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Cornus mas L. Seeds as a Potent Natural Antioxidant Source: Insights from Phenolic Profiling and Antioxidant Capacity Assays

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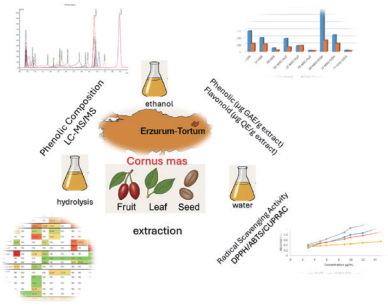

Abstract: In this study, the antioxidant potential and phenolic compound profiles of extracts obtained from the fruit, leaf, and seed parts of *Cornus mas* L. (cornelian cherry) collected from Tortum, Erzurum (Turkey) using ethanol and water solvents were evaluated. Antioxidant capacity was determined by DPPH, ABTS, and CUPRAC assays, while total phenolic content (TPC) and flavonoid content were measured spectrophotometrically. In addition, phenolic profiles were analyzed using LC-MS/MS technique. Among the extracts, those labeled "SD-MAS-H_2O" and "SD-MAS-EtOH" exhibited the highest antioxidant activities, with IC_{50} values of 2.16±0.11 and 2.27±0.09 µg/mL for DPPH, and 1.35 and 1.47±0.05 µg/mL for ABTS, respectively. In the CUPRAC assay, these same extracts showed the strongest reducing power, with absorbance values of 0.866 and 0.828. The highest total phenolic content was found in the "SD-MAS-ETOH" extract (157.80 µg GAE/g), followed by "LF-MAS-ETOH" (113.09 µg GAE/g) and "SD-HDR" (110.76 µg GAE/g). The "SD-MAS-ETOH" extract also contained the highest total flavonoid content (586.47 µg QE/g). LC-MS/MS analysis revealed that the "SD-MAS-H_2O" extract contained high concentrations of gallic acid (443.62 mg/L), vanillic acid (484.35 mg/L), hydroxybenzaldehyde (250.52 mg/L), and hesperidin (34.74 mg/L). These compounds are considered major contributors to the observed antioxidant activity. Pearson correlation analysis showed significant negative correlations between total phenolic content and IC_{50} values for DPPH (r = –0.7535) and ABTS (r = –0.7353), indicating a strong relationship between phenolic composition and antioxidant capacity. These findings highlight the potential of *Cornus mas* L. as a valuable natural antioxidant source for applications in functional foods and pharmaceutical products.

Key words: *Cornus mas*, antioxidant activity, phenolic/flavonoid compounds, LC-MS/MS

1 Introduction

Medicinal plants, which have been used in traditional medicine for centuries, continue to gain value in modern pharmaceutical and food industries due to their rich bioactive content. One such plant is *Cornus mas* L., commonly known as cornelian cherry, which belongs to the *Cornaceae* family. Naturally distributed from Southeastern Europe to Western Asia, this species is especially prevalent in the flora of countries such as Turkey, Bulgaria, Greece, Iran, and Ukraine. The identification of natural bioactive compounds has become increasingly important for the food, pharmaceutical, and cosmetic industries. In this context, investigating the chemical composition and biological activity of medicinal and aromatic plants contributes not only to public health but also guides the development of sustainable natural products. *Cornus mas* L., widely used in traditional medicine, is known for its high antioxidant potential and has become a focus of scientific research in recent years. Studies have reported its antioxidant, antimicrobial, anti-inflammatory, and anticancer effects. Its fruits are especially rich in phenolic compounds, flavonoids, organic acids, and vitamin C[1,2].

However, despite numerous studies on the fruits of *Cornus mas*, there is a notable lack of research focusing

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on the seeds, which are often overlooked as by-products. The phenolic content, antioxidant capacity, and potential industrial applications of the seeds remain underexplored. Additionally, comparative assessments using different solvent systems for phenolic extraction from various plant parts including seeds, leaves, and fruits are limited.

Anthocyanins and flavonols in these fruits provide protective effects against cardiovascular diseases, metabolic syndrome, and age-related degenerative conditions[3, 4]. Among these effects, antioxidant activity stands out as one of the most significant. Widely used methods for determining antioxidant capacity include DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS [2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)], and CUPRAC (cupric ion reducing antioxidant capacity). These assays allow for the quantification of the ability of compounds to neutralize free radicals and compare antioxidant strength. In addition to antioxidant testing, the determination of total phenolic content is essential for evaluating the biological value of plant-based samples. This is commonly measured using the Folin–Ciocalteu reagent and expressed as gallic acid equivalents (GAE). Numerous studies on *Cornus mas* have confirmed its high phenolic content and strong antioxidant activity[5]. For detailed identification of these phenolic compounds, LC-MS/MS (liquid chromatography–mass spectrometry) is widely used. This advanced analytical technique allows for precise qualitative and quantitative detection of phenolic acids, flavonoids, and other secondary metabolites in complex plant extracts[6].

Traditional uses of *Cornus mas* in Anatolian and Balkan folk medicine, such as treatments for diarrhea, fever, and immune system support, are increasingly supported by modern research[7]. Furthermore, its extracts have shown inhibitory effects against both Gram-positive and Gram-negative bacteria, highlighting its potential use as a natural preservative or therapeutic agent[8].

In this study, the fruit, leaf, and seed parts of *Cornus mas* L. collected from Tortum (Erzurum, Turkey) were extracted using different solvent systems. The antioxidant capacities were evaluated by DPPH, ABTS, and CUPRAC assays. Total phenolic content was determined using the Folin–Ciocalteu method, and phenolic profiles were analyzed by LC-MS/MS. The aim was to evaluate the potential of *Cornus mas* as a functional food and natural antioxidant source, contributing to its pharmacological and industrial utilization.

This study aims to evaluate the antioxidant activities and phenolic profiles of *Cornus mas* L. fruit, leaf, and seed extracts collected from Tortum (Erzurum, Turkey), using various solvent systems. Special emphasis is placed on the seed extracts, which have been less studied. Our goal is to contribute to the scientific understanding of *Cornus mas* L. as a natural antioxidant source and support its utilization in pharmaceutical and food industries.

