



## Comparative assessment of antioxidant capacity and phytochemical profiles of *Tilia*, *Mentha* and *Salvia* herbal teas

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### İhlamur, nane ve adaçayılarının antioksidan kapasiteleri ve fitokimyasal profillerinin karşılaştırmalı değerlendirilmesi

**Abstract:** Herbal teas prepared from *Tilia* (linden), *Mentha* (mint), and *Salvia* (sage) species are widely consumed due to their potential health benefits and their traditional use in the management of respiratory disorders and cold-related symptoms. These plants contain various bioactive compounds, including phenolics, flavonoids, and carotenoids, which may contribute to their antioxidant properties. The present study aimed to comparatively evaluate the antioxidant potential and phytochemical composition of methanol and water extracts obtained from *Tilia*, *Mentha* and *Salvia* sp. The antioxidant activities of the extracts were assessed using DPPH radical scavenging activity, reducing power, and metal chelating activity assays. In addition, the levels of important bioactive compounds, including total phenolics, total flavonoids,  $\beta$ -carotene, and lycopene, were determined to investigate their relationship with antioxidant activity. The results revealed notable differences among the tested plant species and extraction solvents. The water extract of *Salvia* sp. exhibited the strongest DPPH radical scavenging activity ( $IC_{50}$ : 0.157 mg/ml), while *Mentha* sp. extracts demonstrated the highest reducing power ( $EC_{50}$ : 0.305 mg/ml) and metal chelating activity ( $IC_{50}$ : 0.756 mg/ml). Phytochemical analyses showed that the methanol extract of *Mentha* sp. contained the highest  $\beta$ -carotene and lycopene levels, whereas the highest total phenolic content was detected in the water extract of *Mentha* sp. In contrast, *Salvia* sp. extracts, particularly the methanol extract, displayed the highest flavonoid content. These findings suggest that phenolic and flavonoid compounds play a major role in the antioxidant activity of the extracts, while carotenoids may further enhance their antioxidant potential. Overall, the findings highlight the importance of *Tilia*, *Mentha*, and *Salvia* sp. as natural sources of antioxidant compounds. Among the tested plants, *Mentha* and *Salvia* extracts demonstrated comparatively stronger antioxidant properties, indicating their potential value as functional ingredients in nutraceuticals and herbal beverages.

**Keywords:** Herbal tea, antioxidant activity, phenolic compounds, traditional medicine

**Özet:** *Tilia* (ihlamur), *Mentha* (nane) ve *Salvia* (adaçayı) türlerinden hazırlanan bitki çayları, potansiyel sağlık yararları ve solunum yolu rahatsızlıkları ile soğuk algınlığına bağlı semptomların yönetimindeki geleneksel kullanımları nedeniyle yaygın olarak tüketilmektedir. Bu bitkiler, antioksidan özelliklerine katkıda bulunabilecek fenolikler, flavonoidler ve karotenoidler dâhil olmak üzere çeşitli biyoaktif bileşikler içermektedir. Bu çalışma, *Tilia*, *Mentha* ve *Salvia* türlerinden elde edilen metanol ve su ekstraktlarının antioksidan potansiyelini ve fitokimyasal bileşimini karşılaştırmalı olarak değerlendirmeyi amaçlamıştır. Ekstraktların antioksidan aktiviteleri DPPH radikal giderme aktivitesi, indirgeme gücü ve metal şelatlama aktivitesi testleri kullanılarak değerlendirilmiştir. Ayrıca, antioksidan aktivite ile ilişkilerini araştırmak amacıyla toplam fenolikler, toplam flavonoidler,  $\beta$ -karoten ve likopen olmak üzere önemli biyoaktif bileşiklerin düzeyleri belirlenmiştir. Sonuçlar, test edilen bitki türleri ve ekstraksiyon çözücülerinde dikkate değer farklılıklar olduğunu ortaya koymuştur. *Salvia* sp.'nin su ekstraktı en güçlü DPPH radikal giderme aktivitesini gösterirken ( $IC_{50}$ : 0,157 mg/ml), *Mentha* sp. ekstraktları en yüksek indirgeme gücü ( $EC_{50}$ : 0,305 mg/ml) ve metal şelatlama aktivitesini ( $IC_{50}$ : 0,756 mg/ml) göstermiştir. Fitokimyasal analizler, *Mentha* sp.'nin metanol ekstraktının en yüksek  $\beta$ -karoten ve likopen düzeylerini içerdiğini, buna karşılık en yüksek toplam fenolik içeriğini *Mentha* sp.'nin su ekstraktında tespit edildiğini göstermiştir. Buna karşın, özellikle metanol ekstraktı olmak üzere *Salvia* sp. ekstraktları en yüksek flavonoid içeriğini göstermiştir. Bu bulgular, fenolik ve flavonoid bileşiklerin ekstraktların antioksidan aktivitesinde önemli bir rol oynadığını, karotenoidlerin ise antioksidan potansiyellerini daha da artırabileceğini göstermektedir. Genel olarak, bulgular *Tilia*, *Mentha* ve *Salvia* türlerinin doğal antioksidan bileşik kaynakları olarak önemini vurgulamaktadır. Test edilen bitkiler arasında *Mentha* ve *Salvia* ekstraktları karşılaştırmalı olarak daha güçlü antioksidan özellikler göstermiş olup, bu durum bu bitkilerin nutrasötikler ve bitkisel içeceklerde fonksiyonel bileşenler olarak potansiyel değerlerini göstermektedir.

**Anahtar Kelimeler:** Bitki çayı, antioksidan aktivite, fenolik bileşikler, geleneksel tıp

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## 1. Introduction

People are constantly searching for natural substances that can enhance biological functions and promote better health and well-being. In recent years, increasing attention has been directed toward plant-based products as important sources of bioactive compounds with potential health benefits (El-Saadony et al. 2025). Among these natural

resources, medicinal and aromatic plants have long been used in traditional medicine and are increasingly recognized for their nutritional and pharmacological properties. Herbal infusions prepared from various plant species are widely consumed around the world not only for their flavor but also for their potential therapeutic effects (Benabderrahim et al. 2019).

Medicinal plants are rich sources of diverse bioactive compounds, including phenolics, flavonoids, terpenoids, vitamins, and other secondary metabolites that contribute to their biological activities. These compounds are known to possess a wide range of beneficial properties such as antioxidant, anti-inflammatory, antimicrobial, antiviral, and anticancer effects (Sofowora et al. 2013). Because of these properties, medicinal plants are increasingly considered valuable sources of nutraceuticals and functional food ingredients that may help prevent or manage various chronic diseases.

