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Evaluation of Antioxidant Activity and Phenolic Content of Medicinal Plants Extracted by Ethanol, Methanol, and Water

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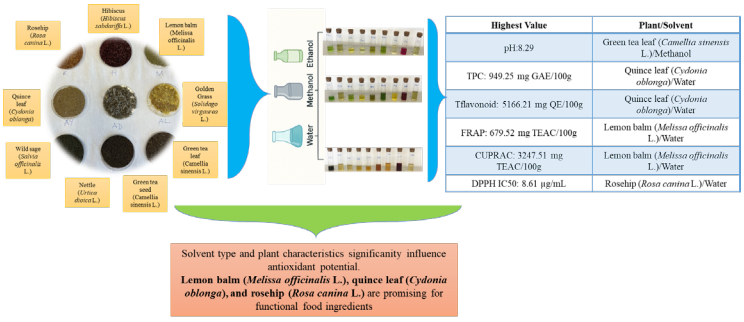
Abstract: The aim of this study was to compare both the efficiency of different extraction solvents and the phenolic content of various plant species. Analyses included pH, total phenolic content (TFC), total flavonoid content (TFC), antioxidant capacity (FRAP, CUPRAC), and DPPH IC_{50}. Methanol extract pH ranged from hibiscus (*Hibiscus sabdariffa* L.) at 3.71 to 8.29 green tea leaf (*Camellia sinensis* L.). Quince leaf (*Cydonia oblonga*) water extract had the highest TPC (949.25 mg GAE/100 g) and flavonoid content (5166.21 mg QE/100 g). Lemon balm (*Melissa officinalis*) showed the highest FRAP and CUPRAC values in both methanol and water extracts. Rosehip (*Rosa canina* L.) water extract had the strongest DPPH activity (IC_{50}: 8.61 µg/mL). These findings suggest that lemon balm (*Melissa officinalis*), quince leaf (*Cydonia oblonga*), and rosehip (*Rosa canina*) have strong potential as functional food ingredients, owing to their rich phenolic and flavonoid content and potent antioxidant activities, which could be beneficial in preventing oxidative stress-related health conditions.



Keywords: plant extracts, antioxidant activity, phenolic compounds, extraction methods

1 Introduction

Herbal medicines have been utilized throughout the ages in different cultures as treatments for disease and have in recent times started gaining strong scientific interest as natural sources of antioxidants. One of the key groups of compounds implicated in the bioactivity of these crops, phenolic compounds, are synthesized as secondary metabolites within plants and play a role in many physiological processes such as color formation, odor, environmental stress response and plant defense[1-3]. As regards human health, phenolic compounds play important functions in the prevention of cardiovascular illness, diabetes, cancer, and other chronic illnesses involving oxidative stress due to their antioxidant, antimicrobial, anticarcinogenic, and anti-inflammatory effects[1, 4].

Phenolic compounds are very widely distributed in the majority of plant food sources such as fruits, vegetables, grains, legumes, tea, spices, and nuts, and have protective effects in the prevention of diseases associated with oxidative stress[3]. Phenolic content and biological activities, however, significantly differ based on many agricultural factors such as plant variety, genotype, and species differences, and environmental factors such as plants' developmental stage, plant cultivation practices, and harvest time[5].

The phenolic content and antioxidant activity of plant extracts not only differ according to the plant material used but also according to the procedure of extraction utilized and the chemical nature of the solvent[1]. Due to differences in the chemical structures and polarities of antioxidant molecules, it is impossible for one solvent to effectively extract all the

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constituents[6, 7]. In this respect, while methanol provides high recovery for the extraction of low molecular weight phenolics, ethanol is the most preferred solvent in food application due to it being non-toxic[8].

Because, in the evaluation of antioxidant activity of extracts, one method of test provides limited information, while the collection of total phenolic content, DPPH, FRAP, CUPRAC, and flavonoid analysis provides more profound results[2, 4]. However, there is no comprehensive study in the literature that compares both the total phenolic content and different antioxidant analyses (DPPH, FRAP, CUPRAC, flavonoid) of plant extracts obtained using three different solvents (ethanol, methanol, water).

