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Antioxidant profiling of *Anethum graveolens*: Insights into phenolic and flavonoid-rich extracts

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Anethum graveolens'in antioksidan profili: Fenolik ve flavonoid bakımından zengin ekstraktlara dair bulgular

Abstract: This study investigates the total phenolic and flavonoid contents, antioxidant activity, and metal chelation capacity of acetone and water extracts from *Anethum graveolens* L.. The total phenolic content of the acetone extract was significantly higher (173.49 ± 4.91 µg GAE/mg extract) than the water extract (98.52 ± 3.62 µg GAE/mg extract). Similarly, the flavonoid content of the acetone extract (72.81 ± 1.15 µg QE/mg extract) exceeded that of the water extract (27.69 ± 1.72 µg QE/mg extract). Concentration-dependent responses revealed higher antioxidant activity for the acetone extract across all tested concentrations (12.5–400 µg/mL), with a sharper increase in response at higher concentrations. The IC_{50} values for DPPH radical scavenging and metal chelation activities further confirmed the acetone extract's superior performance, with lower IC_{50} values for DPPH scavenging (51.56 µg/mL) and metal chelation (113.46 µg/mL) compared to the water extract (192.44 µg/mL and 268.95 µg/mL, respectively). Hierarchical clustering and 3-D surface plot analyses demonstrated strong correlations between DPPH scavenging and metal chelation activities for both extracts, with Pearson correlation coefficients of $r = 0.94$ for the acetone extract and $r = 0.99$ for the water extract. While the acetone extract displayed higher bioactivity, the water extract exhibited a more tightly linked relationship between its antioxidant and metal chelation properties. These findings highlight the potential of *A. graveolens* extracts as natural antioxidants and metal chelators, offering promising applications for oxidative stress mitigation and metal toxicity management.

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

Özet: Bu çalışma, *Anethum graveolens* L.'den elde edilen aseton ve su ekstraktlarının toplam fenolik ve flavonoid içeriklerini, antioksidan aktivitelerini ve metal şelatlama kapasitesini incelemektedir. Aseton ekstraktının toplam fenolik içeriği (173,49 ± 4,91 µg GAE/mg ekstrakt), su ekstraktından (98,52 ± 3,62 µg GAE/mg ekstrakt) anlamlı derecede daha yüksek bulunmuştur. Benzer şekilde, aseton ekstraktının flavonoid içeriği (72,81 ± 1,15 µg QE/mg ekstrakt), su ekstraktının flavonoid içeriğinden (27,69 ± 1,72 µg QE/mg ekstrakt) daha yüksektir. Konsantrasyona bağlı tepkiler, tüm test edilen konsantrasyonlarda (12,5–400 µg/mL) aseton ekstraktının daha yüksek antioksidan aktiviteye sahip olduğunu ve özellikle yüksek konsantrasyonlarda daha keskin bir artış gösterdiğini ortaya koymuştur. DPPH radikal süpürme ve metal şelatlama aktiviteleri için IC_{50} değerleri, aseton ekstraktının üstün performansını daha da doğrulamış, DPPH süpürme için daha düşük IC_{50} değeri (51,56 µg/mL) ve metal şelatlama için (113,46 µg/mL) değerleri bulunmuştur. Buna karşılık, su ekstraktı için DPPH süpürme (192,44 µg/mL) ve metal şelatlama (268,95 µg/mL) değerleri daha yüksektir. Hiyerarşik kümeleme ve 3 boyutlu yüzey grafiği analizleri, her iki ekstrakt için DPPH süpürme ve metal şelatlama aktiviteleri arasında güçlü korelasyonlar göstermiştir. Aseton ekstraktı için Pearson korelasyon katsayısı $r = 0,94$ iken, su ekstraktı için $r = 0,99$ olarak hesaplanmıştır. Aseton ekstraktı daha yüksek biyoaktivite sergilerken, su ekstraktı, antioksidan ve metal şelatlama özellikleri arasında daha sıkı bir ilişki ortaya koymuştur. Bu bulgular, *A. graveolens* ekstraktlarının doğal antioksidanlar ve metal şelatörler olarak potansiyelini vurgulamakta ve oksidatif stresin azaltılması ve metal toksisitesinin yönetimi için umut verici uygulamalar sunduğunu göstermektedir.