Oxidative stress, resulting from an imbalance between the production of reactive oxygen species and the body's antioxidant defense mechanisms, plays a key role in the development of many diseases, including cardiovascular disorders, neurodegenerative diseases, diabetes, and cancer. Therefore, maintaining oxidative balance is essential for human health. In addition to endogenous antioxidant systems, dietary antioxidants derived from plant-based foods and beverages play a crucial role in protecting cells against oxidative damage and maintaining physiological homeostasis (Prakash et al. 2007; Dai and Mumper 2010). Consequently, considerable research efforts have focused on identifying natural plant sources with high antioxidant potential.

Among commonly consumed medicinal plants, linden (*Tilia* sp.), mint (*Mentha* sp.), and sage (*Salvia* sp.) are widely used in traditional herbal medicine, particularly for the management of respiratory disorders such as colds, cough, and sore throat. Herbal teas prepared from these plants are popular in many cultures due to their pleasant aroma and perceived health benefits (Atoui et al. 2005). Previous studies have shown that these plants contain various bioactive compounds, including phenolic acids, flavonoids, and essential oils, which contribute to their antioxidants and therapeutic properties. For instance, linden flowers are known to contain flavonoids and phenolic compounds with antioxidant and anti-inflammatory activities (Ziaja et al. 2020). Similarly, mint species are rich in phenolic constituents and essential oils such as menthol, which are associated with antimicrobial and antioxidant effects (Dorman et al. 2003; Song et al. 2026). Sage species are also recognized for their high phenolic content and strong antioxidant capacity, largely attributed to compounds such as rosmarinic acid and flavonoids (Maral 2023).

Despite the widespread consumption of these herbal plants, comparative studies evaluating their antioxidant potential and bioactive compounds remain limited. Understanding the relative antioxidant capacity of commonly consumed medicinal plants is important for identifying their potential as functional foods and natural sources of health-promoting compounds.

Therefore, the aim of the present study was to compare the antioxidant properties of three widely consumed medicinal plants: *Tilia*, *Mentha* and *Salvia* sp. In addition, the levels of important bioactive compounds, including total phenolics, total flavonoids, and carotenoids, were evaluated to better understand the relationship between their phytochemical composition and antioxidant activity. By comparing these commonly used herbal plants, this study aims to provide valuable insights into their potential as natural antioxidant sources and to contribute to the growing

body of research on plant-derived nutraceuticals and functional foods.

## 2. Materials and Method

### 2.1. Collection and identification of plant samples

The plant materials used in this study, namely linden (*Tilia* sp.), mint (*Mentha* sp.), and sage (*Salvia* sp.), were obtained in dried form from local herbal shops in Karaman, Türkiye. The samples were carefully examined to ensure their quality and authenticity. Subsequently, the plant materials were subjected to taxonomic verification by a qualified botanist based on their morphological characteristics and comparison with relevant taxonomic literature. Following this evaluation, the samples were identified as *Tilia tomentosa*, *Mentha spicata* L. (*Lamiaceae*), and *Salvia fruticosa* Mill. The authenticated plant samples were then used for subsequent experimental analyses.

### 2.2. Extraction of bioactive ingredients

The dried plant materials, consisting of a mixture of flowering parts and leaves of *Tilia*, *Mentha* and *Salvia* sp. were first cleaned to remove possible impurities and then ground into a fine powder using a laboratory grinder. For each plant sample, 10 g of the powdered material was accurately weighed and subjected to extraction. The bioactive compounds were extracted using 300 ml of solvent (either distilled water or methanol) in a Soxhlet extraction apparatus for 48 h to ensure exhaustive recovery of compounds, as shorter durations resulted in lower yields. Water and methanol were chosen to extract compounds of different polarities, enabling broader phytochemical coverage.

After the extraction process, the obtained extracts were concentrated using a rotary evaporator under reduced pressure to remove the solvents. The concentrated extracts were then freeze-dried to obtain dry extract powders. Prior to the experimental analyses, the dried extracts were dissolved in a minimal amount of sterile distilled water and diluted to the desired concentrations according to the requirements of each assay.

### 2.3. DPPH radical scavenging activity

The free radical scavenging activity of the *Tilia*, *Mentha* and *Salvia* sp. extracts were evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical reduction assay (Sharma and Bhat 2009). In this method, gallic acid (0.005-0.2 mM) was used as the reference antioxidant. For the assay, 20 µl of the standard solutions or plant extracts prepared at different concentrations (0.25-10 mg/ml) were added to the wells of a 96-well microplate. Subsequently, 180 µl of DPPH solution (0.06 mM in methanol) was added to each well and the reaction mixtures were incubated in the dark at room temperature for 1 hour.

Control wells containing DPPH solution without extract or standard were also included to obtain blank measurements. After the incubation period, the absorbance values were measured at 517 nm using a microplate reader. The radical scavenging activity was calculated as the percentage reduction of DPPH based on the decrease in absorbance, using the following formula:

$$\% \text{ RSA} = 100 \times \frac{\text{DPPH abs.} - \text{DPPH and extract abs.}}{\text{DPPH abs.}}$$

The radical scavenging activity (RSA) versus extract amount plot was used to calculate the IC<sub>50</sub> value of each extract, and the results were utilized for comparison of different extract as we described earlier (Sadi et al. 2015).

#### 2.4. Determination of reducing power

The total reducing power of the plant extracts was evaluated according to the method described by Oyaizu (1986), using gallic acid as the reference standard. In brief, 50 µl of gallic acid solutions (0.05-1 mM) or plant extracts prepared at different concentrations (1-40 mg/ml) were mixed with 75 µl of phosphate buffer (0.2 M, pH 6.6) and 75 µl of potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>] solution (1% w/v). The reaction mixtures were incubated at 50°C for 20 minutes. After incubation, 75 µl of trichloroacetic acid (10% w/v) was added to terminate the reaction, and the samples were centrifuged at 1000 g for 10 minutes. Subsequently, 75 µl of the resulting supernatant was transferred into a new microplate well and mixed with 75 µl of distilled water and 15 µl of ferric chloride (FeCl<sub>3</sub>) solution (0.1% w/v). The absorbance of the reaction mixtures was measured at 700 nm using a microplate reader. The reducing power of the extracts was expressed as the effective concentration (EC<sub>50</sub>), defined as the concentration of extract required to reach an absorbance value of 0.5.

#### 2.5. Determination of metal chelating activity

The metal chelating activity of the plant extracts was determined according to the method described in previous studies, using EDTA as a reference chelating agent (Dinis et al. 1994). Briefly, 50 µl of extract solutions prepared at different concentrations (2, 4, 6, 8, and 10 mg/ml) or EDTA standard solutions (0.1-5 mM) were transferred into the wells of a microtiter plate. Subsequently, 10 µl of ferrozine solution (5 mM), 5 µl of iron (II) chloride (2 mM), and 185 µl of absolute methanol were added to each well and gently mixed.