The plants researched in this study are species widely used in traditional medicine and are recognized to have various health effects. Rosehip (*Rosa canina* L.) is rich in antioxidant activity and immune-stimulating properties due to its rich content of vitamin C and phenolic compounds[9]. Hibiscus (*Hibiscus sabdariffa* L.), on the other hand, possesses antioxidant, antihypertensive, antibacterial, hypolipidemic, and anti-inflammatory activity, primarily attributed to the high phenolic content, including anthocyanins[10, 11]. Lemon balm (*Melissa officinalis* L.) is distinguished by its flavonoid compounds and volatile oils and is of interest with its antioxidant, antimicrobial, neuroprotective, and anxiolytic activity and has potential protective effects against diseases such as Alzheimer's disease and cardiovascular diseases[12].

Quince leaf (*Cydonia oblonga*) is richer in phenolic compound than ever its fruit and possesses protective effects against obesity and neurodegenerative diseases due to antioxidant activity and lipase and cholinesterase enzyme inhibition[13]. Wild sage (*Salvia fruticosa*) has anticancer, anti-inflammatory, antimicrobial, hypoglycemic, and antioxidant activity and is a functional rich species[14]. *Solidago virgaurea* L. (Golden grass) is rich in antioxidant activity due to its rich flavonoids and phenolic acid content and protective effect against oxidative stress-related diseases[15].

Nettle (*Urtica dioica* L.) is a plant with multiple uses that possess anticancer, antioxidant, antimicrobial, and anti-inflammatory properties and are reported to be effective against metabolic and immune system disorders[16]. Green tea leaves and seeds (*Camellia sinensis* L.) are rich in bioactive components such as catechins, tocopherols, and saponins and are of outstanding importance in the prevention of various chronic diseases due to their effective antioxidant, anti-inflammatory, and cardiovascular protective action[17].

This study aims to determine the most effective solvent for each plant by comparing the yield of bioactive compounds and antioxidant activity across ethanol, methanol, and water, providing a comprehensive evaluation of multiple solvents and analytical methods that are currently lacking in the literature.

2 Materials and Methods

2.1 Materials

The plant materials used in this study comprised species naturally growing in various regions of Turkey and traditionally consumed, including rosehip (*Rosa canina* L.), hibiscus (*Hibiscus sabdariffa* L.), lemon balm (*Melissa officinalis* L.), quince leaf (*Cydonia oblonga*), Wild sage (*Salvia officinalis* L.), golden grass (*Solidago virgaurea* L.), nettle (*Urtica dioica* L.), green tea leaf (*Camellia sinensis* L.), and green tea seed (*Camellia sinensis* L.). All samples were obtained in bulk and additive-free from a local herbal supplier in Kocaeli, Turkey, in June 2024. Upon receipt, samples were cleaned of foreign matter, visually inspected, and ground into powder to prepare homogeneous samples for analysis. They were stored in airtight glass containers in a dark environment at room temperature (25 ± 2 °C) to protect against light, moisture, and oxygen.

2.2 Methods

2.2.1 Extraction procedure

One gram of each powdered plant sample was weighed and mixed with 20 mL of solvent (ethanol, methanol, or distilled water) for extraction. The extraction process was carried out by shaking the mixtures at room temperature (25 ± 2 °C) for 30 minutes. Following extraction, the mixtures were centrifuged at 5000 rpm for 5 minutes (Hettich Zentrifugen Universal 320 R), and the supernatants were filtered through Whatman No. 1 filter paper to obtain clear extracts. The resulting extracts were stored in glass containers at room temperature until further analyses.

2.2.2 pH

The pH values of the extracts were measured using a pH meter, following the method described by Cemeroğlu[18], with slight modifications. Briefly, 10 g of sample was homogenized in 100 mL of distilled water prior to measurement.

2.2.3 Total phenolic content (TPC)

Total phenolic content (TPC) was determined according to the Folin–Ciocalteu method as reported by Binici et al.[19]. Samples were reacted with 0.2 N Folin–Ciocalteu reagent and 10% (w/v) sodium carbonate, incubated in the dark for 30 minutes, and absorbance was measured at 760 nm.

2.2.4 Total flavonoid content (TFC)

The total flavonoid content (TFC) was measured using an aluminum chloride colorimetric method modified from Koçak et al.[20]. Samples were reacted sequentially with $NaNO_2$, 10% $AlCl_3$, and NaOH, and the final volume was adjusted to 2.5 mL with distilled water. After incubation in the dark for 15 minutes, absorbance was recorded at 510 nm.