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

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1. Introduction

Plants are a rich source of natural compounds that support human health. Among these compounds, antioxidants play a crucial role in reducing oxidative stress caused by free radicals and preventing cellular damage (Akbari et al., 2022; Göldağ and Doğan, 2024). Natural antioxidants have gained increasing interest in the food and pharmaceutical industries because of their potential to extend the shelf life of foods and protect human health (Costa et al., 2021). Free radicals are at the root of numerous health problems, including aging, chronic diseases, cancer, diabetes, and cardiovascular disorders (Kumar and Pandey, 2015). Plant-based antioxidants, including phenolic compounds, flavonoids, and vitamins, have the potential to mitigate these harmful effects (Akbari et al., 2022). Therefore, investigating the antioxidant properties of plants is of great importance for discovering new natural therapeutic compounds and developing healthy and sustainable products for the food, pharmaceutical, and cosmetic industries (Diniz do Nascimento et al., 2020; Doğan, 2020). Additionally, determining the antioxidant properties of

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plants contributes to understanding the biochemical variations caused by environmental factors, cultivation conditions, and species differences (Shen et al., 2022). In recent years, many researchers have conducted studies on the antioxidant properties of plants (Kok et al., 2023; Tang et al., 2023; Collins et al., 2024; Tokgoz et al., 2024).

Commonly known as dill, *Anethum graveolens* L. is not only appreciated as a flavorful herb in culinary practices but also recognized as a healing source with significant health benefits (Al Masoody et al., 2023; Mujović et al., 2024). Its flavonoids and phenolic compounds can neutralize free radicals, reduce oxidative stress, and enhance immune function (Mohammed et al., 2019; Khan et al., 2020). Traditionally, dill has been used to alleviate digestive issues, reduce bloating, and regulate appetite (Singh et al., 2024). Furthermore, it has shown potential in regulating blood sugar levels and lowering cholesterol, positively impacting metabolic health (Haidari et al., 2020). Its antimicrobial properties may also reduce the risk of infections (Ghoname et al., 2023). These characteristics highlight dill's importance in both traditional and modern health practices.

Total phenolic compounds play a significant role in the antioxidant activity of plant-based extracts. These compounds are critical for both food preservation and human health due to their ability to neutralize free radicals (Asif, 2015; Gutiérrez-del-Río et al., 2021). Similarly, flavonoids, a group of polyphenolic compounds widely present in plants, exhibit antioxidant properties. Flavonoids act through diverse mechanisms, such as scavenging free radicals, chelating metal ions, and protecting against oxidative stress (Cotelle, 2001; Cherrak et al., 2016).

One of the common methods for determining antioxidant properties is 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay (Gulcin and Alwasel, 2023). This test is used to quantitatively measure a substance's capacity to neutralize free radicals and is considered an important indicator of the antioxidant activity of plant extracts (Nićiforović et al., 2010; Gupta, 2015). Additionally, metal chelating activity reflects a plant's ability to reduce the effects of metal ions that generate reactive oxygen species (Hassinen et al., 2011). Quantitative determination of total phenol and flavonoid contents is also crucial for understanding dill's antioxidant capacity.

Dill has potential applications as a natural preservative in the food industry and as an anti-inflammatory, and anticancer agent in pharmacology (Mohamed et al., 2024). However, a better understanding of factors such as environmental influences and extraction methods that affect dill's antioxidant properties is necessary. In this context, our study aims to determine the antioxidant activity of *A. graveolens* and to elucidate the relationship between this activity and its total phenol and flavonoid contents.

This study aims to provide a comprehensive analysis of the antioxidant capacity of *A. graveolens* using different solvent fractions. The findings will enhance our understanding of this plant's potential applications in food and pharmaceutical industries and serve as a foundation for future research.

