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Unveiling the antioxidant secrets: Phytochemical profiling and bioactive potential of water extracts from Muğla propolis samples

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Abstract

Propolis, a resinous substance collected by bees, is known for its diverse biological activities, including antioxidant properties, which are largely attributed to its phenolic and flavonoid content. This study aimed to investigate the chemical composition and antioxidant activities of water extracts from propolis samples collected from different locations in Muğla, Turkey. Total phenolic and flavonoid contents were quantified, and antioxidant activities were evaluated using various assays, including β-carotene bleaching, superoxide anion radical scavenging, DPPH radical scavenging, reducing power, and ferrous ion chelation. The total phenolic content of the samples ranged from 27.72 to 91.57 mg PEs/g extract, with Arpacık showing the highest phenolic content (91.57 mg PEs/g). In contrast, flavonoid content ranged from 9.33 to 25.78 mg QEs/g extract, with Fethiye exhibiting the highest value (25.78 mg QEs/g). The antioxidant assays revealed that the Fethiye extract consistently demonstrated the strongest activity, with a β-carotene inhibition rate of 96.73% and an IC50 of 49.50 µg/mL in the DPPH assay. Arpacık also showed considerable antioxidant capacity, albeit slightly lower than Fethiye, while the Dalaman and Döğüşbelen samples exhibited weaker activities. Correlation analysis indicated that total phenolic content had a strong positive correlation with DPPH scavenging (r = 0.994) and reducing power (r = 0.986), while flavonoid content was significantly correlated with superoxide anion scavenging (r = 0.931) and ferrous ion chelation (r = 0.894). These results suggest that phenolic and flavonoid compounds are key contributors to the antioxidant mechanisms in propolis. Future studies should explore the effects of different extraction methods and expand the geographical scope to better understand the factors influencing the bioactive composition of propolis.

Keywords: Propolis, Antioxidant activity, Muğla, Phenolic content.

1. Introduction

Propolis, a natural resinous substance that honeybees collect from the buds and secretions of specific plants and trees, is believed to serve as a defensive shield in the hive against potential threats. Traditionally, it has been employed in folk medicine across various cultures [1]. Numerous studies have reported its wide range of biological activities, including antibacterial [2], antiviral [3], anti-inflammatory [4], and anticancer [5,6] effects. Consequently, propolis is frequently incorporated into foods and beverages as a functional ingredient, aiming to promote health and help prevent conditions like inflammation, cardiovascular diseases, diabetes, and cancer [7].

Propolis typically comprises an array of chemical constituents, including polyphenols (such as flavonoids, phenolic acids, and their esters), terpenoids, steroids, and amino acids. The specific composition of propolis is largely influenced by the local plant life in the area from which it is collected [7]. Due to these geographical variations, the chemical makeup of propolis differs between samples from Europe, South America, and Asia [8]. European and Chinese propolis are rich in flavonoids and phenolic acid esters [9], whereas Brazilian propolis predominantly contains terpenoids and prenylated derivatives of *p*-coumaric acids [10,11]. These compositional differences result in varying biological activities depending on the region of origin. For instance, Miyataka et al. [12] found significant differences in the ability of Brazilian and Chinese propolis to inhibit hyaluronidase and release histamine from rat peritoneal mast cells when stimulated by compound 48/80 or concanavalin A [13]. In another study, Hegazi et al. [14] demonstrated that German propolis exhibited strong

antimicrobial effects against *Staphylococcus aureus* and *Escherichia coli*, while Austrian propolis was highly effective against *Candida albicans*. Additionally, Banskota et al. [15] reported that methanol extracts from Dutch and Chinese propolis showed potent cytotoxic effects on murine colon 26-L5 carcinoma cells, whereas the activity of Brazilian propolis varied between samples.

Propolis has been widely recognized for its antioxidant properties, as highlighted by numerous studies. In this study, we aim to assess the antioxidant potential of water extracts of propolis from different geographic regions of Muğla-Türkiye using *in vitro* methods. We employed assays such as superoxide anion and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, β-carotene bleaching, ferrous ion chelation, and reducing power assays to evaluate antioxidant performance. Additionally, total phenolic and flavonoid contents of the extracts were determined spectrophotometrically and the relationship between phenolic/flavonoid compounds contained in the extracts and antioxidant activity tests was revealed using statistical methods.