2.2.5 Ferric (III) reducing ability of plasma (FRAP)

The FRAP assay was carried out by mixing 10 μL of the sample with 2.49 mL of FRAP reagent (prepared from sodium acetate buffer, TPTZ, and $FeCl_3 \cdot 6H_2O$ in an 8:1:1 ratio), and absorbance was measured at 593 nm after 30 minutes of incubation at room temperature[20].

2.2.6 Copper (II) reducing antioxidant capacity (CUPRAC)

For the CUPRAC assay, 0.1 mL of appropriately diluted extract was mixed with 1 mL each of 0.01 mM $CuCl_2 \cdot 2H_2O$, 7.5×10^{-3} mM neocuproine, and 1 M ammonium acetate buffer (pH 7), along with 1 mL of distilled water, as described by Koçak et al. (20). The mixture was incubated for 30 minutes in the dark, and absorbance was read at 450 nm.

2.2.7 DPPH Radical Scavenging Activity

DPPH radical scavenging activity was determined using a method modified from Koçak et al.[20]. A 0.02 mM DPPH solution in ethanol was prepared, and 2.5 mL of sample solution at different concentrations was added. After incubation in the dark for 30 minutes, absorbance was measured at 517 nm using a UV–VIS spectrophotometer.

2.2.8 Statistical Analysis

All analyses were performed in triplicate, and data are presented as mean ± standard deviation. Statistical evaluation was conducted using SPSS version 25.0. Differences between groups were assessed by one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test. The significance level of $p < 0.01$ was considered statistically significant.

3 Results and Discussion

3.1 pH

In this study, the pH values of extracts obtained from nine different plant samples using various solvents (methanol, ethanol, and water) are presented in **Table 1**. Statistically significant differences were observed in the pH values depending on the type of solvent used ($p < 0.01$).

In methanol extracts, pH values ranged between 3.71 and 8.29. The highest pH was recorded in green tea leaf (*Camellia sinensis* L.) at 8.29, followed by nettle (*Urtica dioica* L.) at 8.07, green tea seed (*Camellia sinensis* L.) at 8.05, and lemon balm (*Melissa officinalis* L.) at 7.38. The lowest pH was observed in the hibiscus (*Hibiscus sabdariffa*) extract

Table 1 pH Values of plant samples.

Plant Samples	pH		
	Methanol	Ethanol	Water
Wild sage (*Salvia officinalis* L.)	7.33 ± 0.00^{aE}	5.91 ± 0.00^{bF}	5.31 ± 0.00^{cC}
Golden grass (*Solidago virgaurea* L.)	6.42 ± 0.00^{aG}	5.06 ± 0.00^{bH}	4.95 ± 0.00^{cF}
Quince leaf (*Cydonia oblonga*)	7.16 ± 0.00^{aF}	5.99 ± 0.00^{bE}	5.19 ± 0.00^{cE}
Hibiscus (*Hibiscus sabdariffa* L.)	3.71 ± 0.00^{aI}	3.59 ± 0.00^{bI}	2.79 ± 0.00^{cI}
Nettle (*Urtica dioica* L.)	8.07 ± 0.00^{aB}	6.55 ± 0.00^{cB}	7.32 ± 0.00^{bA}
Rosehip (*Rosa canina* L.)	5.50 ± 0.00^{bH}	5.55 ± 0.00^{aG}	4.02 ± 0.00^{cH}
Lemon balm (*Melissa officinalis* L.)	7.38 ± 0.00^{aD}	6.61 ± 0.00^{bA}	6.01 ± 0.00^{cB}
Green tea seed (*Camellia sinensis* L.)	8.05 ± 0.00^{aC}	6.48 ± 0.00^{bC}	4.87 ± 0.00^{cG}
Green tea leaf (*Camellia sinensis* L.)	8.29 ± 0.00^{aA}	6.18 ± 0.00^{bD}	5.25 ± 0.00^{cD}