2. Materials and Method

2.1. Obtaining Plant Samples and Their Extraction

Anethum graveolens samples were freshly obtained from a local producer in Konya, Türkiye for use in our research. The samples were kept under cool conditions during transportation to prevent spoilage. Before being used in the study, the plants were carefully cleaned and prepared appropriately for analysis. Plant samples were air-dried at room temperature and then transformed into powder form using an ultra-centrifuge grinder. The extraction process involved obtaining acetone and water extracts of *A. graveolens* using a Soxhlet extraction apparatus with 250 mL solvent systems. Acetone and water extracts of *A. graveolens* yielded 2.5% and 23.3% (w/w) of plant substances, respectively. The resulting crude extract from the plant sample was filtered using a Whatman No. 1 filter paper. The solvents were subsequently removed by evaporation through a rotary evaporator under vacuum conditions, leading to complete drying. The extract was further lyophilized to yield ultra-dry powders.

2.2. Determination of Total Phenol Content

To quantify the overall phenolic content in the extracts, we utilized a modified Folin-Ciocalteu method with gallic acid as a reference standard. In separate wells of a microplate, we added 20 µL of each extract (400 µg/mL) and the gallic acid standard to analyze. We then introduced 20 µL of the Folin-Ciocalteu reagent into each well and allowed the mixture to incubate in the dark for 3 min. This incubation is essential for the reduction of the Folin reagent by the phenolic compounds. Next, we added 20 µL of sodium carbonate to each well to neutralize the mixture, followed by the addition of 140 µL of distilled water (dH_2O). This step helps develop the color indicative of phenolic content. The wells were kept in the dark for an additional 10 min to allow color development. After the incubation period, we measured the absorbance at 725 nm using a spectrophotometer. The intensity of the color formed is directly proportional to the phenolic content in the samples. The phenolic content was expressed as gallic acid equivalents (GAE), calculated using a standard calibration curve generated from known concentrations of gallic acid (Kok et al., 2023).

2.3. Determination of Total Flavonoid Content

To quantify the overall flavonoid content in the extracts, we utilized a modified aluminum chloride colorimetric method with quercetin as a reference standard. In distinct wells of a microplate, we added 50 µL of each extract (400 µg/mL) and the quercetin standard. To each well, we then introduced 215 µL of 80% ethyl alcohol to help dissolve the flavonoid compounds and facilitate the reaction. Following this, 5 µL of the aluminum nitrate solution and 5 µL of potassium acetate were added to the mixture in each well. The plates were incubated at room temperature for 40 min in the dark to allow the formation of the aluminum-flavonoid complex, which is crucial for accurate detection. After incubation, the absorbance of the solutions was measured at 415 nm using a spectrophotometer. The absorbance correlates with the flavonoid content in the samples. The flavonoid content was expressed as quercetin equivalents (QE). This was calculated using a standard calibration curve created with known concentrations of quercetin (Kok et al., 2023).

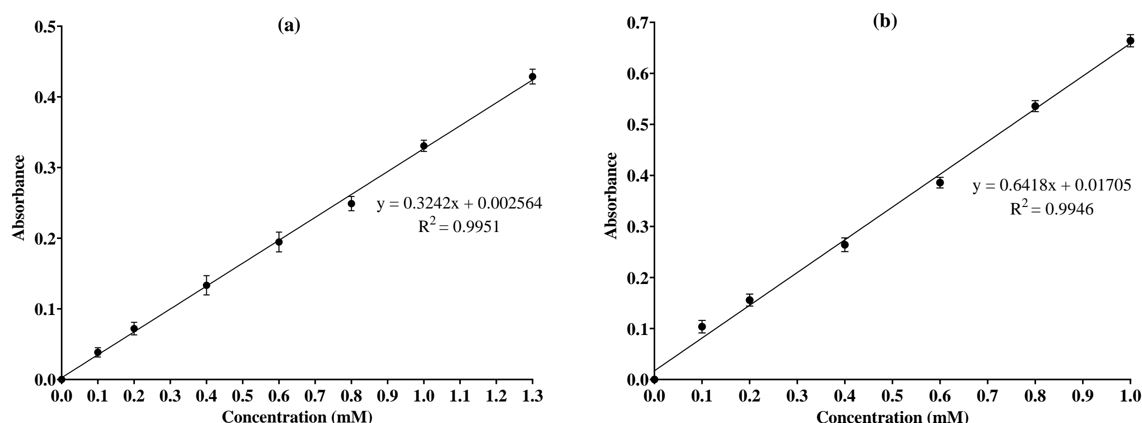

Figure 1. (a) Linear regression graphs for gallic acid, used as a standard in calculating total phenolic content, and **(b)** quercetin, used as a standard in calculating total flavonoid content (n = 3).