2. Materials and Methods

2.1. Sample collection and preparation of water extracts

Propolis samples were collected from four distinct locations in Muğla, Turkey: Arpacık, Dalaman, Döğüşbelen, and Fethiye. A 25 g sample of propolis was extracted in 250 mL of boiling deionized water for 30 minutes. Following extraction, each sample was centrifuged at 4000 rpm for 10 minutes. The resulting extracts were then filtered through filter paper. The water extracts of propolis from Arpacık, Dalaman, Döğüşbelen, and Fethiye were subsequently dried using a freeze-dryer, yielding extract percentages of 5.90%, 3.11%, 5.18%, and 5.79% (w/w), respectively.

2.2. Assay for total phenolics and flavonoids

Total phenolic constituent of the extracts were determined by employing the methods given in the literature [16]. Pyrocatechol was used as a standard agent and the results were calculated as pyrocatechol equivalents (mg PEs/g extract). Total flavonoid constituent was determined using the Dowd method as adapted by Zengin et al. [17]. The results were given as quercetin equivalents (mg QEs/g extract). Both test conditions were provided in the supplementary file.

2.3. Antioxidant Activity

The antioxidant activity of propolis extracts was evaluated using five different test systems: superoxide anion and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, β-carotene bleaching, ferrous ion chelation, and reducing power.

The antioxidant capacity is assessed by quantifying the inhibition of volatile organic compounds and conjugated diene hydroperoxides generated during the oxidation of linoleic acid [18]. The reducing power was assessed using the method described by Oyaizu [19]. Radical scavenging activities were measured using two different assays including DPPH radical [17] and superoxide anion radical [20] according to the procedure in literature. Ferrous ion chelating activity was determined by the method described by Aktumsek et al. [21].

The results are given as inhibition at 2 mg/mL and 0.2 mg/mL concentration for β-carotene/linoleic acid test and superoxide anion radical scavenging, and as IC_{50} (mg/mL) for DPPH radical scavenging, ferrous ion chelation, and reducing power. All antioxidant activity test conditions were conducted as detailed in the supplementary file.

2.4. Statistical Analysis

Detailed information on the Relative Antioxidant Capacity Index (RACI) [22] and the statistical analyses performed is available in the supplementary file.

3. Results and Discussion

3.1. Chemical composition of the propolis samples

The total phenolic and flavonoid contents of the water extracts from propolis samples collected from different locations in Muğla (Turkey) exhibited considerable variation (Figure 1). The total phenolic content ranged from 27.72 mg PEs/g extract to 91.57 mg PEs/g extract, with statistically significant differences observed among the samples ($p < 0.05$). The Arpacık sample displayed the highest total phenolic content at 91.57 mg PEs/g extract, significantly different from all other samples. Fethiye followed with 85.18 mg PEs/g extract, also distinct from the others but lower than Arpacık. The Dalaman and Döğüşbelen samples showed similar phenolic contents, at 27.72 mg PEs/g extract and 28.20 mg PEs/g extract respectively and were statistically grouped together.

For total flavonoid content, Fethiye had the highest value at 25.78 mg QEs/g extract, significantly differing from the other samples. The Arpacık sample, although showing lower flavonoid content at 16.81 mg QEs/g extract, was still statistically different from the remaining locations. Döğüşbelen exhibited a total flavonoid content of 14.90 mg QEs/g extract, which was not significantly different from Arpacık, but it was distinct from Dalaman, which had the lowest flavonoid content at 9.33 mg QEs/g extract (Figure 2).

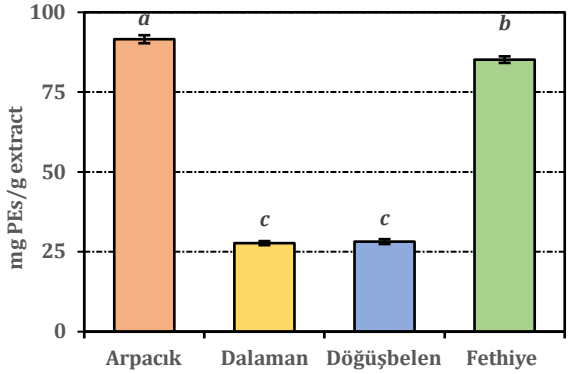

Figure 1. Total phenolic contents of propolis water extracts. PEs: Pyrocatechol equivalents. Values indicated by the same superscripts are not different from the honestly significant difference after Tukey's hoc test at 5% significance level.