Different letters within the same row or column indicate statistically significant differences ($p < 0.05$)

with a value of 3.71. In ethanol extracts, pH values varied from 3.59 to 6.61. The highest value again belonged to lemon balm (*Melissa officinalis* L.) at 6.61, while nettle (*Urtica dioica* L.), green tea seed (*Camellia sinensis* L.), and green tea leaf (*Camellia sinensis* L.) followed closely with values of 6.55, 6.48, and 6.18, respectively. The lowest pH was once again found in hibiscus (*Hibiscus sabdariffa* L.) at 3.59. Water extracts generally showed the lowest pH values among the three solvents. The highest pH in this group was observed in nettle at 7.32, followed by lemon balm (*Melissa officinalis* L.) at 6.01 and quince leaf (*Cydonia oblonga*) at 5.19. The lowest pH in water extracts was recorded for hibiscus (*Hibiscus sabdariffa* L.) at 2.79. Overall, hibiscus consistently showed the lowest pH values across all three solvents, whereas nettle (*Urtica dioica* L.), green tea leaf (*Camellia sinensis* L.), and green tea seed (*Camellia sinensis* L.) stood out with the highest pH values, particularly in methanol and water extracts. Previous studies have reported similar ranges: Saad et al.[21] found pH values between 2.66 and 5.58 for ten herbal extracts, and Tak and Kakde[22] reported values between 2.9 and 6.7 for selected plant extracts. These findings suggest that the choice of extraction solvent significantly influences the chemical characteristics of the extracted compounds, thereby affecting the pH of the final plant extract.

3.2 TPC and TFC

The total phenolic content (TPC) of the plant samples varied significantly depending on the extraction solvent used (**Table 2**). Similarly, total flavonoid content (TFC) varied depending on the plant sample and solvent type (**Table 2**).

Table 2 Total phenolic and total flavonoid contents of plant samples.

Plant Samples	TPC (mg GAE/100g)		
	Methanol	Ethanol	Water
Wild sage (*Salvia officinalis* L.)	259.81 ± 0.17^{bG}	219.62 ± 4.91^{cD}	311.47 ± 0.30^{aF}
Golden grass (*Solidago virgaurea* L.)	537.29 ± 0.19^{aC}	294.13 ± 7.99^{cA}	363.72 ± 0.09^{bD}
Quince leaf (*Cydonia oblonga*)	787.62 ± 0.22^{bA}	244.49 ± 3.78^{cC}	949.25 ± 0.30^{aA}
Hibiscus (*Hibiscus sabdariffa* L.)	309.54 ± 6.30^{aD}	166.72 ± 0.73^{cF}	260.38 ± 0.51^{bI}
Nettle (*Urtica dioica* L.)	191.71 ± 1.05^{bI}	157.11 ± 0.37^{cG}	320.47 ± 0.23^{aE}
Rosehip (*Rosa canina* L.)	270.02 ± 2.12^{bF}	196.02 ± 1.10^{cE}	283.04 ± 3.82^{aH}
Lemon balm (*Melissa officinalis* L.)	569.39 ± 0.14^{bB}	280.30 ± 3.84^{cB}	701.19 ± 0.21^{aB}
Green tea seed (*Camellia sinensis* L.)	248.57 ± 0.36^{bH}	163.94 ± 1.11^{cH}	293.18 ± 0.21^{aG}
Green tea leaf (*Camellia sinensis* L.)	297.69 ± 0.29^{bE}	163.04 ± 0.37^{cH}	420.42 ± 0.25^{aC}
Plant Samples	TFC (mg QE/100g)		
	Methanol	Ethanol	Water
Wild sage (*Salvia officinalis* L.)	2440.53 ± 7.53^{aE}	2410.31 ± 0.25^{bC}	1696.58 ± 0.11^{cG}
Golden grass (*Solidago virgaurea* L.)	3719.36 ± 0.35^{aC}	980.25 ± 0.03^{cE}	1785.28 ± 7.22^{bF}
Quince leaf (*Cydonia oblonga*)	7287.83 ± 0.22^{aA}	2724.25 ± 0.12^{cB}	5166.21 ± 0.33^{bA}
Hibiscus (*Hibiscus sabdariffa* L.)	2364.21 ± 0.18^{bF}	409.18 ± 1.18^{cF}	3425.89 ± 7.39^{aC}
Nettle (*Urtica dioica* L.)	332.28 ± 0.28^{bI}	30.64 ± 0.12^{cI}	2634.44 ± 14.69^{aD}
Rosehip (*Rosa canina* L.)	2916.24 ± 0.06^{bD}	3782.01 ± 0.01^{aA}	968.90 ± 0.13^{cH}
Lemon balm (*Melissa officinalis* L.)	4079.38 ± 0.03^{aB}	2342.50 ± 0.23^{cD}	4054.36 ± 1.50^{bB}
Green tea seed (*Camellia sinensis* L.)	827.12 ± 0.15^{aH}	286.49 ± 0.25^{cH}	804.45 ± 0.45^{bI}
Green tea leaf (*Camellia sinensis* L.)	1276.56 ± 0.02^{bG}	341.73 ± 0.03^{cG}	2179.92 ± 0.03^{aE}