2.4. Free Radical Scavenging Activity

In the measurement of DPPH scavenging activity of acetone and water extracts obtained from the plants, applications were carried out with the final concentrations of the extracts in the plate wells of 12.5, 25, 50, 100, 200, and 400 µg/mL. According to the method, 20 µL of the extracts were placed in each microplate well, and 180 µL of DPPH (0.06 mM in methanol) was added. The reduction of DPPH free radical was determined by measuring the absorbance values at 517 nm after 60 minutes in the dark. The free radical scavenging activities of the extracts were calculated as a percentage using the following formula (1): Radical scavenging activity = [(Control absorbance – Extract absorbance) / Control absorbance)] × 100 (Kok et al., 2023).

2.5. Chelation of Metals

To assess the metal-binding capacity, we followed a spec-trophotometric method using $FeCl_2$ and ferrozine. Various concentrations (12.5–400 µg/mL) of acetone and water extracts from both allelopathic and control plants were added to wells in a microplate. Each well received 50 µL of the plant extract, 185 µL of distilled water, 5 µL of $FeCl_2$ (2 mM), and 10 µL of ferrozine (5 mM). After 10 min incuba- tion at room temperature, the absorbance was measured at 562 nm to determine the Fe^{2+}-binding ability of the extracts. The metal chelating activities of the extracts were calculated as a percentage using the formula (2): Metal chelating activity = [(Control absorbance – Sample absorbance) / Control absorbance)] × 100 (Kok et al., 2023).

2.6. Statistical Analyses

The activities of the extracts were evaluated through a one-way ANOVA followed by the Duncan test. Probit regression analysis was applied to calculate the median inhibitory concentration (IC_{50}) values. To examine the relationships among DPPH scavenging and metal chelating activities across different extracts, three-dimensional (3D) density analysis was performed. These statistical analyses were conducted using SPSS software (version 27.0, IBM Corporation, Armonk, NY, USA). Additionally, heatmap and hierarchical cluster analyses, employing Ward's minimum variance method, were used to identify similarities and differences among DPPH scavenging and metal chelating activities. These analyses were performed in the RStudio console using the pheatmap package in R software (version 4.1.0).

3. Results and Discussion

3.1. Analysis of Antioxidant Compounds of the Extracts

The total phenolic and flavonoid contents of acetone and water extracts were determined and expressed as GAE and QE, respectively. The acetone extract demonstrated a significantly greater phenolic content (1.76 times higher than the water extract), highlighting its superior antioxidant potential. For total flavonoid content, the acetone extract also demonstrated higher values, with 72.81 ± 1.15 µg QE/mg of extract, compared to the water extract, which had a total flavonoid content of 27.69 ± 1.72 µg QE/mg of extract (Table 1). These results suggest that the acetone extract has higher concentrations of both phenolic and flavonoid compounds compared to the water extract, indicating its greater potential as an antioxidant source.

Phenolic compounds, such as flavonoids, have been widely recognized for their antioxidant properties due to their ability to neutralize free radicals and chelate metal ions (Vuolo et al., 2019; Parcheta et al., 2021). The higher phenolic and flavonoid content in the acetone extract is likely a result of acetone's ability to dissolve a wider range of non-polar and semi-polar bioactive compounds. Acetone is a less-polar solvent compared to water and is particularly effective in extracting compounds such as flavonoids, phenolic acids, and other secondary metabolites that have antioxidant and anti-inflammatory properties (Tzanova et al., 2020; Eid et al., 2023). These compounds have been associated with a broad range of biological activities, including anti-cancer, anti-inflammatory, and antioxidant effects, highlighting the acetone extract's greater potential for use in pharmaceutical and nutraceutical applications.