The reaction mixtures were incubated at room temperature for 10 minutes to allow the formation of the ferrozine-Fe<sup>2+</sup> complex. After incubation, the absorbance values were measured at 562 nm using a microplate reader. The metal chelating activity of the extracts was calculated based on the decrease in absorbance relative to the control, and the IC<sub>50</sub> values (the concentration required to chelate 50% of the Fe<sup>2+</sup> ions) were determined accordingly.

#### 2.6. Determination of total phenolic contents

The total phenolic content of the plant extracts was determined using the Folin-Ciocalteu colorimetric method based on the procedure described previously (Taga et al. 1984), with minor modifications suitable for microplate analysis. Gallic acid (0.02-1.00 mM) was used as the reference standard to construct the calibration curve. For the assay, 20 µl of each extract solution (10 mg/ml) or gallic acid standard was transferred into the wells of a microtiter plate and mixed with 20 µl of Folin-Ciocalteu reagent (2N). The mixture was allowed to react in the dark for 3 minutes.

Subsequently, 20 µl of 35% sodium carbonate solution (w/v) and 140 µl of distilled water were added to each well. The reaction mixtures were then incubated for an additional 10 minutes at room temperature. Following incubation, the absorbance was recorded at 725 nm using a microplate reader. The total phenolic content of the samples was

calculated from the gallic acid standard curve and expressed as microgram of gallic acid equivalents per milligram of extract (µg GAE/mg extract). All measurements were performed in triplicate, and the results were presented as mean ± standard error of the mean (SEM).

#### 2.7. Determination of total flavonoid contents

The total flavonoid content (TFC) of the plant extracts was determined according to the method described by Pal et al. (2010) with slight modifications. In brief, 50 µl of each extract solution (10 mg/ml) was transferred into the wells of a microtiter plate. Subsequently, 215 µl of 80% (v/v) ethanol, 5 µl of 1 M potassium acetate, and 5 µl of 10% (w/v) aluminum nitrate were added to each well and thoroughly mixed. The reaction mixtures were incubated at room temperature for 40 minutes to allow the formation of flavonoid-aluminum complexes. After the incubation period, the absorbance of the samples was measured at 415 nm using a microplate reader. The total flavonoid content was calculated as the amount of flavonoids per milligram of extract according to the equation given below.

$$\text{TFC } (\mu\text{g / mg extract}) = \frac{(A_{415} + 0.01089)}{0.002108}$$

#### 2.8. Determination of β-carotene and lycopene contents

The β-carotene and lycopene contents of the methanol and aqueous extracts were determined following the method described by Pal et al. (2010). Briefly, 1 ml of each extract solution (10 mg/ml) was mixed with 1 ml of an acetone:hexane (4:6, v/v) solvent mixture. The resulting solution was thoroughly vortexed and subsequently filtered through Whatman No. 4 filter paper to remove any particulate matter. The absorbance of the clear filtrate was then recorded at 453, 505, and 663 nm using a spectrophotometer. The concentrations of β-carotene and lycopene in the samples were calculated using the equations provided below, as described in the referenced method.

$$\beta \text{ carotene (mg/100 mg)} = 0.216 A_{663} - 0.304 A_{505} + 0.452 A_{453}$$

$$\text{Lycopene (mg/100 mg)} = -0.0458 A_{663} + 0.372 A_{505} - 0.0806 A_{453}$$

#### 2.9. Statistical analyses

All experiments were conducted at least in triplicate, and the data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed to evaluate differences among the plant extracts. Normality of the data distribution was evaluated using the Shapiro–Wilk test, and homogeneity of variances was tested using Levene's test. The results indicated that the data met the required assumptions for the application of parametric tests. Analysis of Variance (ANOVA) was applied, followed by Duncan's multiple range test to determine statistically significant differences between groups at a significance level of p < 0.05. IC<sub>50</sub> and EC<sub>50</sub> values together with their 95% confidence intervals were calculated using Probit Regression Analysis. Relationships among the measured variables were examined using Bivariate Correlation Analysis. All statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS®) software, version 21.0 (IBM Corporation, Armonk, NY, USA). In addition, hierarchical clustering analysis was performed to evaluate the similarity patterns among the extracts. Heat map visualizations of the variables were generated using GraphPad software, version 8.4.3 (San Diego, CA, USA).

### 3. Results and Discussions

In this study, the antioxidant capacities and phenolic contents of *Tilia*, *Mentha* and *Salvia* sp. extracts were investigated to better understand their potential as natural sources of bioactive compounds.

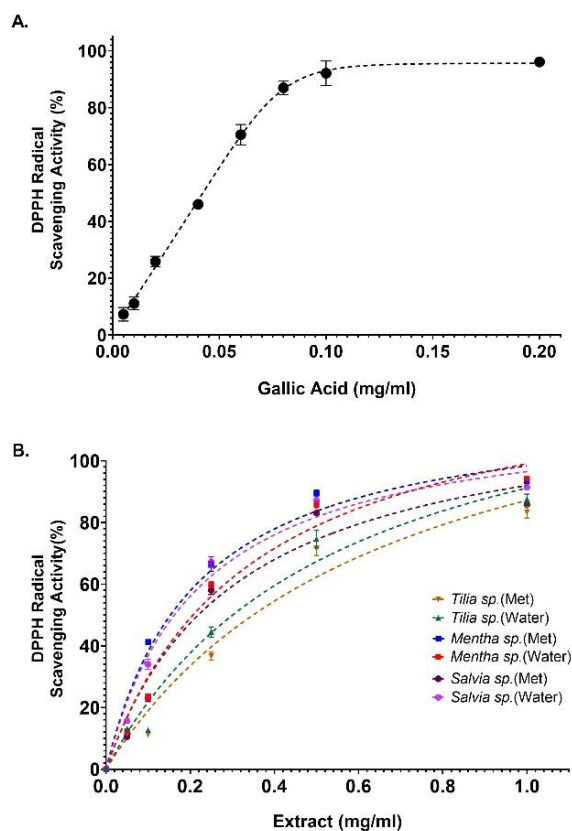
In addition, the relationship between antioxidant activity and the levels of major bioactive compounds, including total phenolics, total flavonoids, lycopene, and  $\beta$ -carotene was investigated. The overall antioxidant capacity of the plant extracts was assessed using DPPH radical scavenging activity, reducing power, and metal chelating activity assays and the results are summarized in Table 1.