Different letters within the same row or column indicate statistically significant differences ($p < 0.05$)

In general, aqueous extracts exhibited higher TPC values. The highest TPC was found in the aqueous extract of quince leaf (*Cydonia oblonga*), reaching 949.25 mg GAE/100 g. This was followed by lemon balm (*Melissa officinalis* L.) with 701.19 mg GAE/100 g and green tea leaf (*Camellia sinensis* L.) with 420.42 mg GAE/100 g. Among the methanol extracts, the highest TPC was also observed in quince leaf (*Cydonia oblonga*) (787.62 mg GAE/100 g), whereas in ethanol extracts, the highest value was recorded in wild sage (*Salvia officinalis* L.) at 294.13 mg GAE/100 g. The lowest TPC was detected in the ethanol extract of nettle (*Urtica dioica* L.), measured at 157.11 mg GAE/100 g. These findings indicate that total phenolic content is significantly influenced by both the plant species and the solvent used, with water proving to be a more effective solvent for phenolic compound extraction in many cases. This is largely attributed to the polar nature of phenolic compounds, which have higher solubility in polar solvents like water. The same results were noted by Alara et al.[2], where water was reported to provide high yields during the extraction of polyphenol-rich plants. Extraction efficiency, nonetheless, is not solely dependent on solvent polarity but also on the plant matrix structure, extraction time, and temperature[8]. Therefore, variations in compound profiles and tissue types may be able to account for why methanol yielded higher TPC in some plants and water in others. In the methanol extracts, the highest flavonoid content was measured in quince leaf (*Cydonia oblonga*) at 7287.83 mg QE/100 g, followed by lemon balm (*Melissa officinalis* L.) (4079.38 mg QE/100 g) and golden grass (*Solidago virgaurea* L.) (3719.36 mg QE/100 g). In aqueous extracts, quince leaf (*Cydonia oblonga*) (5166.21 mg QE/100 g), lemon balm (*Melissa officinalis* L.) (4054.36 mg QE/100 g), and hibiscus (*Hibiscus sabdariffa* L.) (3425.89 mg QE/100 g) showed prominent flavonoid levels. In ethanol extracts, the highest flavonoid content was observed in rosehip (*Rosa canina* L.) with 3782.01 mg QE/100 g, while the lowest was again detected innettle (*Urtica dioica* L.) (30.64 mg QE/100 g). These results highlight that both quince leaf (Cydonia oblonga) and lemon balm (*Melissa officinalis* L.) exhibit high flavonoid contents in methanol and water extractions, suggesting strong potential as functional ingredients. Moreover, the choice of solvent clearly plays a crucial role in the efficiency of flavonoid extraction. These findings suggest conformity with the report of Do et al.[7] that methanol and water are effective solvents for flavonoid extraction from various plants. Similarly, methanolic extracts contained higher TFC in *Camellia sinensis* and *Hibiscus sabdariffa* than ethanol or aqueous counterparts[23].

3.3 FRAP and CUPRAC

The FRAP values of the plant extracts showed significant differences depending on the extraction solvent used (**Table 3**). CUPRAC assay results followed a similar trend to FRAP.