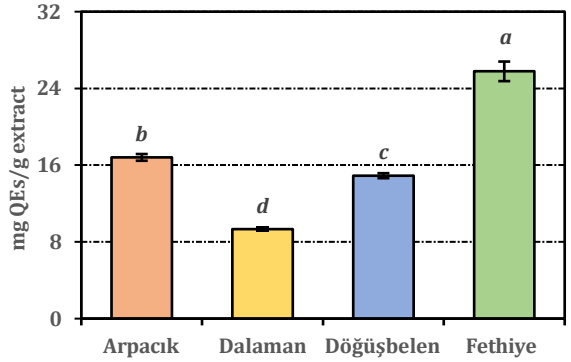

Figure 2. Total flavonoid contents of propolis water extracts. QEs: Quercetin equivalents. Values indicated by the same superscripts are not different from the honestly significant difference after Tukey's hoc test at 5% significance level.

When comparing the two groups of compounds, the Fethiye sample consistently showed high levels of both total phenolics and flavonoids, suggesting that this sample may have a more balanced and rich composition of bioactive compounds. In contrast, the Arpacık sample had the highest phenolic content but a comparatively lower flavonoid content, which may indicate a phenolic-dominant profile. On the other hand, both the Dalaman and Döğüşbelen samples exhibited low phenolic contents, but Döğüşbelen contained a relatively higher amount of flavonoids, showing a different bioactive profile compared to Dalaman.

The statistical analysis reveals that the phenolic and flavonoid compositions of these propolis samples vary significantly across locations, indicating that geographical factors might influence the biosynthesis of these compounds in propolis. Furthermore, the distinct statistical groupings in phenolic and flavonoid contents highlight the diversity in the chemical makeup of the samples, which may reflect different ecological and botanical conditions influencing the resin sources for the bees. Such variations in phenolic and flavonoid profiles could lead to differing biological activities, which warrants further investigation into the bioactivity of these regionally diverse propolis samples.

The present study demonstrated significant variation in the total phenolic and flavonoid contents of water extracts from propolis samples collected from different locations in Muğla, Turkey. These variations are consistent with previous findings in the literature, where the phenolic and flavonoid profiles of propolis have been shown to depend on geographic, botanical, and climatic factors [23,24]. For instance, the total phenolic content in this study ranged from 27.72 mg PEs/g extract (Dalaman) to 91.57 mg PEs/g extract (Arpacık), with Fethiye showing a similarly high phenolic content (85.18 mg PEs/g extract). These values align with studies such as those by Bouchelaghem et al. [25] and Sulaeman et al. [26], where propolis samples from different regions displayed total phenolic contents ranging from 20 to 100 mg PEs/g, depending on their geographical origins.

The total flavonoid content in this study also exhibited location-based variability, ranging from 9.33 mg QEs/g extract (Dalaman) to 25.78 mg QEs/g extract (Fethiye). The high flavonoid content in the Fethiye sample is comparable to results from Indonesian and Malaysian propolis, which showed flavonoid concentrations between 10 and 30 mg QEs/g extract [23,26]. In the present study, while Arpacık exhibited the highest phenolic content, its flavonoid content was lower compared to Fethiye, which suggests a phenolic-dominant composition. This phenolic-flavonoid distribution is in line with the observation by Wang et al. [24], where distinct propolis profiles were observed depending on regional and botanical diversity.

The differences in phenolic and flavonoid compositions across the Muğla samples are likely influenced by the local flora that bees source for resin collection. As noted by Wang et al. [24], environmental factors such as altitude, vegetation, and climate significantly impact the chemical makeup of propolis. The present data showing that Arpacık, with its higher altitude and distinct flora, yielded the highest phenolic content, while Fethiye's sample was more balanced in both phenolics and flavonoids, support this hypothesis. Such botanical influences have been widely documented, with similar trends observed in Indonesian and South Korean propolis samples [24,26].