The radical scavenging activities of the methanol and water extracts of *Tilia*, *Mentha* and *Salvia* sp. were evaluated using the DPPH assay. Among the tested samples, the water extract of *Salvia* sp. exhibited the strongest radical scavenging activity with the lowest  $IC_{50}$  value (0.157 mg/ml), indicating a higher antioxidant capacity compared to the other plant extracts. This was followed by the methanol extract of *Mentha* sp. ( $IC_{50}$  = 0.199 mg/ml) and the methanol extract of *Salvia* sp. ( $IC_{50}$  = 0.197 mg/ml), which also demonstrated considerable radical scavenging potential.

In contrast, the methanol extract of *Tilia* sp. showed the weakest activity among the tested samples ( $IC_{50}$  = 0.305 mg/ml), suggesting a comparatively lower antioxidant capacity. When the solvent effect was considered, water extracts of *Salvia* and *Mentha* sp. generally displayed stronger radical scavenging activities than their corresponding methanol extracts, whereas the difference between solvent types for *Tilia* sp. was less pronounced (Fig. 1). Overall, the results indicate that *Salvia* sp. extracts, particularly the water extract, possess the highest radical scavenging potential among the tested plant species.

The reducing power capacities of the methanol and water extracts of *Tilia*, *Mentha* and *Salvia* sp. are presented in Table 1 and Figure 2 as  $EC_{50}$  values, where lower  $EC_{50}$  values indicate stronger reducing activity. Among the tested extracts, the methanol extract of *Mentha* sp. exhibited the highest reducing power ( $EC_{50}$  = 0.303 mg/ml), closely followed by its water extract ( $EC_{50}$  = 0.305 mg/ml), indicating that both solvent extracts of *Mentha* sp. possess comparable and relatively strong electron-donating capacity. Similarly, the methanol extract of *Tilia* sp. showed notable reducing activity ( $EC_{50}$  = 0.305 mg/ml),

while its water extract demonstrated slightly weaker activity ( $EC_{50}$  = 0.354 mg/ml). In contrast, *Salvia* sp. extracts exhibited comparatively lower reducing power, particularly the water extract which had the highest  $EC_{50}$  value among all tested samples (0.443 mg/ml). The methanol extract of *Salvia* sp. ( $EC_{50}$  = 0.329 mg/ml) showed moderate reducing activity but remained weaker than the corresponding extracts of *Mentha* sp. Overall, the results indicate that *Mentha* sp. extracts possess the strongest reducing power among the tested plant species, whereas *Salvia* sp., especially its water extract, displayed the lowest reducing capacity (Fig. 2b).

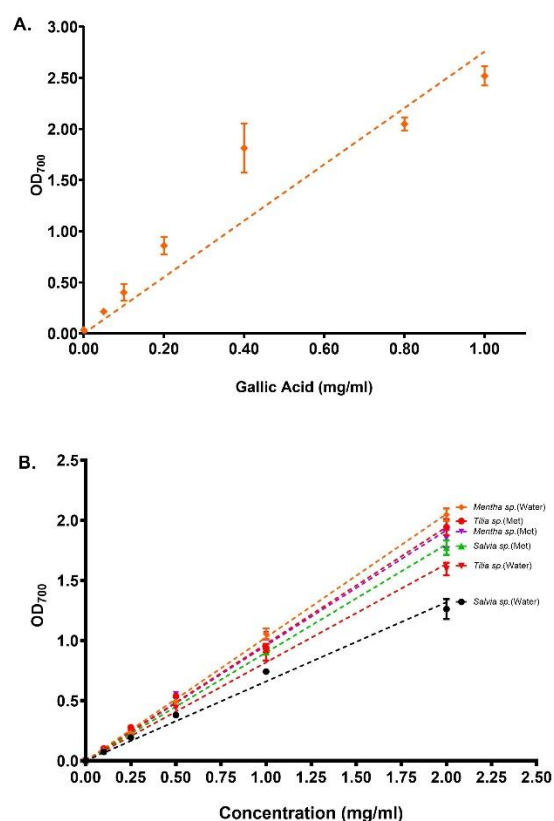


**Figure 1.** DPPH radical scavenging activities of gallic acid and plant extracts. (A) Concentration-dependent DPPH radical scavenging activity of gallic acid used as the reference antioxidant. (B) Radical scavenging activities of methanol and water extracts of *Tilia*, *Mentha* and *Salvia* sp. at different extract concentrations (0-1 mg/ml)

**Table 1.** Antioxidant activities of methanol and water extracts of *Tilia*, *Mentha* and *Salvia* sp. The table presents DPPH radical scavenging activity ( $IC_{50}$ ), reducing power ( $EC_{50}$ ), and metal chelating activity ( $IC_{50}$ ) values together with their 95% confidence intervals (95% CI)

	Radical Scavenging $IC_{50}$ (%95 CI)	Reducing Power $EC_{50}$ (%95 CI)	Metal Chelating $IC_{50}$ (%95 CI)
<i>Tilia</i> sp. (Met)	0.305 (0.282 to 0.331)	0.305 (0.247 to 0.363)	3.185 (2.598 to 4.520)
<i>Mentha</i> sp. (Met)	0.199 (0.164 to 0.236)	0.303 (0.235 to 0.371)	6.298 (5.847 to 6.549)
<i>Salvia</i> sp. (Met)	0.197 (0.176 to 0.217)	0.329 (0.269 to 0.389)	5.182 (3.523 to 6.641)
<i>Tilia</i> sp. (Water)	0.282 (0.255 to 0.318)	0.354 (0.297 to 0.411)	3.140 (2.724 to 3.785)
<i>Mentha</i> sp. (Water)	0.216 (0.203 to 0.230)	0.305 (0.249 to 0.361)	0.756 (0.694 to 0.821)
<i>Salvia</i> sp. (Water)	0.157 (0.130 to 0.180)	0.443 (0.376 to 0.510)	1.663 (1.549 to 1.804)
Gallic Acid (mg/mL)	0.041 (0.036 to 0.045)	0.093 (0.076 to 0.110)	ND
EDTA (mg/mL)	ND	ND	0.059 (0.052 to 0.069)

\*Gallic acid and EDTA were used as reference standards for antioxidant and metal chelating assays, respectively. ND: Not determined.