Generally, higher FRAP values were observed in water and methanol extracts, whereas ethanol extracts exhibited lower antioxidant capacities. Among the water extracts, the highest FRAP value was recorded in lemon balm (*Melissa officinalis* L.) at 679.52 mg TEAC/100 g, followed closely by quince leaf (*Cydonia oblonga*) at 673.23 mg TEAC/100 g and green tea leaf (*Camellia sinensis* L.) at 650.76 mg TEAC/100 g. In methanol extracts, lemon balm (*Melissa officinalis* L.) again showed the highest value at 672.49 mg TEAC/100 g. For ethanol extracts, lemon balm (*Melissa officinalis* L.) demonstrated the highest antioxidant capacity with 304.51 mg TEAC/100 g. The lowest FRAP value was found in the ethanol extract of nettle, measuring only 4.81 mg TEAC/100 g. These results indicate that lemon balm (*Melissa officinalis* L.) and quince leaf (*Cydonia oblonga*) possess strong reducing power and that water is a more effective solvent for extracting antioxidant compounds. These results corroborate earlier reports indicating that polar solvents extract reducing compounds more effectively than less polar ones[24,25,26]. The particularly strong reducing power observed in lemon balm (*Melissa officinalis* L.) and quince leaf (*Cydonia oblonga*) aligns with studies reporting high levels of hydrophilic phenolic acids—such as rosmarinic, caffeic, and chlorogenic acids—in these species. These compounds are preferentially extracted by polar solvents, especially water[27-29]. Interestingly, the comparable FRAP values found in the water and methanol extracts of lemon balm (*Melissa officinalis* L.)suggest that moderately polar solvents like methanol can be just as effective as water, especially when extraction parameters—such as temperature, time, and solvent-to-solid ratio—are optimized[30].

The highest CUPRAC value was observed in the water extract of lemon balm (*Melissa officinalis* L.), with 3247.51 mg TEAC/100 g. Lemon balm (*Melissa officinalis* L.) also showed a high CUPRAC value in the methanol extract at 3268.82 mg TEAC/100 g. Quince leaf (*Cydonia oblonga*) ranked second among water extracts, with a CUPRAC value of 3083.47 mg TEAC/100 g. In ethanol extracts, lemon balm (*Melissa officinalis* L.) again stood out with a value of 2363.40 mg TEAC/100 g. The lowest CUPRAC value was detected in the ethanol extract of nettle, at 84.69 mg TEAC/100 g, indicating a notably limited antioxidant capacity in this extract. These results further confirm that lemon balm (*Melissa officinalis* L.) and quince leaf (*Cydonia oblonga*) possess strong antioxidant potential, regardless of the solvent used, although water and methanol appear to be more effective in extracting active compounds. The exceptionally high CUPRAC activity of lemon balm (*Melissa officinalis* L.) in both aqueous and methanolic extracts reflects its richness in hydrophilic phenolic acids such as rosmarinic and caffeic acid, which are highly soluble in polar solvents. These findings are in line with

Table 3 FRAP and CUPRAC antioxidant capacity values of plant samples.

Plant Samples	FRAP (mg TEAC/100g)		
	Methanol	Ethanol	Water
Wild sage (*Salvia officinalis* L.)	293.53±0.03^{bG}	192.77±0.14^{cB}	491.48±0.41^{aD}
Golden grass (*Solidago virgaurea* L.)	299.15±2.78^{bF}	127.86±0.40^{cD}	333.41±0.33^{aG}
Quince leaf (*Cydonia oblonga*)	602.60±1.06^{bB}	181.64±1.58^{cC}	673.23±1.56^{aB}
Hibiscus (*Hibiscus sabdariffa* L.)	502.56±0.14^{aC}	44.28±2.68^{cH}	466.91±0.00^{bE}
Nettle (*Urtica dioica* L.)	10.42±0.28^{bI}	4.81±1.55^{cI}	255.94±1.35^{aI}
Rosehip (*Rosa canina* L.)	275.62±0.18^{bH}	84.85±0.00^{cE}	292.84±1.20^{aH}
Lemon balm (*Melissa officinalis* L.)	672.49±0.80^{bA}	304.51±0.29^{cA}	679.52±0.34^{aA}
Green tea seed (*Camellia sinensis* L.)	312.11±0.02^{bE}	48.35±0.55^{cG}	453.24±0.13^{aF}
Green tea leaf (*Camellia sinensis* L.)	490.72±0.07^{bD}	57.66±0.78^{cF}	650.76±1.77^{aC}
Plant Samples	CUPRAC (mg TEAC/100g)		
	Methanol	Ethanol	Water
Wild sage (*Salvia officinalis* L.)	1288.26±5.43^{bH}	934.32±0.05^{cE}	2129.44±5.82^{aD}
Golden grass (*Solidago virgaurea* L.)	2132.92±2.73^{aE}	995.54±0.69^{bD}	226.32±2.60^{cI}
Quince leaf (*Cydonia oblonga*)	2765.49±3.63^{bB}	1169.88±4.08^{cC}	3083.47±0.00^{aB}
Hibiscus (*Hibiscus sabdariffa* L.)	2319.70±8.00^{aD}	359.38±0.12^{cH}	2103.38±0.41^{bE}
Nettle (*Urtica dioica* L.)	349.15±0.63^{bI}	84.69±0.14^{cI}	1900.81±0.04^{aF}
Rosehip (*Rosa canina* L.)	2327.91±1.91^{aC}	1817.10±3.43^{bB}	1774.93±0.01^{cH}
Lemon balm (*Melissa officinalis* L.)	3268.82±3.44^{aA}	2363.40±4.14^{cA}	3247.51±0.00^{bA}
Green tea seed (*Camellia sinensis* L.)	1342.46±0.221^{bG}	433.14±0.67^{cG}	1870.71±2.04^{aG}
Green tea leaf (*Camellia sinensis* L.)	1954.62±5.78^{bF}	480.95±2.02^{cF}	2711.75±0.29^{aC}