2 Materials and Methods

In this study, various parts of *Cornus mas* L. (leaf, fruit, and seed) were subjected to different extraction methods, including maceration with ethanol or water and acid hydrolysis. To facilitate clarity and consistency throughout the manuscript, abbreviations were used to denote each sample type. Leaf extracts obtained by maceration with ethanol and water were labeled as LF-MAS-EtOH and LF-MAS-H_2O, respectively, while the leaf extract prepared via acid hydrolysis was referred to as LF-HDR. Similarly, fruit extracts obtained through ethanol and water maceration were designated as FR-MAS-EtOH and FR-MAS-H_2O, with FR-HDR representing the hydrolyzed fruit extract. Seed extracts were labeled SD-MAS-EtOH and SD-MAS-H_2O for ethanol and water maceration, respectively, and SD-HDR for the hydrolyzed seed extract. These standardized abbreviations were used consistently across tables, figures, and results discussions to enable clear comparison of phytochemical and antioxidant properties among the different extraction types and plant parts.

2.1 Preparation of plant extracts

Cornus mas L. samples were collected from the Tortum district of Erzurum, Turkey (40.37° N, 41.46° E). Samples of *Cornus mas* L. were collected in August 2023, during the fruit ripening period, from wild-growing plants in Tortum (Erzurum, Turkey). This region is characterized by a continental climate with hot summers and cold winters. No artificial irrigation or fertilization was applied, and the samples reflect the phytochemical content of naturally grown plants under moderate water stress conditions. The fruit, seed, and leaf parts were separated and dried under suitable conditions. The collected plant materials (leaves, fruits, and seeds of *Cornus mas* L.) were first dried in a ventilated oven at 40℃ for 48 hours to prevent degradation of thermolabile phenolic compounds. After drying, the samples were ground into a fine powder using a mechanical grinder. For each extract, 20 g of powdered material was mixed with 250 mL of either ethanol or deionized water and subjected to maceration at room temperature (～25℃) for 72 hours During maceration, the mixtures were manually shaken 3 to 4 times daily to enhance solvent penetration and extraction efficiency. Extraction was performed via maceration and acid hydrolysis. For hydrolyzed extracts, the method of Hertog et al. (1992) was followed. A 10 g sample was placed in a flask, to which 40 mL of 62.5% methanol and 10 mL of 6 M HCl were added. The mixture was incubated in a water bath at 80℃ for 2 hours[9, 10]. After cooling, the extract was filtered using Whatman No. 4 filter paper, then passed through a 0.45 μm membrane filter. This hydrolysis process was intended to release phenolics conjugated to sugars or cell wall polymers, thereby allowing a broader evaluation of the phenolic content in *Cornus mas* L. plant parts. Comparisons between hydrolyzed and

non-hydrolyzed (macerated) extracts were conducted to assess both free and bound phenolic profiles and their contributions to antioxidant activity. All extracts were stored at +4℃ in the dark for no longer than seven days (≤ 7 days) prior to analysis to minimize potential degradation of phenolic compounds and preserve antioxidant activity. The abbreviations of extraction samples used in the study are given in Table 1.

2.2 DPPH and ABTS radical scavenging activity

The DPPH radical scavenging activity was evaluated with minor modifications of the method by Blois (1958)[11]. Stock extract solutions (1 mg/mL) were prepared, and 10, 20, and 30 μL aliquots were taken and diluted with deionized water and ethanol to a final volume of 2 mL. Subsequently, 0.5 mL of DPPH solution was added, and the mixture was vortexed and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm. The scavenging activity was calculated using the following equation[12].

The ABTS radical scavenging activity was determined using the method by Re et al. (1999)[13] with slight modifications. $ABTS^{\cdot+}$ was generated by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the solution to stand in the dark for 12–16 hours. For the assay, 3, 6, and 9 μL of extract were added to ethanol and adjusted to 2 mL, then mixed with 0.5 mL of the $ABTS^{\cdot+}$ solution and incubated for 30 minutes in the dark. Absorbance was measured at 734 nm[14].

$$\text{DPPH/ABTS removal activity}(\%) = [(A_0 - A_1)/A_0] \times 100$$

A_0: Absorbance value measured for control, A_1: Absorbance value measured for extract. All measurements were performed in triplicate (n = 3).

2.3 Cu^{2+} - Cu^{+} reduction activity

The reducing power of the extracts was determined based on the method described by Apak et al. (2004)[15]. Extracts (3, 6, and 9 μL) were mixed with 247–241 μL of deionized water, 0.25 mL of copper (II) chloride solution, 0.25 mL of neocuproine solution, and 0.25 mL of ammonium acetate buffer. The mixture was incubated in the dark for 30 minutes, and absorbance was measured at 450 nm. All measurements were performed in triplicate (n = 3).

2.4 Total phenolic content (TPC) and total flavonoid content (TFC)

TPC was determined using the Folin–Ciocalteu method (Singleton & Rossi, 1965)[16]. Extracts (50 μL) were mixed with 1,150 μL of distilled water and 25 μL of Folin–Ciocalteu's reagent. After 3 minutes, 75 μL of 7% Na_2CO_3 was added, and the solution was incubated at room temperature in the dark for 2 hours. Absorbance was measured at 760 nm. Results were expressed as μg gallic acid equivalent (GAE)/g extract[17, 18]. TFC was measured using the aluminum chloride colorimetric method[19, 20]. Briefly, 50 μL of extract was added to a tube along with 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$ solution. After 5 minutes, 0.3 mL of 10% $AlCl_3$ was added, followed by 2 mL of 1 M NaOH. The total volume was adjusted to 10 mL with water. Absorbance was measured at 510 nm, and results were expressed as μg quercetin equivalent (QE)/g extract. Each assay was conducted in triplicate (n = 3) to ensure reproducibility.

2.5 LC-MS/MS analysis

Phenolic compounds were identified and quantified using LC-MS/MS. Shimadzu LCMSMS-8030 equipped with a SUPELCO Discovery C18 column (5 cm × 2.1 mm, 5 μm) was used. Mobile phases A and B consisted of 0.1% formic

Table 1 Linear regression equation for DPPH/ABTS, coefficient of determination (R^2) and half maximal inhibition concentration.