**Figure 2.** Reducing power of gallic acid and plant extracts. (A) Concentration-dependent reducing power of gallic acid used as the reference antioxidant. (B) Reducing power of methanol and water extracts of *Tilia*, *Mentha* and *Salvia* sp. at different concentrations

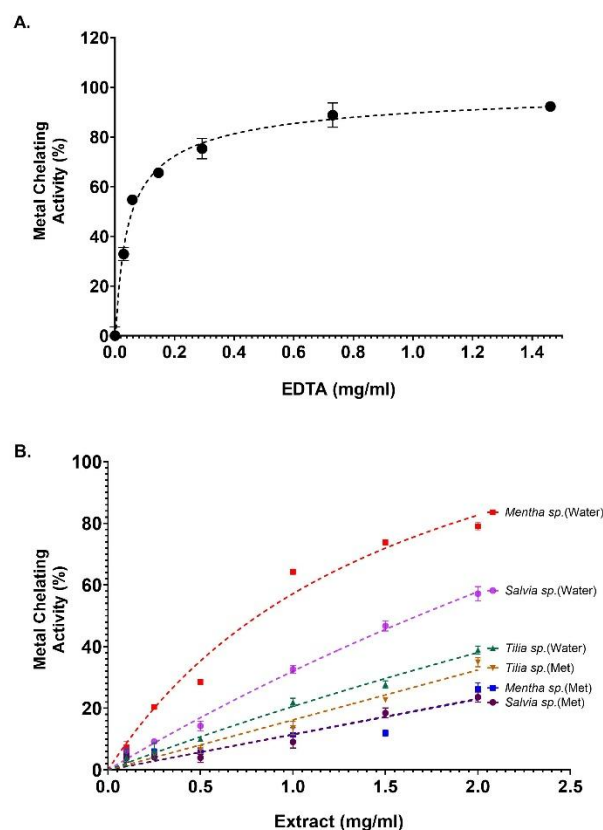
The metal chelating activities of the methanol and water extracts of *Tilia*, *Mentha* and *Salvia* sp. are presented in Table 1 and Figure 3 as IC<sub>50</sub> values, where lower IC<sub>50</sub> values indicate stronger metal chelating capacity. Among the tested samples, the water extract of *Mentha* sp. exhibited the strongest metal chelating activity with the lowest IC<sub>50</sub> value (0.756 mg/ml), suggesting a high ability to bind metal ions compared to the other plant extracts. This was followed by the water extract of *Salvia* sp. (IC<sub>50</sub> = 1.663 mg/ml), which also demonstrated considerable chelating potential. In contrast, both methanol extracts of *Mentha* sp. (IC<sub>50</sub> = 6.298 mg/ml) and *Salvia* sp. (IC<sub>50</sub> = 5.182 mg/ml) showed relatively weak metal chelating activity.

The extracts of *Tilia* sp. displayed moderate chelating capacity, with similar IC<sub>50</sub> values for both methanol (3.185 mg/ml) and water extracts (3.140 mg/ml). Overall, the results indicate that water extracts, particularly from *Mentha* sp., exhibit stronger metal chelating activity compared to methanol extracts (Fig. 3b), highlighting the importance of extraction solvent in determining the chelating potential of these medicinal plants.

### 3.2. Bioactive components

Previous studies have demonstrated that medicinal plants contain a variety of antioxidant compounds, including polyphenols, flavonoids and carotenoids such as lycopene and  $\beta$ -carotene, which are responsible for many of their biological activities (Pandey and Rizvi 2009; Dai and Mumper 2010).

In the present study, important antioxidant-related phytochemicals,

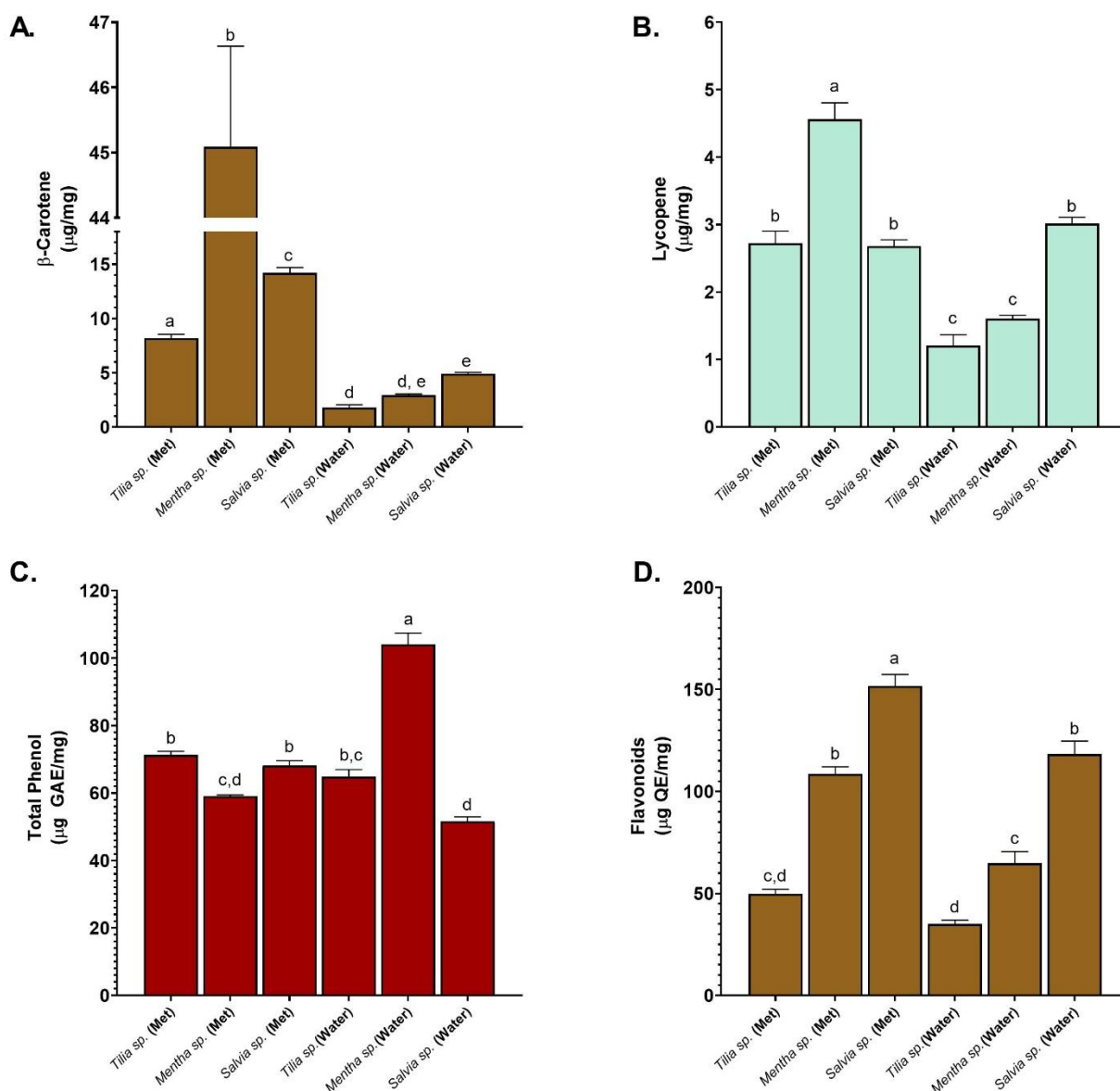


**Figure 3.** Metal chelating activities of EDTA and plant extracts. (A) Concentration-dependent metal chelating activity of EDTA used as the reference chelating agent. (B) Metal chelating activities of methanol and water extracts of *Tilia*, *Mentha* and *Salvia* sp. at different concentrations