Different letters within the same row or column indicate statistically significant differences ($p < 0.05$)

previous findings of greater CUPRAC activity in water and methanol extracts of lemon balm (*Melissa officinalis* L.) than in ethanol[31]. Teofilović et al.[32] also indicated that Lamiaceae species aqueous infusions, particularly lemon balm (*Melissa officinalis* L.), always have high CUPRAC values because these are rich in water-soluble phenolics. Conversely, the strongly low CUPRAC value of nettle ethanol extract indicates its poor compatibility with less polar solvents, as was also previously noted in FRAP and CUPRAC-based research[33]. Overall, the CUPRAC assay confirms that solvent polarity plays an important role in antioxidant compound extraction and suggests that optimized water or methanol extractions—or blends thereof—can further enhance antioxidant recovery in phenolic-rich species like lemon balm (*Melissa officinalis* L.) and quince leaf (*Cydonia oblonga*).

3.4 DPPH

The IC_{50} values determined by the DPPH method inversely reflect the samples' ability to scavenge free radicals; thus, a lower IC_{50} indicates higher antioxidant activity.

In this context, the lowest IC_{50} value among the aqueous extracts was observed in rosehip (*Rosa canina* L.)" at 8.61 µg/mL, demonstrating its strong radical scavenging capacity. This was followed by hibiscus (*Hibiscus sabdariffa* L.) with 8.70µg/mL and golden grass (*Solidago virgaurea* L.) with 10.99 µg/mL (**Table 4**). Overall, aqueous extracts exhibited lower IC_{50} values compared to other solvents, indicating superior antioxidant capacity. Among methanol extracts, the lowest IC_{50} was found in golden grass (*Solidago virgaurea* L.) at 15.16 µg/mL, followed by hibiscus (*Hibiscus sabdariffa*

Table 4 DPPH radical scavenging activity IC_{50} values of plant samples.

Plant Samples	DPPH IC_{50} (μg/mL)		
	Methanol	Ethanol	Water
Wild sage (*Salvia officinalis* L.)	74.69±2.44^{aE}	73.39±0.56^{bB}	44.56±0.63^{cB}
Golden grass (*Solidago virgaurea* L.)	15.16±0.09^{bI}	39.27±0.69^{aE}	10.99±0.02^{cG}
Quince leaf (*Cydonia oblonga*)	149.29±3.46^{aC}	30.78±0.09^{bG}	28.72±0.43^{cD}
Hibiscus (*Hibiscus sabdariffa* L.)	27.33±1.34^{bH}	34.43±0.05^{aF}	8.70±0.44^{cH}
Nettle (*Urtica dioica* L.)	832.37±2.22^{aA}	377.80±2.32^{bA}	164.55±1.90^{cA}
Rosehip (*Rosa canina* L.)	72.60±1.88^{aG}	12.91±0.09^{bI}	8.61±0.28^{cH}
Lemon balm (*Melissa officinalis* L.)	207.59±1.55^{aB}	26.32±1.45^{cH}	36.87±1.61^{bC}
Green tea seed (*Camellia sinensis* L.)	89.10±1.06^{aD}	41.31±0.56^{bD}	20.62±1.04^{cF}
Green tea leaf (*Camellia sinensis* L.)	73.42±1.37^{aF}	52.60±0.19^{bC}	27.08±0.36^{cE}