	DPPH			ABTS		
		R^2	IC_{50} μg/mL		R^2	IC_{50} μg/mL
SD-MAS-EtOH	y = −0.151x + 0.6656	0.9937	2.269 ± 0.09	y = −0.2458x + 0.7115	0.9985	1.47 ± 0.05
SD-HDR	y = −0.0182x + 0.6446	0.9916	17.176 ± 0.73	y = −0.0375x + 0.6733	0.9929	8.621 ± 0.52
SD-MAS-H_2O	y = −0.137x + 0.6279	0.9957	2.16 ± 0.11	y = −0.2498x + 0.687	0.992	1.349 ± 0.06
FR-MAS-EtOH	y = −0.0032x + 0.6327	0.9879	96.156 ± 4.62	y = −0.0027x + 0.7278	0.9966	139.926 ± 6.29
FR-HDR	y = −0.0015x + 0.6396	0.9811	209.733 ± 11.26	y = −0.0029x + 0.7125	0.992	125 ± 5.69
FR-MAS-H_2O	y = −0.0023x + 0.6359	0.9822	97.156 ± 3.85	y = −0.0008x + 0.6479	0.9795	372.375 ± 12.56
LF-MAS-EtOH	y = −0.0413x + 0.6565	0.9961	7.857 ± 0.28	y = −0.075x + 1.15	0.9868	10.666 ± 0.13
LF-HDR	y = −0.019x + 0.6431	0.9901	16.374 ± 0.54	y = −0.0362x + 0.6533	0.9932	8.378 ± 0.98
LF-MAS-H_2O	y = −0.0148x + 0.6538	0.9946	21.743 ± 0.94	y = −0.0256x + 0.668	0.9971	12.421 ± 1.01
Ascorbic acid	y = −0.1074x + 0.6596	0.9926	3.05 ± 0.12	y = −0.0788x + 1.0221	0.9898	6.62 ± 0.75
Trolox	y = −0.0892x + 0.6438	0.9916	3.49 ± 0.17	y = −0.1175x + 0.9844	0.9862	4.12 ± 0.36

acid in 80:20 methanol:water and 0.1% formic acid in 20:80 methanol:water, respectively. The system operated in binary gradient mode with a flow rate of 0.4 mL/min at 40℃. Each compound's retention time, limit of detection (LOD), limit of quantification (LOQ), and mass transitions were determined. Quantitative analysis was expressed in mg/L. All these results are given in Table 2. Each compound was identified using retention time and mass transitions compared with analytical standards. Calibration curves were constructed using external standards at five concentration levels ($R^2 > 0.995$). Limits of detection (LOD) and quantification (LOQ) were calculated based on signal-to-noise ratios of 3:1 and 10:1, respectively.

2.6 Statistical analysis

All experimental results were expressed as mean ± standard deviation (SD). Statistical analyses were performed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons ($p < 0.05$ considered statistically significant). Analyses were conducted using SPSS v25.0 (IBM, USA).

3 Results

3.1 DPPH and ABTS radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a simple, rapid, and reliable spectrophotometric method frequently used to evaluate antioxidant capacity. DPPH is a stable free radical that appears deep purple in solution. Antioxidant compounds donate hydrogen atoms or electrons to DPPH, reducing it to a colorless or pale yellow form. This reduction leads to a measurable change in absorbance at 517 nm.

Table 2 LC-MS/MS Retention time. LOD. LOQ and Main ion (*m/z*).

	Retention time (min.)	LOD (Limit of Detection) (mg/L)	LOQ (Limit of Quantification) (mg/L)	Main ion (*m/z*)
Alizarin (+)	5.518	6.90	23.01	240.90＞139.00
Thymoquinone (+)	1.806	2.05	6.84	164.90＞137.00
Vanilic acid (+)	0.860	84.78	282.61	168.95＞65.20
Acetohydroxamic acid (+)	0.438	2.88	9.61	75.95＞57.90
Catechinhyrate (+)	0.549	7.64	25.47	291.00＞139.10
Syringic acid (+)	0.926	41.83	139.43	198.90＞140.10
Resveratrol (+)	3.815	7.91	26.38	229.00＞135.00
Kaempferol (+)	4.940	3.92	13.06	286.90＞153.00
Ellagic acid_neg	2.655	2.87	9.58	300.80＞145.10
Silymarin (-)	4.884	7.33	24.44	481.00＞125.00
Chlorogenic acid	0.426	8.92	29.74	353.00＞191.20
Fumaric acid (-)	0.469	2.76	9.20	115.00＞70.90
Gallic acid (-)	0.429	22.88	76.25	169.00＞125.00
Protocatechuik acid (-)	0.548	7.17	23.90	153.20＞108.00
Caffeic acid (-)	0.822	81.80	272.67	178.90＞135.10
Oleuropein (-)	4.364	2.07	6.91	539.10＞307.10
Phloridzindyhrate (-)	4.211	4.34	14.45	434.90＞273.10
Myricetin_neg	4.117	237.42	791.40	316.80＞151.00
Hydoxycinamic acid (-)	2.907	7.79	25.98	163.00＞119.10
Naringenin (-)	4.872	38.5	128.2	270.90＞151.00
Quercetin (-)	4.768	68.4	228.1	300.80＞151.00
2-Hyroxy1,4 naphthaquinone (-)	2.449	8.0	26.7	173.00＞144.90
Luteolin (-)	4.956	6.4	21.4	284.80＞133.00
Salicylic acid (-)	2.633	3.9	13.0	137.20＞93.10
4-Hydroxybenzoic acid (-)	0.687	15.3	51.1	137.20＞92.90
Curmin (-)	6.473	12.8	42.7	366.90＞217.10

In this study, extracts prepared using four different solvents and methods were analyzed for DPPH radical scavenging activity. For each extract, linear regression equations, determination coefficients (R^2), and half-maximal inhibitory concentrations (IC_{50}) were calculated. These parameters allow for quantitative comparison of antioxidant potential among the extracts. "Significant differences ($p < 0.05$) were observed between IC_{50} values of extracts, confirming that the variations in antioxidant activity are statistically meaningful.