3.2. Antioxidant activity of the propolis samples

The antioxidant activity of water extracts from propolis samples collected in various regions of Muğla (Turkey) was evaluated using multiple assays, revealing distinct variations in their efficacy. These assays provide insight into the capacity of the extracts to inhibit lipid peroxidation, scavenge reactive oxygen species, and reduce or chelate ions, which together give a comprehensive view of their antioxidant potential.

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The β-carotene bleaching assay showed that all propolis extracts exhibited considerable antioxidant activity, with Fethiye extract demonstrating the highest inhibition at 96.73%, statistically comparable to the positive control, BHT (97.44%) (Figure 3). Arpacık and Dalaman extracts also showed high inhibition rates, 92.19% and 92.44% respectively, with no significant difference between them ($p > 0.05$). Döğüşbelen, however, showed a lower activity at 86.10%, significantly different from the other samples ($p < 0.05$). The superior performance of the Fethiye extract in this assay suggests its strong capability in preventing oxidative degradation of β-carotene.

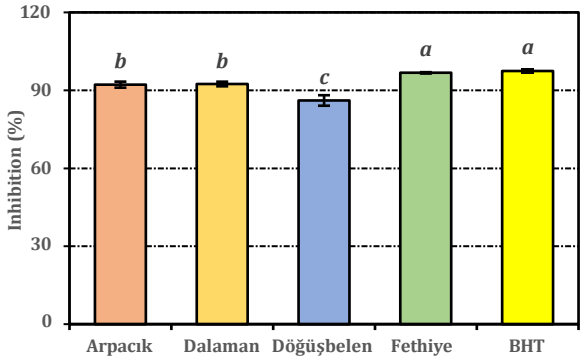

Figure 3. Antioxidant activity (%) of propolis water extracts at 2 mg/mL concentration measured by β-carotene bleaching method. Values indicated by the same superscripts are not different from the honestly significant difference after Tukey's hoc test at 5% significance level.

When evaluating the scavenging effect on superoxide anion radicals, Fethiye extract again outperformed the others, with a high inhibition rate of 72.59%, significantly surpassing even the reference compound quercetin (68.22%) (Figure 4). Arpacık exhibited moderate activity at 44.21%, which was significantly higher than both Dalaman (38.03%) and Döğüşbelen (38.42%), which displayed similar and lower scavenging effects ($p < 0.05$). This data indicates that the Fethiye extract has a much stronger capacity for neutralizing superoxide radicals, a key reactive species involved in oxidative stress.

The DPPH assay showed Fethiye and Arpacık extracts as the most effective scavengers, with IC_{50} values of 49.50 µg/mL and 51.30 µg/mL respectively, which are not statistically different ($p > 0.05$) (Figure 5). These values are also significantly lower than those for the other two samples, Dalaman (145.50 µg/mL) and Döğüşbelen (151.40 µg/mL), indicating their inferior ability to neutralize DPPH radicals. Fethiye's performance was even stronger than BHT (IC_{50} = 73.00 µg/mL), suggesting that this extract may possess potent free radical scavenging properties.

In the reducing power assay, Fethiye and Arpacık samples again demonstrated strong activity with IC_{50} values of 10.59 µg/mL and 11.01 µg/mL, respectively, both significantly better than the Dalaman and Döğüşbelen extracts, which showed higher IC_{50} values of 28.97 µg/mL and 42.15 µg/mL ($p < 0.05$) (Figure 6). However, BHT displayed the best performance with an IC_{50} of 9.82 µg/mL, although the difference between Fethiye, Arpacık, and BHT was not statistically significant (Figure 6).

The ferrous ion chelating ability test revealed a significant difference among the samples (Figure 7). The Fethiye extract showed the strongest chelating activity with an IC_{50} of 413 µg/mL, outperforming even BHT (IC_{50} = 866 µg/mL). Arpacık (IC_{50} = 887 µg/mL) showed moderate chelating activity, similar to BHT ($p > 0.05$). On the other hand, Dalaman (IC_{50} = 1960 µg/mL) and Döğüşbelen (IC_{50} = 4651 µg/mL) exhibited much weaker chelating capacities, significantly different from the other samples ($p < 0.05$).