On the other hand, the water extract, while exhibiting lower levels of both phenolic and flavonoid compounds, may still offer beneficial bioactive properties. Water is a highly polar solvent and may extract a different spectrum of compounds, such as water-soluble vitamins, sugars, and other hydrophilic antioxidants, which may still contribute to its overall bioactivity. However, the lower concentrations of phenolic and flavonoid compounds in the water extract suggest that its potential as a potent antioxidant source is less than that of the acetone extract (Fatima et al., 2019; Shi et al., 2022). The findings also suggest that the acetone

extract may be more effective as a natural antioxidant due to the higher concentrations of these bioactive compounds. Since phenolic compounds and flavonoids are known for their ability to scavenge free radicals and reduce oxidative stress (Huyut et al., 2017), the acetone extract's superior levels of these compounds could potentially offer more robust protection against oxidative damage. This is consistent with previous research that has shown acetone extracts to possess stronger antioxidant activity compared to water extracts (Zhao et al., 2006; Ahmad et al., 2020).

In conclusion, the acetone extract of *A. graveolens* is a more promising source of antioxidants due to its higher phenolic and flavonoid content. While the water extract may have potential, particularly for applications requiring less potent antioxidant activity, the acetone extract is better suited for industries such as cosmetics, food preservation, and pharmaceuticals, where strong antioxidant properties are highly valued. Further studies are needed to investigate the specific antioxidant mechanisms of these extracts and to explore their applications in various fields.

3.2. Assessing DPPH Scavenging Abilities of the Extracts

The effects of acetone and water extracts were evaluated at different concentrations (12.5, 25, 50, 100, 200, and 400 µg/mL) on the measured responses.

For the acetone extract, the observed values increased with concentration. At the lowest concentration (12.5 µg/mL), the response was 14.17, which increased progressively to 75.5 at the highest concentration (400 µg/mL). Notably, the acetone extract exhibited a marked rise in response, especially at concentrations above 50 µg/mL, with values of 64.56 (50 µg/mL), 70.69 (100 µg/mL), and 72.80 (200 µg/mL), before stabilizing at 75.5 at 400 µg/mL. This could be attributed to the higher concentrations of phenolic and flavonoid compounds, which are known for their antioxidant and metal chelation properties (Chanda et al., 2015). The higher concentrations of these bioactive compounds in the acetone extract likely contribute to its greater ability to neutralize free radicals and chelate metal ions, which in turn leads to a stronger overall bioresponse at elevated concentrations.

In contrast, the water extract showed a more gradual increase in response across the concentrations. At 12.5 µg/mL, the response was 4.43, which increased steadily to 63.54 at 400 µg/mL. The response at 50 µg/mL was 22.52, rising to 30.61 at 100 µg/mL, and peaked at 57.60 at 200 µg/mL before reaching the highest value at 63.54 at 400 µg/mL. The gradual rise in response suggests that the bioactivity of the water extract is less concentration-dependent compared to the acetone extract. This behavior could be due to the different chemical profiles of the water extract, which may contain a lower concentration of phenolic and flavonoid compounds (Ahmad et al., 2020).

Table 1. Antioxidant compounds of the plant extracts (µg/mg)

Treatment	Total phenol (Gallic acid equivalent)	Total flavonoid (Quercetin equivalent)
Acetone extract	173.49 ± 4.91	72.81 ± 1.15
Water extract	98.52 ± 3.62	27.69 ± 1.72

Each value is expressed as mean ± standard deviation (n = 3)

The water extract may still possess antioxidant and metal chelation properties, but the presence of lower levels of bioactive compounds may result in a more gradual increase in biological response.

The data indicate a higher overall response for the acetone extract across all concentrations compared to the water extract. Additionally, the acetone extract demonstrated a sharper increase in response at higher concentrations, while the water extract exhibited a more gradual, linear rise. These findings suggest that the acetone extract may be more potent or effective at promoting the observed response, particularly at higher concentrations. The letters above the bars indicate statistical differences among the groups. Groups sharing the same letter are not significantly different ($p > 0.05$), while those with different letters indicate statistically significant differences ($p < 0.05$). For the acetone extract, the scavenging activity increased significantly with concentration, reaching the highest value at 100 µg/mL, which was not significantly different from 50 µg/mL. We found that there was no difference ($p > 0.05$) between 200 and 400 µg/mL applications of acetone extract (Fig. 2). This effect is common in biological systems, where increasing concentrations beyond a certain threshold may not result in a proportional increase in activity due to saturation of the biological response mechanism (Xie and Schaich, 2014).