The results, summarized in Table 1, showed that the extracts labeled "SD-MAS-H_2O" and "SD-MAS-EtOH" exhibited the lowest IC_{50} values (2.16 ± 0.11 and 2.27 ± 0.09 μg/mL, respectively), indicating the strongest DPPH scavenging activity. In contrast, the "FR-HDR", "FR-MAS-H_2O", and "me" extracts had significantly higher IC_{50} values (209.73 ± 11.26, 97.16 ± 3.85, and 96.16 ± 4.62 μg/mL, respectively), reflecting weak antioxidant activity.

All samples demonstrated high R^2 values (0.9811–0.9961), suggesting strong linearity and reliability of the assay. These findings support the conclusion that variations in antioxidant activity among the samples are closely related to differences in their phenolic and flavonoid compositions. In particular, the strong activity of "SD-MAS-H_2O" and "SD-MAS-EtOH" suggests their potential utility in functional food or pharmaceutical formulations. However, as the DPPH assay is an in vitro method, further in vivo studies are needed to confirm these results. The differences in IC_{50} values reflect the variations in phenolic and flavonoid content among the extracts. These results suggest that the differences in antioxidant activity between the samples are due to the differences in phenolic and flavonoid content in their compositions. Since the DPPH test measures the protective effects against free radicals, it is used especially in determining the antioxidant potential of natural compounds. The different IC_{50} values observed in the study indicate the variability of phenolic and flavonoid contents in the composition of the samples. For example, SD-MAS-H_2O and SD-MAS-EtOH samples may have a higher concentration of phenolic compounds or more effective antioxidant molecules.

The ABTS assay is widely used for assessing antioxidant capacity by measuring the ability of antioxidants to scavenge the $ABTS^{\cdot+}$ radical cation. In this method, the blue-green $ABTS^{\cdot+}$ radical is reduced to a colorless form in the presence of antioxidant compounds, resulting in a decrease in absorbance at 734 nm. One of the advantages of this assay is its applicability to both hydrophilic and lipophilic antioxidants. Antioxidant capacity is typically expressed as IC_{50}, where lower values indicate stronger activity. In the current study, all extracts were evaluated for their ABTS scavenging activity by calculating linear regression equations, determination coefficients (R^2), and IC_{50} values. The results (see Table 1) showed excellent linearity for all samples ($R^2 > 0.97$), confirming the reliability of the method. Among the tested samples, the "SD-MAS-H_2O" and "SD-MAS-EtOH" extracts demonstrated the strongest antioxidant capacity, with IC_{50} values of 1.35 and 1.47 μg/mL, respectively. Notably, the SD-MAS-H_2O extract exhibited an ABTS IC_{50} value of 1.35 ± 0.06 μg/mL, which was significantly lower than that of the standard antioxidants Trolox (4.12 ± 0.36 μg/mL) and ascorbic acid (6.62 ± 0.75 μg/mL), indicating a stronger radical scavenging capacity. Similarly, its DPPH IC_{50} value (2.16 ± 0.11 μg/mL) was markedly lower than both standards, supporting the conclusion of exceptionally high antioxidant potential relative to known reference compounds. These values were significantly lower than those of other samples, indicating high ABTS radical scavenging activity. In contrast, "FR-MAS-H_2O", "FR-HDR", and "FR-MAS-EtOH" extracts displayed considerably higher IC_{50} values (372.38 ± 12.56, 125 ± 5.69, and 139.93 ± 6.29 μg/mL, respectively), suggesting weak antioxidant potential. Intermediate activity was observed in "SD-HDR", "LF-HDR", "LF-MAS-EtOH", and "LF-MAS-H_2O" extracts, indicating moderate antioxidant properties. These findings suggest that the extraction method and plant part significantly influence the antioxidant efficacy of *Cornus mas* extracts. In comparison with standards, ascorbic acid and Trolox exhibited IC_{50} values of 6.62 ± 0.75 μg/mL and 4.12 ± 0.36 μg/mL, respectively. Interestingly, the "SD-MAS-H_2O" extract showed even stronger scavenging activity than ascorbic acid in the ABTS assay. This highlights the potential of certain *Cornus mas* extracts as powerful natural antioxidants. ABTS shows that it reliably represents the relationship between radical scavenging capacity and concentration. IC_{50} values quantitatively express antioxidant activity, and low values indicate that the radical scavenging capacity of the extract is high. As a result, ABTS radical scavenging capacity data obtained in the study provided important information in comparing the antioxidant activities of different extracts. The strong antioxidant properties of SD-MAS-EtOH and SD-MAS-H_2O extracts increase the potential of these extracts to be used as natural antioxidant agents in the pharmaceutical or food industry. In future studies, identification of the active components of these extracts and detailed examination of their mechanisms will contribute to a better understanding of their antioxidant activities. The findings obtained show that ce and cs extracts have strong antioxidant activity and can be used in potential biomedical applications. The fact that other extracts have relatively lower antioxidant capacity suggests that the extraction method and the chemical composition of the plant source are effective. Future studies should be supported by structural analyses of the active components of these extracts and their in vivo activities should be examined. In addition, since the use of natural antioxidants can provide safer alternatives for health compared to synthetic antioxidants, the importance

of research in this area is great.

3.2 Cu^{2+} - Cu^{+} reduction activity

Cu^{2+} – Cu^{+} reduction activity is an analysis method that measures the ability of antioxidant compounds in samples to reduce copper(II) ion(Cu^{2+}) to lower value copper(I) ion (Cu^{+}). This method is generally known as CUPRAC (Cupric Reducing Antioxidant Capacity) test. In the CUPRAC test, when Cu^{2+} ions are reduced by reacting with neocuproine, a Cu^{+}–neocuproine complex is formed and this complex gives a stable yellow-orange color that can be measured at a wavelength of 450 nm. High absorbance indicates that the substances in the sample have a strong reducing(antioxidant) capacity. The CUPRAC method is advantageous in that it is suitable for the analysis of both hydrophilic and lipophilic antioxidants. It can cover a wide range of antioxidant types. It gives a stable and sensitive absorbance value. Among the tested samples, SD-MAS-ETOH and SD-HDR exhibited the highest reducing power, consistent with their high TPC and TFC values (Figs. 1–4).