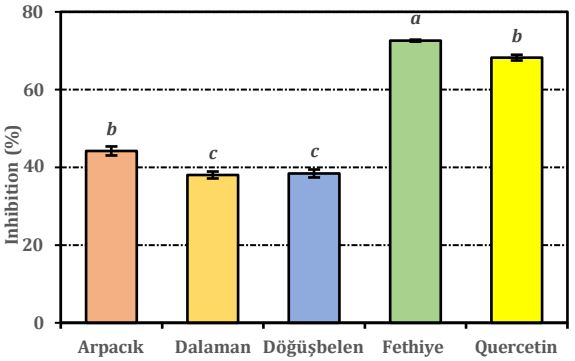

Figure 4. Scavenging effect (%) on superoxide anion radicals of propolis water extracts at 0.2 mg/mL concentration. Values indicated by the same superscripts are not different from the honestly significant difference after Tukey's hoc test at 5% significance level.

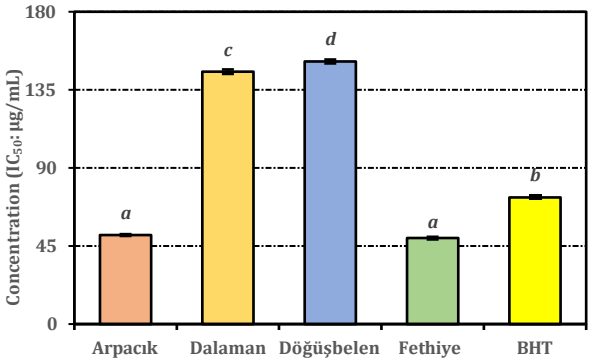

Figure 5. Scavenging effect on DPPH radicals of propolis water extracts. Values indicated by the same superscripts are not different from the honestly significant difference after Tukey's hoc test at 5% significance level.

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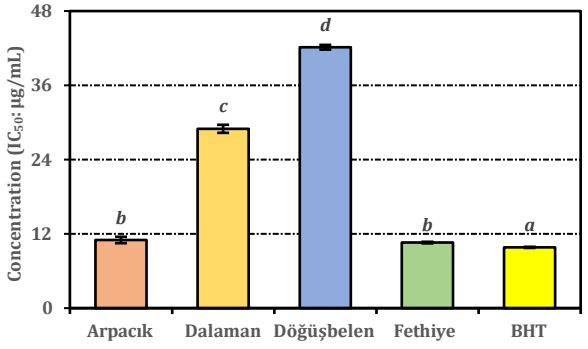

Figure 6. Reducing power of propolis water extracts. Values indicated by the same superscripts are not different from the honestly significant difference after Tukey's hoc test at 5% significance level.

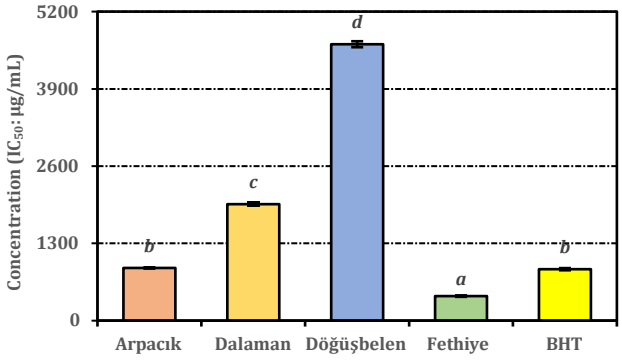

Figure 7. Ferrous ion chelating activity of propolis water extracts. Values indicated by the same superscripts are not different from the honestly significant difference after Tukey's hoc test at 5% significance level.

Fethiye consistently showed the highest antioxidant activity across all assays, which correlates with its high total phenolic (85.18 mg PEs/g) and flavonoid content (25.78 mg QEs/g). The rich phenolic and flavonoid composition likely contributes to its superior radical scavenging, reducing, and chelating abilities. In contrast, Arpacık, which also exhibited strong antioxidant performance in most assays, had the highest phenolic content (91.57 mg PEs/g) but a lower flavonoid concentration (16.81 mg QEs/g), suggesting that phenolics may be the primary contributors to its antioxidant activity.