3.3. Chelation of Metals by the Extracts

The effects of concentrations ranging from 12.5–400 µg/mL of acetone and water extracts were evaluated. Both extracts exhibited concentration-dependent increases in bioactivity, although the acetone extract consistently showed higher responses across most concentrations, especially at higher levels, suggesting it might be more effective in delivering antioxidant or other bioactive effects. This finding is consistent with previous studies where solvent polarity has been found to affect the extraction efficiency and bioactive potential of plant compounds (Tripathi et al., 2013).

For the acetone extract**,** the values ranged from 4.29 at 12.5 µg/mL to 67.68 at 400 µg/mL. Notably, the acetone extract showed an increasing trend in its response with higher concentrations, with the highest values observed at the 400 µg/mL concentration. The variability in the acetone extract response, as indicated by the second set of measurements, ranged from 1.31 at 12.5 µg/mL to 1.66 at 400 µg/mL. Previous studies have shown that acetone is an effective

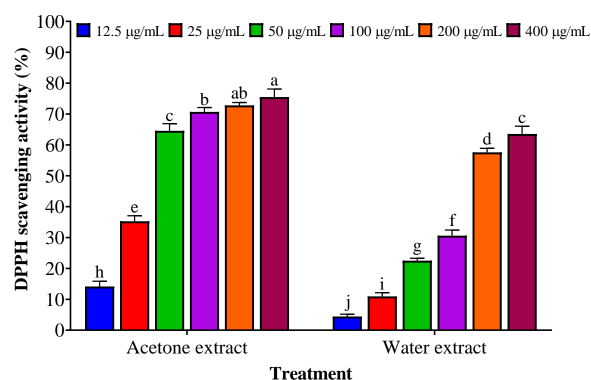

Figure 2. DPPH radical scavenging activity of different plant extracts is presented as mean ± standard deviation (n = 3). Distinct letters indicate statistically significant differences at $p < 0.05$.

solvent for extracting phenolic compounds, which are known for their antioxidant and metal chelation activities (Wang et al., 2009). The lower variability in the acetone extract also suggests that acetone-soluble compounds are more consistent in their bioactive interactions, which could be advantageous in applications requiring a predictable and reliable antioxidant effect (Salak et al., 2013).

In comparison, the water extract exhibited a similar concentration-dependent increase, with values ranging from 3.47 at 12.5 µg/mL to 56.40 at 400 µg/mL. The variability for the water extract response ranged from 1.17 at 12.5 µg/mL to 2.09 at 400 µg/mL. Overall, the water extract displayed more significant variations in response compared to the acetone extract, particularly at higher concentrations. This could be due to the more complex mixture of compounds in the water extract, including both hydrophilic and less bioavailable substances, leading to more variability in biological responses (Verma et al., 2012). Water extracts tend to contain a greater variety of compounds, but these compounds may not all contribute equally to antioxidant activity resulting in less predictable outcomes compared to acetone extracts. At all concentrations, the acetone extract generally demonstrated higher values than the water extract, with the exception of the 100 µg/mL and 200 µg/mL concentrations where water extract values were comparable or slightly higher. These findings are in line with research suggesting that water extracts can sometimes outperform organic solvent extracts at lower concentrations, particularly when hydrophilic bioactive compounds are present in substantial amounts (Matalka et al., 2007). These findings suggest that the acetone extract might be more effective at higher concentrations, whereas the water extract, although exhibiting some variability, also shows potential efficacy at different concentration levels.