3.3 Determination of total phenolic and flavonoid contents in plant extracts and their relation to antioxidant activity

The total phenolic content of plant extracts prepared using different solvents was determined spectrophotometrically and expressed as gallic acid equivalents(GAE). The calculations were performed using the calibration equation $y = 0.0684x + 0.1172$ with a high linearity($R^2 = 0.9987$). Similarly, the total flavonoid content of the extracts was measured spectrophotometrically and expressed as quercetin equivalents(QE), based on the calibration curve $y = 0.0176x + 0.0589$($R^2 = 0.9981$). The obtained results are presented in Fig. 5. The total phenolic contents of the analyzed samples were quantitatively expressed as μg GAE/g extract. Among the tested samples, the highest phenolic content was observed in sample SD-MAS-ETOH(157.80 μg GAE/g), followed by LF-MAS-ETOH(113.09 μg GAE/g), SD-HDR(110.76 μg GAE/g), and LF-HDR(104.91 μg GAE/g). The high phenolic content in these samples may be attributed to their rich phenolic profiles, which could have significantly contributed to their antioxidant activities. Samples such as LF-MAS-H_2O(81.22 μg GAE/g) and SD-MAS-H_2O(61.90 μg GAE/g) showed moderate phenolic

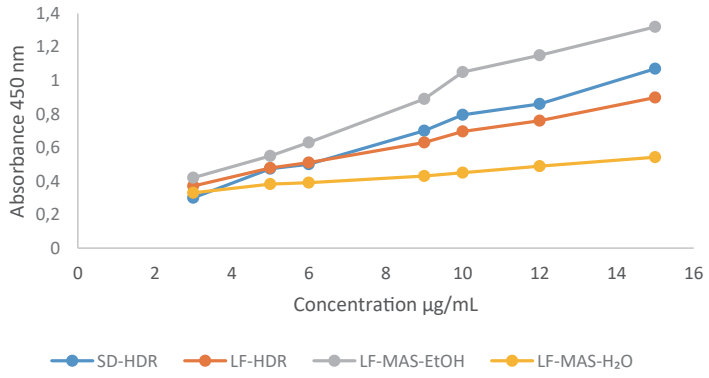

Fig. 1 Metal-reducing capacity with CUPRAC assays of SD-HDR, LF-HDR, LF-MAS-EtOH and LF-MAS-H_2O fractions in different concentrations.

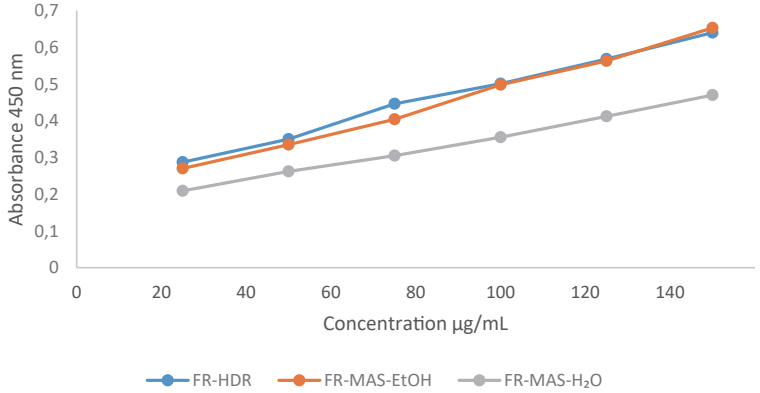

Fig. 2 Metal-reducing capacity with CUPRAC assays of FR-HDR,FR-MAS-EtOH and FR-MAS-H_2O fractions in different concentrations.

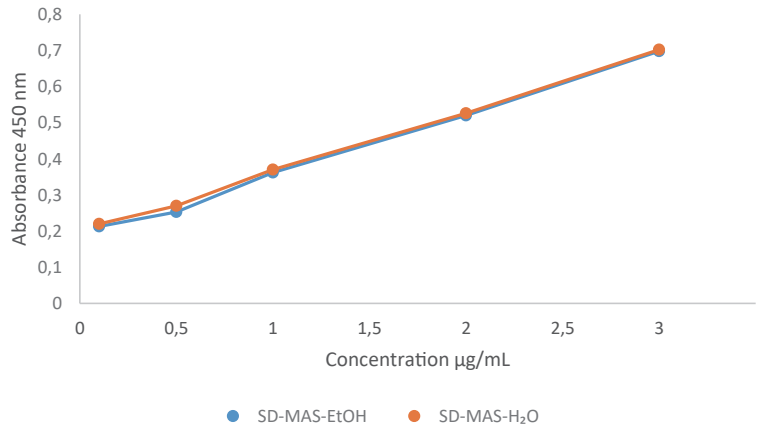

Fig. 3 Metal-reducing capacity with CUPRAC assays of SD-MAS-EtOH and SD-MAS-H_2O fractions in different concentrations.

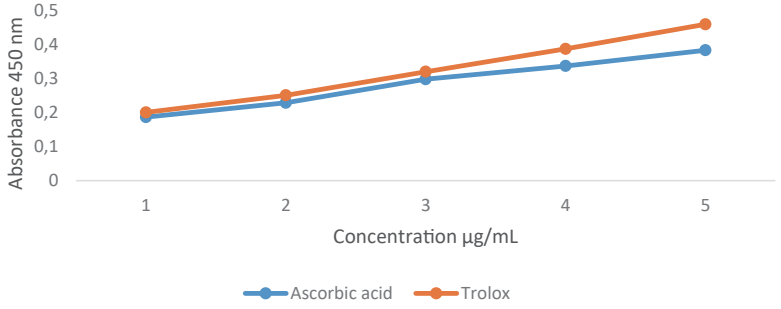

Fig. 4 Metal-reducing capacity with CUPRAC assays of ascorbic acid and trolox fractions in different concentrations.