including  $\beta$ -carotene, lycopene, total phenolics, and total flavonoids, were determined in the methanol and water extracts of *Tilia*, *Mentha* and *Salvia* sp., and the results are presented in Figure 4. Among the analyzed samples, the methanol extract of *Mentha* sp. exhibited the highest  $\beta$ -carotene content (45.09  $\mu\text{g}/\text{mg}$ ), followed by the methanol extract of *Salvia* sp. (14.21  $\mu\text{g}/\text{mg}$ ), whereas the methanol extract of *Tilia* sp. contained comparatively lower levels (8.18  $\mu\text{g}/\text{mg}$ ) (Fig. 4a). In contrast, all water extracts showed substantially lower  $\beta$ -carotene concentrations, with *Salvia* sp. (water) displaying the highest level among them (4.89  $\mu\text{g}/\text{mg}$ ), followed by *Mentha* sp. (water) (2.93  $\mu\text{g}/\text{mg}$ ) and *Tilia* sp. (water).

Regarding lycopene content, the methanol extract of *Mentha* sp. showed the highest concentration (4.56  $\mu\text{g}/\text{mg}$ ), while the methanol extracts of *Tilia* and *Salvia* sp. exhibited similar intermediate levels (2.72 and 2.68  $\mu\text{g}/\text{mg}$  respectively). Among the water extracts, *Salvia* sp. displayed relatively higher lycopene content (3.01  $\mu\text{g}/\text{mg}$ ) compared with *Mentha* sp. (1.61  $\mu\text{g}/\text{mg}$ ) and *Tilia* sp. (1.20  $\mu\text{g}/\text{mg}$ ) (Fig. 4b).

The total phenolic contents also varied among the plant extracts. The highest phenolic level was detected in the water extract of *Mentha* sp. (104.09  $\mu\text{g}$  GAE/mg), followed by the methanol extract of *Tilia* sp. (71.24  $\mu\text{g}$  GAE/mg) and the methanol extract of *Salvia* sp. (68.13  $\mu\text{g}$  GAE/mg). The lowest phenolic content was observed in the water extract of *Salvia* sp. (51.64  $\mu\text{g}$  GAE/mg). These results indicate that, in general, water extracts tended to show relatively



**Figure 4.** Bioactive compound contents of methanol and water extracts of *Tilia*, *Mentha* and *Salvia* sp. (A)  $\beta$ -carotene content ( $\mu\text{g}/\text{mg}$ ), (B) lycopene content ( $\mu\text{g}/\text{mg}$ ), (C) total phenolic content and (D) total flavonoid content. Values are presented as mean  $\pm$  SEM of at least three independent experiments. Different letters above the bars indicate statistically significant differences among groups ( $p < 0.05$ )

higher phenolic levels for certain species, particularly in *Mentha* sp. (Fig. 4c). Similarly, the flavonoid contents differed significantly among the tested extracts. The methanol extract of *Salvia* sp. exhibited the highest flavonoid concentration ( $151.67 \mu\text{g QE}/\text{mg}$ ), followed by the water extract of *Salvia* sp. ( $118.38 \mu\text{g QE}/\text{mg}$ ) and the methanol extract of *Mentha* sp. ( $108.67 \mu\text{g QE}/\text{mg}$ ). In contrast, the lowest flavonoid level was detected in the water extract of *Tilia* sp. ( $34.98 \mu\text{g QE}/\text{mg}$ ) (Fig. 4d).

Overall, the comparison of the bioactive compound contents revealed that methanol extracts generally contained higher levels of carotenoids such as  $\beta$ -carotene and lycopene, whereas phenolic compounds were relatively abundant in certain water extracts, particularly in *Mentha* sp. Moreover, *Salvia* sp. extracts, especially the methanol extract, were characterized by the highest flavonoid concentrations. These findings suggest that both the plant species and the extraction solvent play important roles in

determining the phytochemical composition and antioxidant potential of the analyzed herbal extracts.

When the bioactive contents are considered together with the antioxidant activity results, several relationships between phytochemical composition and antioxidant mechanisms become apparent. In the DPPH radical scavenging assay, the strongest activity was observed in the water extract of *Salvia* sp., followed by the methanol extracts of *Mentha* sp. and *Salvia* sp. This result is consistent with the relatively high phenolic and flavonoid contents detected in these extracts, since phenolic compounds are well known for their hydrogen-donating capacity and radical neutralization potential. In particular, the high phenolic level of the *Mentha* sp. water extract and the elevated flavonoid concentration of the *Salvia* sp. methanol extract may have contributed to their strong free radical scavenging activity. Similarly, the reducing power results showed that *Mentha* sp. extracts exhibited the

highest electron-donating capacity among the tested samples. This observation correlates well with the comparatively high phenolic and carotenoid contents measured in *Mentha* sp., suggesting that these compounds might play a role in the reduction of oxidized intermediates.

In terms of metal chelating activity, the water extract of *Mentha* sp. demonstrated the strongest chelating capacity, while *Tilia* sp. extracts exhibited moderate activity and methanol extracts of *Mentha* sp. and *Salvia* sp. showed weaker effects. Metal chelation is often associated with the presence of specific phenolic structures capable of binding transition metals. Therefore, the relatively high phenolic content detected in the *Mentha* sp. water extract may explain its superior chelating performance. Overall, the combined evaluation of antioxidant assays and phytochemical profiles indicates that phenolic and flavonoid compounds likely play a major role in the antioxidant properties of the tested plant extracts, while carotenoids such as  $\beta$ -carotene and lycopene may further contribute to their radical scavenging and reducing activities. These findings highlight a clear association between the phytochemical composition of the extracts and their observed antioxidant capacities.