Different letters within the same row or column indicate statistically significant differences ($p < 0.05$)

L.) at 27.33 μg/mL and rosehip (*Rosa canina* L.) at 72.60 μg/mL. The highest IC_{50} value was recorded in nettle at 832.37 μg/mL, indicating relatively low antioxidant activity. For ethanol extracts, rosehip (*Rosa canina* L.) again showed the lowest IC_{50} value of 12.91 μg/mL. Lemon balm (*Melissa officinalis* L.) and quince leaf (*Cydonia oblonga*) also exhibited high antioxidant activities with IC_{50} values of 26.32 μg/mL and 30.78 μg/mL, respectively. The highest IC_{50} value in ethanol extracts was observed in nettle (*Urtica dioica* L.) at 377.80 μg/mL. In general, antioxidant capacities of water and methanol extracts were found to be superior to those of ethanol extracts. Rosehip (*Rosa canina* L.), golden grass (*Solidago virgaurea* L.), and hibiscus (*Hibiscus sabdariffa* L.) consistently demonstrated strong antioxidant activities across all solvents with low IC_{50} values. Conversely, nettle showed relatively weak antioxidant capacity, reflected by its high IC_{50} values. Antioxidant activity values derived using the DPPH assay were quite different with respect to plant species and extraction solvents. Highly greater activity of aqueous extracts of rosehip (*Rosa canina* L.)", hibiscus, and yarrow may be due to the extreme polarity of water, which facilitates the extraction of polar hydrophilic phenolic compounds such as gallic acid, chlorogenic acid, and quercetin derivatives. This is consistent with recent studies that have revealed water-based extracts to always have greater total phenolic content (TPC) and better antioxidant activity, particularly in medicinal plants and agro-industrial waste[34]. Furthermore, new extraction techniques like subcritical water extraction have been found to further increase phenolic recovery and antioxidant capacities; for example, optimized subcritical water extraction of coffee residue generated over 200 mg GAE/g TPC values with strong DPPH radical scavenging activity[35]. Similarly, Samid et al.[36] reported that water extracts of certain apple varieties have high contents of polyphenols in line with high antioxidant capacity. Thus, the low IC_{50} values recorded for rosehip (*Rosa canina* L.)", hibiscus, and yarrow water extracts are consistent with these general findings, emphasizing the effectiveness of water as an extracting solvent of phenolic antioxidants in diverse botanical matrices.

4 Conclusions

This comprehensive study revealed that the chemical properties and antioxidant potentials of plant extracts vary significantly based on the type of solvent employed. Analyses demonstrated that water and methanol are more effective than ethanol for the extraction of phenol and flavonoid compounds, a finding directly correlated with the polar nature of these molecules. The study identified three primary plant species distinguished by their robust antioxidant capacities. Quince leaf (*Cydonia oblonga*) emerged as the richest source in terms of both total phenolic and flavonoid content. Lemon balm's (*Melissa officinalis* L.) potent reducing potential was evidenced by its highest values in the FRAP and CUPRAC assays. Conversely, rosehip (*Rosa canina* L.) exhibited the most potent free radical scavenging activity, as indicated by the lowest DPPH IC_{50} value. These findings suggest that plants such as quince leaf (*Cydonia oblonga*), lemon balm (*Melissa officinalis* L.), and rosehip (*Rosa canina* L.) possess significant potential as valuable functional food ingredients for combating oxidative stress within the food and nutrition sector.

Disclosure Statement

No potential conflict of interest was reported by the author(s).

Author Contributions

BÇS conceptualized and initiated the project, which included writing the Methods, Formal analysis, and Writing-original draft of the manuscript along with data collection and analysis. HİB authored the Introduction and Writing-original draft, Formal analysis, Investigation, Project administration and visualization, and reviewed and edited the entire paper. MA assisted with the review process, guided, Resources, and Validation.

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