 0.08 (1.38–1.66)

F-AE: Acetone extract of the fruit of *C. coriaceus*; F-EAE: Ethyl acetate extract of the fruit of *C. coriaceus*; F-WE: Water extract of the fruit of *C. coriaceus*; L-AE: Acetone extract of the leaves of *C. coriaceus*; L-EAE: Ethyl acetate extract of the leaves of *C. coriaceus*; L-WE: Water extract of the leaves of *C. coriaceus*.

dose-dependent scavenging activity. Among the extracts, the acetone extracts of both fruit and leaves demonstrated superior antioxidant capacity, with inhibition rates exceeding 90% at higher concentrations. Particularly, the leaf acetone extract showed the highest DPPH scavenging activity and the lowest IC₅₀ value (10.55 µg/mL), indicating a strong free radical neutralization capacity. These findings are consistent with previous reports that highlighted the high efficacy of acetone as a solvent for extracting polyphenolic compounds due to its intermediate polarity (Rajhard et al., 2021; Palaogiannis et al., 2023).

The IC₅₀ values, which reflect the concentration required to scavenge 50% of DPPH radicals, further supported the DPPH assay results. Lower IC₅₀ values indicate stronger antioxidant capacity, and in this study, the IC₅₀ values were markedly lower in acetone extracts compared to ethyl acetate and water extracts. Similar observations have been made in studies on other medicinal plants, where acetone extracts often outperformed aqueous and semi-polar extracts in radical scavenging assays (Ivesa et al., 2024; Dahmouni et al., 2025).

The strong antioxidant activity of the leaf acetone extract can be attributed to its remarkably high total phenolic (996.41 µg GAE/mg) and flavonoid (403.53 µg QE/mg) contents. Numerous studies have shown a direct correlation between total phenolic and flavonoid contents and antioxidant activity, as these compounds are known to donate electrons and hydrogen atoms to stabilize free radicals (Parcheta et al., 2021; Hassanpour and Doroudi, 2023). In this context, the positive relationship observed in this study between polyphenol content and DPPH scavenging activity aligns well with previous literature.

Interestingly, although the leaf ethyl acetate extract exhibited relatively low phenolic content (265.25 µg GAE/mg), it had a high flavonoid concentration (339.95 µg QE/mg). This suggests that flavonoids may contribute significantly to antioxidant activity, even in the absence of high total phenolic content, as reported in earlier studies (Tumilaar et al., 2024). However, the DPPH scavenging activity of leaf ethyl acetate extract remained modest compared to leaf acetone extract and leaf water extract,

indicating that the flavonoid profile may be as important as the total quantity (Chatepa et al., 2024).

Water extracts of both fruit and leaves also exhibited moderate antioxidant activity. Although water is effective in extracting hydrophilic antioxidants such as tannins and certain phenolic acids (Molnar et al., 2024), its efficacy was lower than that of acetone in this study. Nonetheless, the leaf water extract demonstrated notable antioxidant activity (IC₅₀: 34.03 µg/mL), suggesting the presence of water-soluble phenolics that contribute to radical scavenging.

When comparing plant parts, leaf extracts consistently exhibited higher antioxidant potential than fruit extracts. This may be due to the higher metabolic activity and secondary metabolite accumulation in leaves, which serve as primary sites for photosynthesis and defense compound production (Yeshe et al., 2022; Goura et al., 2025). The significantly higher phenolic and flavonoid contents in leaves, especially in the acetone extract, further support this explanation.

Collectively, these findings highlight the strong antioxidant potential of *C. coriaceous*, particularly in its leaf extracts prepared using acetone. The results also emphasize the importance of solvent selection in maximizing the extraction of bioactive compounds. Given the increasing interest in natural antioxidants for pharmaceutical, cosmetic, and food applications, *C. coriaceous* may represent a valuable source of phenolic and flavonoid compounds with potent free radical scavenging abilities.

Further investigations involving the isolation and structural elucidation of individual bioactive constituents, as well as in vivo and cellular antioxidant assays, would be valuable to confirm the biological relevance and therapeutic potential of these extracts.

Conflict of Interest

There is no conflict of interest in any form between the authors.

Authors' Contributions

The authors contributed equally.

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