### 3.3. Correlational Analysis

The correlation analysis revealed several relationships between antioxidant activities and phytochemical contents (Table 2). DPPH radical scavenging activity showed a strong correlation with flavonoid content ( $r = 0.807$ ), indicating that extracts with higher flavonoid levels tended to exhibit stronger radical scavenging capacity and this relationship was statistically significant ( $p = 0.050$ ). DPPH activity also displayed weak correlations with phenolics,  $\beta$ -carotene, and lycopene. Reducing power exhibited moderate correlations with total phenolics ( $r = 0.562$ ) and  $\beta$ -carotene ( $r = 0.375$ ), suggesting that these compounds may contribute to electron-donating capacity in the extracts.

Metal chelating activity showed a strong positive correlation with  $\beta$ -carotene ( $r = 0.818$ ,  $p < 0.05$ ), indicating a significant association between carotenoid content and metal-binding capacity. Additionally, a moderate positive

relationship was observed between metal chelating activity and lycopene ( $r = 0.657$ ), suggesting that carotenoids may contribute to the metal chelating mechanism of the extracts. A strong and statistically significant correlation was also detected between  $\beta$ -carotene and lycopene levels ( $r = 0.875$ ,  $p < 0.05$ ), indicating that these carotenoids tend to occur together in the plant extracts.

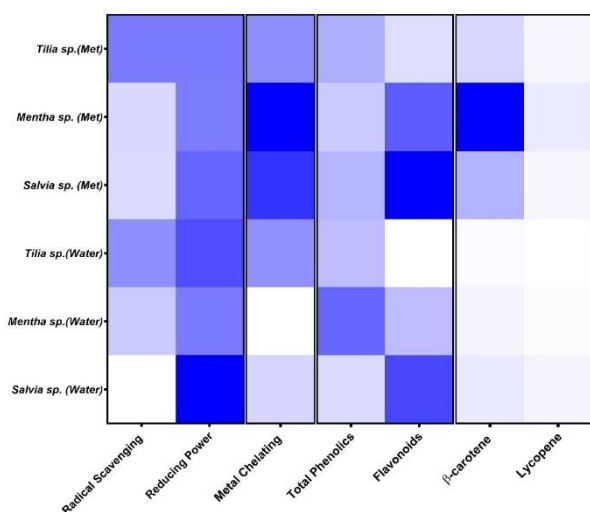
Overall, the correlation results suggest that different classes of phytochemicals contribute differently to the antioxidant mechanisms. While flavonoids appear to be more closely associated with radical scavenging activity, carotenoids such as  $\beta$ -carotene and lycopene seem to play a more important role in metal chelating activity. These findings highlight the complex relationship between phytochemical composition and antioxidant functions in the analyzed plant extracts.

Heat maps are powerful visualization tools that facilitate the interpretation of complex datasets by displaying patterns and relationships among multiple variables simultaneously. They enable the identification of similarities and differences between samples based on their biochemical composition and biological activities. In the present study, a heat map was generated to visualize the relationships between antioxidant activities (DPPH radical scavenging, reducing power, and metal chelating) and bioactive compounds (total phenolics, total flavonoids,  $\beta$ -carotene, and lycopene) in the methanol and water extracts of *Tilia*, *Mentha* and *Salvia* sp. (Fig. 5). The color intensity in the heat map reflects the relative magnitude of the measured parameters across the extracts.

The heat map clearly demonstrates that the methanol extract of *Mentha* sp. forms a distinct pattern characterized by high levels of carotenoids ( $\beta$ -carotene and lycopene) and strong reducing power, indicating a pronounced antioxidant potential. Similarly, the methanol extract of *Salvia* sp. is distinguished by its high flavonoid content, which may contribute to its strong radical scavenging activity. On the other hand, the water extract of *Mentha* sp. exhibits elevated total phenolic content and strong metal chelating capacity, suggesting that phenolic compounds play an important role in its antioxidant mechanisms.

**Table 2.** Pearson correlation coefficients ( $r$ ) and significance values ( $p$ ) showing the relationships between antioxidant activities and phytochemical components of the plant extracts. Asterisks (\*) indicate statistically significant correlations at  $p < 0.05$

		DPPH Scavenging	Reducing Power	Metal Chelating	Total Phenolics	Total Flavonoids	$\beta$ -carotene	Lycopene
<b>DPPH Scavenging</b>	Pearson Correlation ( $r$ )	1	0.486	0.026	0.213	0.807*	0.271	0.430
	Significance ( $p$ )		0.328	0.961	0.686	0.050	0.604	0.395
<b>Reducing Power</b>	Pearson Correlation ( $r$ )	0.486	1	0.370	0.562	-0.250	0.375	0.063
	Significance ( $p$ )	0.328		0.470	0.246	0.632	0.464	0.905
<b>Metal Chelating</b>	Pearson Correlation ( $r$ )	-0.026	-0.370	1	-0.488	0.433	0.818*	0.657
	Significance ( $p$ )	0.961	0.470		0.326	0.391	0.047	0.157
<b>Total Phenolics</b>	Pearson Correlation ( $r$ )	0.213	-0.562	-0.488	1	-0.358	-0.334	-0.518
	Significance ( $p$ )	0.686	0.246	0.326		0.486	0.518	0.292
<b>Total Flavonoids</b>	Pearson Correlation ( $r$ )	0.807*	0.250	0.433	-0.358	1	0.406	0.565
	Significance ( $p$ )	0.050	0.632	0.391	0.486		0.424	0.243
<b><math>\beta</math>-carotene</b>	Pearson Correlation ( $r$ )	-0.271	-0.375	0.818*	-0.334	0.406	1	0.875*
	Significance ( $p$ )	0.604	0.464	0.047	0.518	0.424		0.022
<b>Lycopene</b>	Pearson Correlation ( $r$ )	-0.430	-0.063	0.657	-0.518	0.565	0.875*	1
	Significance ( $p$ )	0.395	0.905	0.157	0.292	0.243	0.022	