Dalaman and Döğüşbelen extracts, which consistently displayed lower antioxidant capacities, also contained lower levels of both phenolics and flavonoids. These results reinforce the role of phenolic and flavonoid compounds in driving the antioxidant activity of propolis. In particular, the correlation between phenolic content and reducing power, as well as flavonoid content and radical scavenging capacity, is evident in these findings.

The relative antioxidant capacity index (RACI) was calculated for each water extract of propolis from different locations in Muğla (Turkey), allowing for a comparative assessment of their overall antioxidant potential across the various assays (Figure 8). RACI, by standardizing the results from distinct antioxidant assays, provides a more holistic evaluation of each extract's antioxidant capacity, making it possible to rank them even though individual tests measure different mechanisms of action.

The RACI values suggest that the Fethiye extract possesses the highest overall antioxidant capacity, with a score of 1.13, significantly outperforming the other samples. This finding aligns well with the earlier assay results, where Fethiye consistently demonstrated superior antioxidant activity across the β-carotene bleaching, superoxide anion radical scavenging, DPPH radical scavenging, and reducing power tests. Its higher phenolic and flavonoid content further supports this elevated activity, as these compounds are well-established contributors to antioxidant defense mechanisms.

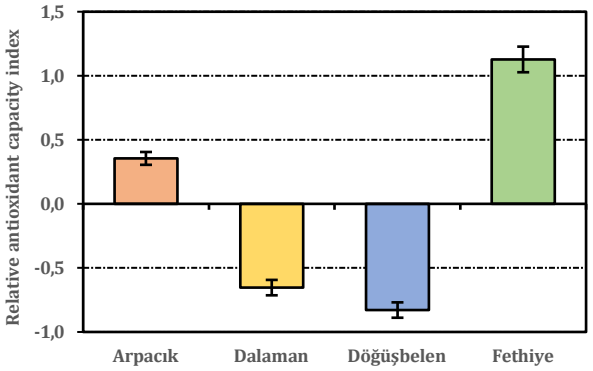

Figure 8. Relative antioxidant capacity index of propolis water extracts

The Arpacık extract follows with a positive RACI value of 0.35, indicating a relatively high antioxidant capacity. This is consistent with the individual assay data, where Arpacık exhibited strong performance, particularly in β-carotene bleaching and reducing power assays. Although its superoxide anion scavenging and DPPH radical scavenging activities were moderate, the overall RACI score reflects its generally good antioxidant profile. This is in accordance with its rich phenolic content, which likely contributes to its substantial antioxidant activity.

In contrast, both Dalaman and Döğüşbelen samples show negative RACI values of -0.65 and -0.83, respectively, indicating lower overall antioxidant capacities. These results also correspond with the previous findings, where both extracts displayed comparatively weaker antioxidant activities across all assays. Their lower phenolic and flavonoid contents provide a plausible explanation for this diminished antioxidant potential. Specifically, their limited ability to scavenge radicals and reduce ions, as shown in the DPPH, reducing power, and

ferrous ion chelating assays, is reflected in their negative RACI values.

In summary, the RACI values provide a comprehensive ranking of the antioxidant capacity of the propolis extracts, with Fethiye and Arpacık demonstrating stronger antioxidant activities, while Dalaman and Döğüşbelen exhibit significantly weaker performances. These results are consistent with the chemical composition data, suggesting that higher phenolic and flavonoid concentrations are closely associated with enhanced antioxidant activity in the propolis extracts.

The correlation analysis between the chemical composition and antioxidant activities of water extracts from propolis samples collected from various locations in Muğla (Turkey) reveals several significant relationships, indicating that the phenolic and flavonoid content plays a crucial role in influencing the antioxidant potential of these extracts (Table 1).

Notably, total phenolic content demonstrates a very strong positive correlation with the DPPH radical scavenging activity (r = 0.994) and reducing power (r = 0.986). This suggests that phenolic compounds are major contributors to these specific antioxidant mechanisms. The strong correlation with both assays implies that the phenolics present in the extracts are highly effective in neutralizing free radicals and promoting electron transfer processes, which are critical to antioxidant defenses.