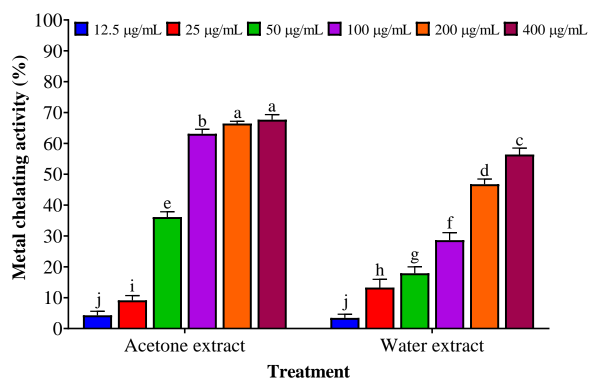

Figure 3. Metal chelating activity of different plant extracts is presented as mean ± standard deviation (n = 3). Distinct letters indicate statistically significant differences at $p < 0.05$.

When the statistical differences between the concentrations were examined, we found that there was no difference ($p > 0.05$) between 200 and 400 µg/mL applications of acetone extract (Fig. 3). Such effects are common in studies of antioxidant activity, where the bioactivity of certain compounds reaches a maximum threshold beyond which higher concentrations do not result in proportional increases in activity (Abd El-Gawad, 2016). This observation may provide valuable insight into optimizing concentrations for applications in which acetone extracts are used, such as in food preservation or therapeutic applications aimed at reducing oxidative stress.

The IC_{50} values of *A. graveolens* extracts for DPPH radical scavenging and ferrous ion chelating activities are presented in Table 2. The acetone extract exhibited a significantly lower IC_{50} value for DPPH scavenging activity (51.56 µg/mL) compared to the water extract (192.44 µg/mL), indicating stronger antioxidant activity in the acetone extract. The slope for the DPPH scavenging activity was 1.11 ± 0.06 for acetone, with a confidence interval of 0.98–1.23, suggesting a moderately steep dose-response curve.

For metal chelation, the acetone extract also showed a lower IC_{50} value (113.46 µg/mL) compared to the water extract (268.95 µg/mL), indicating superior metal chelation ability. The slope for the acetone extract's metal chelating activity was 1.48 ± 0.07 (1.34–1.62), suggesting a slightly steeper dose-response relationship than for the DPPH scavenging activity. In comparison, the water extract showed weaker activity for both DPPH scavenging and metal chelation, as reflected by the higher IC_{50} values and the less pronounced slopes (1.38 ± 0.07 for DPPH scavenging, 1.21 ± 0.07 for metal chelation). These findings demonstrate that the acetone extract of *A. graveolens* is more effective than the water extract in both DPPH radical scavenging and ferrous ion chelation activities.

The lower IC_{50} value for the acetone extract supports its ability to neutralize reactive oxygen species more efficiently, which is consistent with the general observation that acetone tends to extract more potent antioxidant compounds, such as polyphenols and flavonoids, compared to water (Ali et al., 2014). Metal ion chelation is a critical mechanism in antioxidant defense, as metal ions like iron and copper can catalyze the production of harmful free radicals (Gulcin and Alwasel, 2022). These results corroborate earlier findings in the literature, where acetone and other organic solvents were shown to be more efficient than water in extracting bioactive compounds with antioxidant and metal-chelating properties (Petkova et al., 2020).

Table 2. IC_{50} values (µg/mL) of *A. graveolens* extracts for their DPPH radical scavenging and ferrous ion chelating activities

Extract	Activity	IC_{50} (Limits)	Slope ± Standard error (Limits)
Acetone	DPPH scavenging	51.56 (45.01–58.64)	1.11 ± 0.06 (0.98–1.23)
	Metal chelating	113.46 (102.54–126.09)	1.48 ± 0.07 (1.34–1.62)
Water	DPPH scavenging	192.44 (170.10–220.85)	1.38 ± 0.07 (1.24–1.52)
	Metal chelating	268.95 (229.64–323.55)	1.21 ± 0.07 (1.07–1.36)

3.4. Analysis of Heatmap, Cluster, and 3-D Density

A hierarchical clustering analysis was conducted to group the samples based on their DPPH radical scavenging activity and metal chelation capacity. The resulting dendrogram (Fig. 4a) revealed distinct clusters, each represented by a unique color, demonstrating significant variability in bioactivities among the samples. The clustering segregated the samples into 4 groups, highlighting differences in antioxidant and metal chelation properties between the acetone extract and water extract. Applications of 100, 200, and 400 µg/mL acetone extract under Cluster 4 stood out with high DPPH and metal chelation activities. This cluster, representing the highest activity, further corroborates the strong antioxidant and metal-chelating properties of the acetone extract, which are consistent with its lower IC_{50} values for both DPPH scavenging and metal chelation (Çayan et al., 2022).