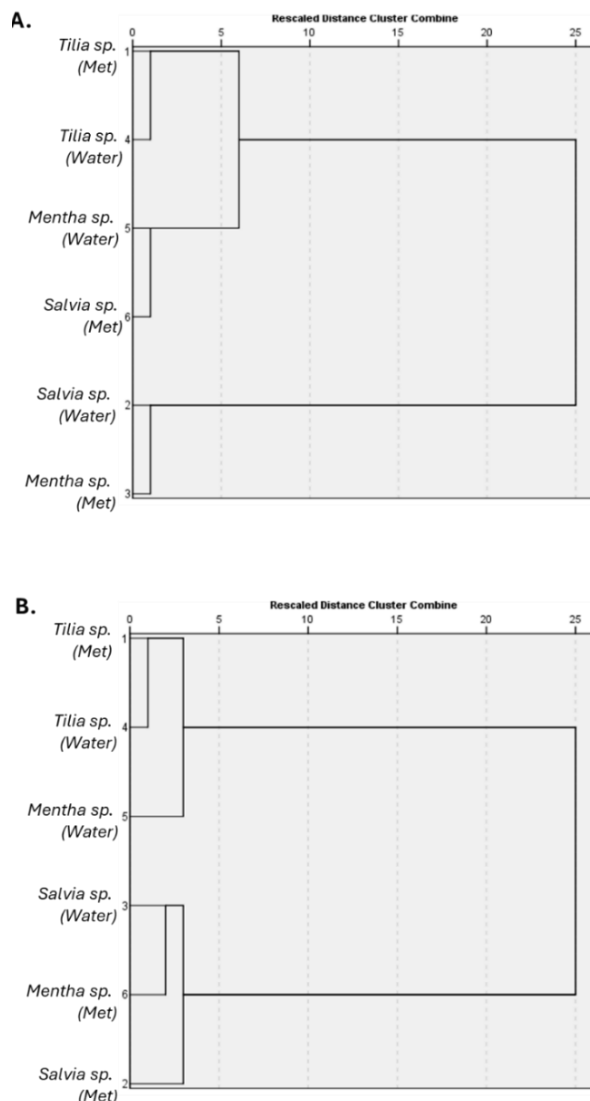


**Figure 5.** Heat maps of antioxidant activities of herbal plants along with their bioactive compounds. The color scales represent the relative amounts of individual components obtained from the extracts listed on the left side.

In contrast, *Tilia sp.* extracts generally display comparatively lower intensities for several antioxidant-related parameters, particularly for flavonoids and carotenoids, indicating a relatively lower antioxidant potential compared to *Mentha sp.* and *Salvia sp.* Overall, the heat map highlights clear differences among the plant extracts and demonstrates that *Mentha sp.* and *Salvia sp.* species possess richer bioactive profiles and stronger antioxidant activities than *Tilia sp.*. These visual patterns support quantitative results and emphasize the close relationship between phytochemical composition and antioxidant capacity in the analyzed herbal extracts.

The hierarchical clustering results obtained in this study are illustrated by the dendrograms presented in Figure 6. The extracts were grouped according to similarities in their antioxidant activities (DPPH radical scavenging, reducing power, and metal chelating) as well as their bioactive compound contents ( $\beta$ -carotene, lycopene, total phenolics, and total flavonoids). In the first dendrogram (Fig. 6a), the extracts were generally separated into two major clusters based on their antioxidant performance. One cluster includes the methanol extracts of *Tilia sp.*, *Mentha sp.*, and *Salvia sp.*, which are grouped together due to their relatively similar antioxidant activity and comparatively higher levels of carotenoids and flavonoids. In contrast, the water extracts form a separate cluster, reflecting differences in antioxidant activity patterns, particularly in terms of reducing power and metal chelating activity. Within this group, the water extract of *Mentha sp.* appears closer to the methanol extracts, which may be associated with its relatively high phenolic content and strong metal chelating activity.

The second dendrogram (Fig. 6b), which is based primarily on bioactive compound composition, reveals additional clustering patterns among the plant extracts. In this analysis, *Mentha* and *Salvia sp.* extracts tend to cluster together due to their relatively high levels of carotenoids, phenolics, and flavonoids. Conversely, *Tilia sp.* extracts form a separate branch, indicating comparatively lower levels of these bioactive components. The clustering pattern also demonstrates that extraction solvent plays a significant



**Figure 6.** Hierarchical cluster analysis (dendrogram) illustrating the similarity patterns among methanol and water extracts of *Tilia*, *Mentha* and *Salvia sp.* based on their (A) antioxidant activities (DPPH radical scavenging, reducing power, and metal chelating) and (B) bioactive compound contents (total phenolics, total flavonoids,  $\beta$ -carotene, and lycopene)

role in determining phytochemical composition, as methanol and water extracts of the same plant species are often separated into different branches. Overall, the dendrogram analysis supports the quantitative findings and highlights that *Mentha* and *Salvia sp.* extracts share similar phytochemical and antioxidant characteristics, while *Tilia sp.* extracts exhibit a comparatively distinct and weaker bioactive profile.

#### 4. Conclusion

In the present study, the antioxidant potential and phytochemical composition of methanol and water extracts obtained from three widely consumed medicinal plants, *Tilia*, *Mentha* and *Salvia sp.*, were comparatively investigated. The results demonstrated that both the plant species and extraction solvent significantly influenced the antioxidant capacity and the levels of bioactive compounds present in the extracts. Overall, methanol extracts generally contained higher concentrations of carotenoids such as  $\beta$ -carotene and lycopene, whereas certain water extracts,

particularly that of *Mentha* sp., exhibited higher levels of total phenolic compounds. In terms of antioxidant activity, the water extract of *Salvia* sp. showed the strongest DPPH radical scavenging activity, while *Mentha* sp. extracts displayed the highest reducing power. In addition, the water extract of *Mentha* sp. demonstrated the most effective metal chelating capacity among the tested samples.

The phytochemical analyses revealed notable variations among the plant species. The methanol extract of *Mentha* sp. contained the highest  $\beta$ -carotene and lycopene levels, whereas *Salvia* sp., particularly its methanol extract, exhibited the highest flavonoid content. The highest total phenolic concentration was detected in the water extract of *Mentha* sp. These findings suggest that phenolic and flavonoid compounds may play a key role in the observed antioxidant activities, especially in radical scavenging and metal chelation processes, while carotenoids such as  $\beta$ -carotene and lycopene may contribute to the reducing power and overall antioxidant performance of the extracts.

In conclusion, the results of this study highlight the importance of commonly consumed herbal plants such as *Tilia*, *Mentha* and *Salvia* sp. as valuable natural sources of antioxidant compounds. Among the species investigated, *Mentha* and *Salvia* sp. extracts exhibited particularly strong antioxidant characteristics due to their rich phenolic,

flavonoid, and carotenoid contents. These findings suggest that these herbal plants may contribute to reducing oxidative stress and could be considered promising candidates for the development of natural antioxidant agents and nutraceutical products. Further studies focusing on the isolation and characterization of individual bioactive compounds may provide deeper insights into their potential health-promoting properties and possible applications in the food, pharmaceutical, and nutraceutical industries.

### Competing of Interest

The authors declare that they have no competing financial, personal, or professional interests that could have influenced the work reported in this manuscript

### Authors' Contribution

İSA conducted experiments, made data analysis and drafted the manuscript. GS designed and initiated the study, evaluated data and made critical revisions to the manuscript.

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