Similarly, a substantial positive correlation is observed between total flavonoid content and superoxide anion radical scavenging activity (r = 0.931), highlighting the significant role of flavonoids in mitigating oxidative stress caused by superoxide radicals. This finding suggests that the flavonoid-rich extracts, such as those from Fethiye and Arpacık, possess enhanced capacity to counteract reactive oxygen species, which aligns with their higher overall antioxidant performance in the superoxide anion radical assay.

Furthermore, the correlation between total flavonoid content and ferrous ion chelation (r = 0.894) is also strong, indicating that flavonoids contribute meaningfully to the metal-chelating properties of the extracts. This relationship is critical, as metal chelation can inhibit the generation of reactive species via Fenton reactions, thus protecting against oxidative damage.

The β-carotene bleaching assay also shows moderate correlations with both phenolic (r = 0.649) and flavonoid (r = 0.562) content, suggesting that these compounds play a role in protecting against lipid peroxidation, though not as strongly as in other assays. The moderate correlation indicates that while phenolics and flavonoids contribute to this mechanism, other compounds in the extracts may also be involved in inhibiting the oxidation of linoleic acid in this specific test.

Ferrous ion chelation exhibits strong correlations with both phenolic (r = 0.790) and flavonoid content (r = 0.894), reinforcing the notion that these compounds are key players in the extracts' ability to bind and neutralize metal ions, which could otherwise catalyze the formation of harmful radicals.

Overall, the correlation data indicate that both phenolic and flavonoid compounds are essential to the antioxidant activity of the propolis extracts, with phenolics more strongly influencing DPPH radical scavenging and reducing power, while flavonoids exhibit a more pronounced effect on superoxide anion scavenging and ferrous ion chelation. These findings highlight the multifaceted nature of the antioxidant mechanisms in propolis extracts, wherein different phytochemicals contribute to distinct antioxidant pathways.

The antioxidant activity of propolis water extracts from different locations in Muğla (Turkey) revealed substantial variability across multiple assays, demonstrating that the chemical composition, particularly phenolic and flavonoid contents, significantly influences their bioactivity. These findings are consistent with existing literature, which supports the strong correlation between the phenolic and flavonoid profiles of propolis and its antioxidant properties [23,27].

Table 1. Correlations among phenolic compounds and assays

	β-Carotene bleaching	Superoxide anion radical	DPPH radical	Reducing power	Ferrous ion chelation
Superoxide anion radical	0.779				
DPPH radical	0.709	0.735			
Reducing power	0.767	0.730	0.994		
Ferrous ion chelation	0.873	0.970	0.854	0.862	
Total phenolic	0.649	0.654	0.994	0.986	0.790
Total flavonoid	0.562	0.931	0.791	0.751	0.894

In the current study, the Fethiye sample consistently exhibited the highest antioxidant activity, which aligns with its rich chemical composition, including a high total phenolic content and the highest flavonoid content. The superior performance of this sample across various assays, such as β-carotene bleaching and superoxide radical scavenging, highlights the critical role these compounds play in its bioactivity. This trend is supported by research conducted by Gregoris and Stevanato [28], who found a strong correlation between polyphenolic content and antioxidant activity in Venetian propolis, emphasizing that high phenolic concentrations contribute to enhanced radical scavenging and lipid peroxidation inhibition.

The relationship between phenolic content and antioxidant capacity was particularly evident in the Arpacık sample, which showed the highest total phenolic content and performed strongly in assays such as DPPH and reducing power. However, despite its lower flavonoid content compared to Fethiye, Arpacık's performance suggests that phenolics are the primary contributors to its antioxidant efficacy, a conclusion also reached by da Silva et al. [27], who reported that phenolic acids significantly drive the antioxidant activity in Brazilian propolis.

On the other hand, the Dalaman and Döğüşbelen samples, which consistently displayed lower antioxidant activities, also contained lower levels of both phenolics and flavonoids, indicating their limited ability to neutralize free radicals. This observation is in line with studies like those by Fathi Hafshejani et al. [29] and Narimane et al. [30], who demonstrated that samples with reduced phenolic and flavonoid contents generally exhibit weaker antioxidant properties. Furthermore, the chelating activity of these extracts was also significantly lower, which again underscores the importance of a rich phenolic and flavonoid composition for robust antioxidant potential.