content. A positive correlation was observed between the phenolic contents and antioxidant activities measured via ABTS and DPPH assays in these samples. In contrast, samples FR-MAS-H_2O (11.33 µg GAE/g), FR-MAS-ETOH (10.31 µg GAE/g), and FR-HDR (19.54 µg GAE/g) exhibited low levels of total phenolics and flavonoids. These low values may explain their weak antioxidant capacities. Specifically, FR-MAS-H_2O and FR-MAS-ETOH showed both low phenolic content and high IC_{50} values, indicating low biological activity. These findings support the hypothesis that phenolic compounds play a critical role in antioxidant mechanisms and suggest a meaningful correlation between the phenolic content and the biological activity of plant-derived extracts. Samples such as SD-MAS-ETOH, LF-MAS-ETOH, and SD-HDR with high phenolic contents may hold potential for use in functional foods or pharmacological formulations. Phenolic compounds are recognized as major contributors to antioxidant activity in plant sources. Their levels may vary depending on plant species, cultivation conditions, harvest time, and extraction methods. Conversely, the low phenolic levels detected in samples FR-MAS-H_2O, FR-HDR, and FR-MAS-ETOH may indicate a poorer chemical composition and limited biological activity. Such differences could be attributed to biological diversity among the samples, environmental conditions, or extraction efficiency. In conclusion, the variability in the distribution of phenolic compounds among the tested samples emphasizes the importance of phenolic content analysis in evaluating the antioxidant potential of plant sources. In this context, further investigation of the biological activities of samples with high phenolic contents could contribute to natural product development efforts.

The total flavonoid contents of the plant extracts were expressed as µg QE/g and showed significant variation among the samples. The highest flavonoid content was recorded in sample SD-MAS-ETOH (586.47 µg QE/g), highlighting its rich matrix in phenolic compounds and potential biological activity. This was followed by SD-HDR (286.47 µg QE/g), LF-MAS-ETOH (229.65 µg QE/g), and LF-HDR (192.15 µg QE/g), all of which also exhibited strong antioxidant activities. Samples SD-MAS-H_2O (180.17 µg QE/g) and LF-MAS-H_2O (78.52 µg QE/g) were found to possess moderate flavonoid levels. Notably, SD-MAS-H_2O stood out for both its flavonoid content and low IC_{50} values, suggesting a direct relationship with its antioxidant capacity. On the other hand, the lowest flavonoid contents were found in samples FR-MAS-H_2O (13.75 µg QE/g), FR-MAS-ETOH (16.53 µg QE/g), and FR-HDR (46.70 µg QE/g), which also demonstrated weak antioxidant activities. In a study conducted by Savaş et al.[21], the antioxidant activity

of garagurt extract was analyzed using ABTS, CUPRAC, and DPPH methods, yielding values of 8.428 ± 1.206 mg TE/100 g, 1.599 ± 41.4 mg TE/100 g, and 773 ± 206 mg TE/100 g, respectively. In summary, samples such as SD-MAS-ETOH, SD-HDR, and LF-MAS-ETOH, which are rich in flavonoids, should be further investigated as potential sources of natural antioxidants and for their applications in functional food, phytotherapy, and cosmetic industries. The results of this study contribute significantly to the literature by suggesting a positive correlation between total flavonoid levels and antioxidant activities. All these results are given in Fig. 5.

The relationships between total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity parameters (DPPH IC_{50}, ABTS IC_{50}, CUPRAC) were analyzed using Pearson correlation coefficients. The resulting correlation matrix provides valuable insights into the connection between antioxidant capacity and major phenolic constituents. A strong positive correlation was observed between TPC and TFC ($r = 0.8916$), supporting the notion that flavonoids, as a subclass of phenolic compounds, significantly contribute to the overall phenolic profile. This finding highlights the complementary roles of phenolics and flavonoids in determining the antioxidant potential of plant-derived extracts. Significant negative correlations were found between the IC_{50} values obtained from DPPH and ABTS assays and both TPC and TFC. The DPPH IC_{50} value exhibited a strong negative correlation with total phenolic content ($r = -0.7535$) and a moderate negative correlation with flavonoid content ($r = -0.5852$). Similarly, the ABTS IC_{50} value showed negative correlations with TPC ($r = -0.7353$) and TFC ($r = -0.5652$). Since lower IC_{50} values indicate stronger antioxidant activity, these negative correlations clearly demonstrate the pivotal role of phenolic and flavonoid compounds in scavenging free radicals, particularly in the DPPH and ABTS assays. On the other hand, the reducing capacity measured by the CUPRAC method was found to be strongly and positively correlated with both total phenolic ($r = 0.7433$) and flavonoid ($r = 0.7636$) contents. This suggests that the CUPRAC assay, which is based on electron transfer capacity, is particularly sensitive to phenolic compounds with reducing properties. Moreover, strong negative correlations were observed between CUPRAC and both DPPH ($r = -0.7397$) and ABTS ($r = -0.7097$) assays, indicating that although these assays are complementary, they evaluate antioxidant capacity through different mechanisms. Overall, these findings demonstrate that phenolic and flavonoid compounds play a central role in the antioxidant activities of plant extracts. The strong correlations observed between these compounds and antioxidant capacities measured by DPPH, ABTS, and CUPRAC assays underscore their biological relevance and provide a scientific basis for assessing the bioactivity potential of the natural products analyzed in this study.

3.4 LC-MS/MS-based content analysis

Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) is an advanced analytical technique that enables the separation, identification, and quantification of components within complex mixtures with high sensitivity and selectivity. In this technique, compounds are first separated through liquid chromatography (LC), followed by ionization and detection based on their mass-to-charge (m/z) ratios using mass spectrometry (MS). Tandem mass spectrometry (MS/MS), which employs sequential mass analyzers, allows for further fragmentation of target molecules, thereby providing detailed structural information. LC-MS/MS is widely employed for the analysis of phenolic compounds, flavonoids, alkaloids, and various metabolites, offering high accuracy and reproducibility in pharmaceutical, food, environmental, and biological sample analyses. In the present study, the analysis of phenolic compounds was conducted using a Shimadzu LCMSMS-8030 system. A SUPELCO Discovery C18 column (5 cm × 2.1 mm, 5 µm particle size) was employed for chromatographic separation. The column temperature was maintained at 40℃,