To explore the relationship between DPPH radical scavenging activity and metal chelation capacity, 3-D surface plots were generated for the acetone extract and water extract, as shown in Figures 4b and 4c, respectively. For the acetone extract, the 3-D surface plot (Fig. 4b) demonstrated a strong positive correlation between DPPH radical scavenging activity and metal chelation capacity, with a Pearson correlation coefficient of r = 0.94. This correlation was observed within the activity range of 10–60% DPPH scavenging activity and 10–50% metal chelation activity, indicating a synergistic relationship where higher antioxidant activity is consistently associated with enhanced metal chelation capacity in the acetone extract.

Similarly, the water extract exhibited an even stronger correlation, as illustrated in Fig. 4c, with a Pearson correlation coefficient of r = 0.99. This correlation was observed within the activity range of 10–70% DPPH scavenging activity and 10–60% metal chelation activity, suggesting an almost linear relationship between the two activities. The near-perfect correlation highlights the robust, tightly linked relationship and consistent, potent bioactive profile of the water extract. This is in line with previous studies indicating that antioxidants capable of scavenging free radicals are often also effective in chelating metal ions, which helps prevent oxidative damage (Di Meo and Venditti, 2020).

When comparing the two extracts, the water extract showed a slightly higher correlation coefficient compared to the acetone extract. This finding suggests that the water extract may possess a more tightly linked mechanism governing its antioxidant and metal chelation activities. The difference emphasizes the potential of water extract as a more effective extract for applications requiring both strong antioxidant and metal chelation properties. The slightly higher correlation coefficient for the water extract could reflect the presence of water-soluble compounds that more directly influence both antioxidant and chelating activities, or a unique bioactive synergy specific to the water extract (Cheng et al., 2021).

These results provide strong evidence of the interplay between antioxidant and metal chelation activities in both extracts, with the water extract demonstrating superior performance. This highlights the potential of these extracts for further bioactive compound characterization and their use in applications targeting oxidative stress and metal toxicity mitigation.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

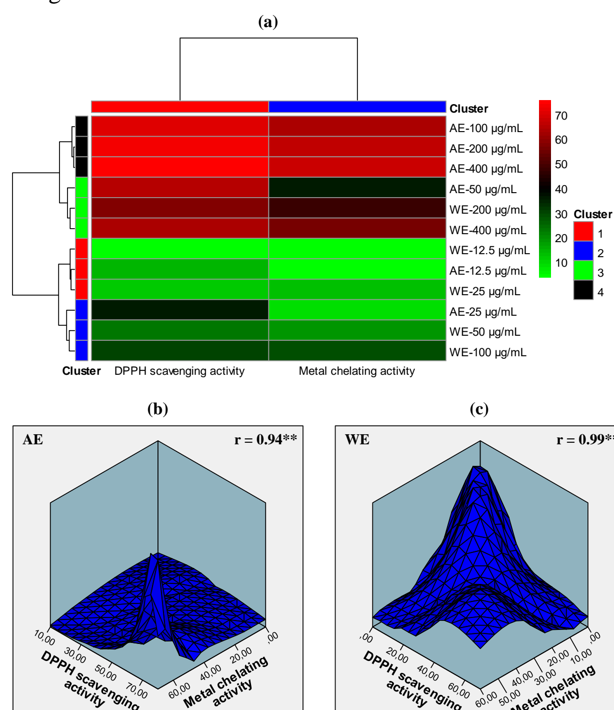

Figure 4. (a) Heatmap and hierarchical clustering analysis of various concentrations of the tested extracts, displaying DPPH scavenging and metal chelating activities, where red indicates high activity and green indicates low activity. **(b, c)** 3-D density analysis was performed to assess the DPPH scavenging and metal chelating activities of the extracts. Correlation coefficients (r) were calculated, with statistical significance denoted by a double asterisk (**) at the 0.01 level. AE: Acetone extract of *A. graveolens*, WE: Water extract of *A. graveolens*.

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