The overall findings from this study strongly support the established view that the antioxidant activity of propolis is largely determined by its phenolic and flavonoid composition. In particular, Fethiye's balanced high levels of both compounds correlate with its superior performance across all antioxidant assays. This mirrors the work of Asem et al. [23], who found that Malaysian propolis with high phenolic and flavonoid contents exhibited enhanced radical scavenging and lipid peroxidation inhibition. Similarly, Gregoris and Stevanato [28] concluded that high phenolic content enhances the reducing power and radical scavenging activity of propolis, a pattern clearly evident in the current data.

In conclusion, the variations in antioxidant activity among the Muğla propolis samples are closely tied to their chemical compositions, particularly their phenolic and flavonoid profiles. Samples with higher concentrations of these compounds, like Fethiye and Arpacık, exhibited stronger antioxidant activity, underscoring the critical role of these bioactive compounds. The present findings are consistent with literature, which consistently highlights the contribution of phenolics and flavonoids to the antioxidant properties of propolis from various regions [23,27,28]. Future studies may benefit from further exploring the specific phenolic compounds responsible for the observed activities, as well as expanding the geographical scope to examine how environmental factors influence the bioactivity of propolis.

4. Conclusion

This study provides compelling evidence that the antioxidant activities of water extracts from propolis samples collected across different locations in Muğla (Turkey) are strongly influenced by their phenolic and flavonoid contents. The Fethiye and Arpacık samples consistently exhibited the highest antioxidant capacities across multiple assays, which correlates with their rich phenolic and flavonoid compositions. Particularly, the Fethiye extract, with its high levels of both phenolics and flavonoids, demonstrated superior radical scavenging, reducing, and chelating abilities, surpassing even standard antioxidant compounds in certain assays. Arpacık, while phenolic-dominant, also showed significant antioxidant performance, although its lower flavonoid content may have limited its activity in assays like superoxide radical scavenging.

The observed correlations between total phenolic and flavonoid content and various antioxidant mechanisms underscore the central role these compounds play in determining the bioactivity of propolis extracts. Phenolics were more closely associated with DPPH radical scavenging and reducing power, while flavonoids demonstrated a stronger influence on superoxide radical scavenging and ferrous ion chelation, indicating distinct contributions of these compounds to different antioxidant pathways.

Despite these promising findings, the study also highlights several limitations. First, the focus on water extracts may not fully capture the antioxidant potential of propolis, as different solvents could extract varying bioactive compounds. Future studies should include a broader range of extraction methods to provide a more comprehensive understanding of propolis' antioxidant capacity. Additionally, while the phenolic and flavonoid content appears to be key drivers of antioxidant activity, other bioactive compounds, such as terpenes and polysaccharides, may also contribute. Further research using advanced analytical techniques like mass spectrometry and nuclear magnetic resonance (NMR)

could identify these additional components and their potential synergies.

Another limitation lies in the geographical scope of the study. While significant variation was observed across the Muğla region, expanding the sampling to include other regions could reveal broader trends and provide insights into how different environmental factors influence the bioactive composition of propolis. Moreover, the ecological and botanical factors driving the observed variations were not fully explored. Detailed studies on the floral sources of the propolis and their seasonal variations could provide critical context for understanding these chemical differences.

In conclusion, this study confirms the significant impact of phenolic and flavonoid compounds on the antioxidant activity of propolis extracts and opens the door for future investigations into the broader chemical composition and ecological influences affecting propolis bioactivity. Further research addressing the noted limitations will help to solidify our understanding of propolis as a potent natural antioxidant and its potential applications in food, pharmaceutical, and cosmetic industries. The main conclusions of the study should be presented, not to summarize information already presented in the results and discussion section.

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Author's Contributions

Cengiz Sarikurkcu: Conceptualization, Methodology, Data curation, Software, Visualization, Investigation, Validation, Writing- original draft, Supervision, Writing-review and editing.

Ethics

There are no ethical issues after the publication of this manuscript.

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