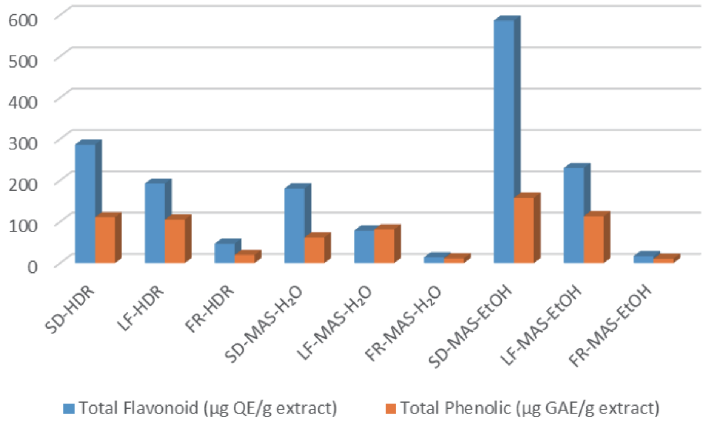

Fig. 5 Total Phenolic (µg GAE/g extract) and Flavonoid (µg QE/g extract) substance amounts.

with a maximum temperature limit of 75℃. The system operated in binary gradient mode. Mobile phase A consisted of 0.1% formic acid, 20% ultrapure water, and 80% methanol, while mobile phase B comprised 0.1% formic acid, 20% methanol, and 80% ultrapure water. The flow rate was set to a constant 0.4000 mL/min. The LC system was equipped with LC-20ADXR pump modules, and sample injections were performed using a SIL-20ACXR autosampler. The maximum system pressure was limited to 400 bar. Under these conditions, the composition and quantitative determination of targeted phenolic compounds were carried out with precision. The table shows the concentrations of various phenolic compounds and flavonoids in different extracts (LF-HDR, LF-MAS-EtOH, LF-MAS-H_2O, FR-HDR, FR-MAS-EtOH, FR-MAS-H_2O, SD-HDR, SD-MAS-EtOH, SD-MAS-H_2O). Retention Time (RT) and reference response (Resp) of each compound are given. Also, "ND" (Not Detected) indicates that the corresponding compound was not detected in the extract. Gallic acid was found in high concentrations in most of the extracts, with particularly dominant levels in SD-MAS-H_2O (443.62 μg/g), SD-HDR (288.02 μg/g), and LF-HDR (208.38 μg/g) extracts. The strong antioxidant properties of gallic acid have been widely reported in the literature, suggesting that its presence may significantly contribute to the antioxidant capacity of these extracts. The high content of this compound is consistent with the elevated radical scavenging capacities observed in these extracts via the ABTS assay. Protocatechuic acid was detected in LF-HDR (60.93 μg/g) and LF-MAS-H_2O (18.41 μg/g) extracts but was not present in the others, indicating a diversity in the phenolic profiles of the extracts. Caffeic acid was especially abundant in the LF-HDR extract (429.22 μg/g), while it was found in lower amounts in other extracts such as LF-MAS-EtOH (not detected) and LF-MAS-H_2O (42.62 μg/g). Considering the known anti-inflammatory and antioxidant activities of caffeic acid, its high concentration may underlie the notable biological potential of the LF-HDR extract. Chlorogenic acid was identified only in LF-MAS-H_2O (277.09 μg/g), FR-MAS-EtOH (44.63 μg/g), and SD-MAS-H_2O (21.13 μg/g) extracts. The presence of this compound in selective extracts may be attributed to differences in plant material or extraction methodology. Vanillic acid and vanillin were found at notably high concentrations in SD-HDR (270.44 / 29.91 μg/g) and SD-MAS-H_2O (484.35 / 19.99 μg/g) extracts, while being low or undetectable in the others, indicating a richness of these extracts in aromatic phenolic compounds. Naringenin was detected in relatively similar amounts across all extracts, ranging approximately from 50 to 65 μg/g, with the highest level observed in SD-MAS-H_2O (65.70 μg/g). Naringenin, a member of the flavonoid class, is known for its antioxidant, anti-inflammatory, and anticancer properties. Hesperidin was exclusively detected in LF-MAS-H_2O (82.39 μg/g) and SD-MAS-H_2O (34.74 μg/g) extracts. The presence of hesperidin may enhance the potential health benefits associated with these specific extracts. Inevitably, several flavonoid and phenolic compounds (e.g., rutin, quercetin, luteolin) were not detected ("ND") in some of the extracts, reflecting the chemical diversity among them and emphasizing that each extract possesses a unique composition of bioactive compounds. In a related study conducted by Savaş et al., the concentrations of major phenolic compounds were reported as follows: gallic acid 33.4 ± 2.24 μg/g, ellagic acid 16.9 ± 2.04 μg/g, catechin derivative 12.6 ± 0.97 μg/g, *p*-coumaric acid 1.40 ± 0.07 μg/g, *p*-coumaric acid derivative 1.00 ± 0.02 μg/g, and rutin 0.70 ± 0.12 μg/g. All these results are given in Table 3. The LC-MS/MS results were statistically validated by recovery tests (average recovery > 90%), ensuring reliable quantification of phenolic compounds.

Previous studies have shown that environmental factors such as drought stress, altitude, sunlight exposure, and harvest time can significantly influence the accumulation of phenolic compounds in medicinal plants[21]. Environmental and seasonal factors such as harvest time, altitude, sunlight exposure, and water stress significantly affect the accumulation of phenolic and flavonoid compounds. For instance, regional variability has been shown to alter phenolic profiles in cornelian cherry fruits and processed products (e.g., jams) across different growing regions and storage times. Therefore, the variability observed among our samples may reflect these environmental influences, especially given the wild collection without agronomic control[22,23]. The variability observed among extracts in this study may also reflect such environmental influences, especially considering the wild collection without agronomic control. The results of this study align with previous reports highlighting the antioxidant potential of *Cornus mas* L. fruit extracts (Sadeghi et al., 2020; Ghosh et al., 2021); however, our work differs by providing a comprehensive comparison across leaf, fruit, and especially seed parts, as well as between hydrolyzed and non-hydrolyzed extracts. Notably, the SD-MAS-H_2O extract exhibited IC_{50} values lower than those of Trolox and ascorbic acid, indicating a stronger antioxidant effect than commonly used reference standards, a finding not previously reported for *Cornus mas* seed extracts. Furthermore, LC-MS/MS analysis revealed that this extract is particularly rich in gallic acid and vanillic acid, consistent with the compounds' known radical scavenging activity. While earlier studies focused predominantly on fruit phenolics, the current study offers novel data on seed phenolic profiles and high-yield antioxidant performance, contributing new insights into underutilized parts of the plant. Overall, this research extends the existing body of knowledge by identifying *Cornus mas* seeds as a promising source of natural antioxidants, with potential applications in nutraceutical, pharmaceutical, and food formulations[24–26].

Table 3 Phenolic compounds and their concentrations (ppb) detected by LC-MS/MS.

Compound	LF-HDR	LF-MAS-EtOH	LF-MAS-H_2O	FR-HDR	FR-MAS-EtOH	FR-MAS-H_2O	SD-HDR	SD-MAS-EtOH	SD-MAS-H_2O
Shikimic acid	ND	ND	ND	ND	115.45	191.30	ND	ND	ND
Gallic acid	208.38	ND	202.93	76.19	39.29	ND	288.02	160.00	443.62
Protocatechuic acid	60.93	ND	18.41	ND	20.66	ND	ND	ND	ND
Epigallocatechin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Catechin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chlorogenic acid	ND	ND	277.09	ND	44.63	ND	ND	ND	21.13
Hydroxybenzaldeyde	14.05	ND	ND	24.10	ND	ND	243.34	109.87	250.52
Vanillic acid	ND	ND	ND	ND	ND	ND	270.44	ND	484.35
Caffeic acid	429.22	ND	42.62	18.16	ND	ND	ND	ND	10.09
Syringic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND
Caffein	ND	ND	ND	0.57	1.46	2.37	2.93	0.32	0.83
Vanillin	ND	ND	ND	12.02	ND	ND	29.91	15.58	19.99
Polydatine	ND	ND	ND	ND	ND	ND	ND	ND	ND
o-Coumaric acid	44.48	ND	12.24	ND	ND	ND	ND	ND	ND
Salicylic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND
Taxifolin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Resveratrol	ND	ND	ND	ND	ND	ND	ND	ND	ND
trans-Ferulic acid	ND	ND	ND	ND	62.49	27.84	ND	ND	ND
Sinapic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND
Scutellarin	ND	ND	3.81	ND	ND	ND	ND	ND	ND
p-Coumaric acid	ND	ND	ND	ND	ND	ND	ND	ND	ND
Coumarin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Protocatehuic ethyl ester	ND	ND	5.46	ND	ND	ND	ND	ND	ND
Hesperidin	ND	ND	82.39	ND	ND	ND	ND	ND	34.74
Isoquercitrin	ND	3.18	10.71	ND	ND	ND	ND	ND	1.05
Rutin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Quarcetin-3-xyloside	ND	ND	ND	ND	ND	ND	ND	ND	ND
Kaempferol-3-glucoside	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fisetin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Baicalin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chrysin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Daidzein	ND	ND	ND	ND	ND	ND	ND	ND	ND
trans-Cinnamic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND
Quercetin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Naringenin	55.36	51.88	55.65	40.19	63.35	61.75	63.20	60.25	65.70
Hesperetin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Morin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Kaempferol	ND	ND	ND	ND	ND	ND	ND	ND	ND
Baicalein	ND	ND	ND	ND	ND	ND	ND	ND	ND
Luteolin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Biochanin A	ND	ND	ND	ND	ND	ND	ND	ND	ND
Capcaicin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dihydrocapcaicin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Diosgenin	10.01	9.90	11.27	12.09	8.32	8.92	ND	10.65	8.09

ND: Not Detected

4 Conclusion

The present study demonstrates that the extracts analyzed are rich in phenolic and flavonoid compounds, with notable differences in their chemical profiles depending on the extract type. These compositional variations significantly influence the biological activities of the samples, particularly their antioxidant capacities. High levels of key phenolic compounds such as gallic acid, caffeic acid, vanillic acid, and naringenin support the strong biological potential of certain extracts. The results clearly indicate that both the quantity and quality of phenolic constituents play a crucial role in determining antioxidant activity. Among the tested samples, the SD-MAS-H_2O extract exhibited the highest antioxidant activity, as evidenced by its lowest IC_{50} values in both ABTS and DPPH assays. This was followed by the SD-MAS-EtOH and SD-HDR samples. In contrast, the FR-MAS-H_2O and FR-MAS-EtOH extracts showed markedly higher IC_{50} values, indicating weaker antioxidant activity. CUPRAC assay results supported these findings, with the SD-MAS-H_2 and SD-MAS-EtOH extracts exhibiting the highest absorbance values, indicative of strong reducing power. Correspondingly, lower absorbance values observed in FR-MAS-H_2O are consistent with diminished antioxidant capacity. The total phenolic content (TPC) analysis further corroborated these findings. The SD-MAS-H_2O extract recorded the highest TPC followed by SD-MAS-EtOH and SD-HDR. A negative correlation was observed between TPC and IC_{50} values supporting the hypothesis that phenolic content is inversely related to antioxidant activity. LC-MS/MS analyses provided detailed insights into the phenolic profiles of the extracts. The SD-MAS-H_2O sample, in particular, contained high levels of gallic acid hydroxybenzaldehyde, vanillic acid and hesperidin all of which are well-documented for their potent antioxidant properties. Similarly, the SD-MAS-EtOH and SD-HDR samples showed elevated concentrations of these compounds. In contrast, the FR-MAS-H_2O and FR-MAS-EtOH samples exhibited limited phenolic diversity and significantly lower concentrations of key bioactive compounds, such as gallic acid. Collectively, these findings underscore the importance of evaluating both the total amount and specific composition of phenolic compounds when assessing the antioxidant potential of natural products. The extraction method and the plant part used are critical determinants of bioactive compound profiles and their biological efficacy. However, the present study is limited to in vitro analyses, and further in vivo investigations are needed to evaluate the bioavailability, metabolism, and potential toxicity of these extracts. Future work should also focus on formulation studies for food or pharmaceutical applications, as well as exploring synergistic interactions among phenolic compounds. The results suggest that extraction methods, plant species, and the intrinsic chemical makeup of the plant materials are key determinants of the phytochemical profile and biological activity of the extracts. Future studies should explore other biological activities and the synergistic interactions among phenolic constituents to better understand and utilize the therapeutic and functional food applications of such natural products.

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