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Comparison of Antioxidant Activity, Metal Chelating Power and Antibacterial Activity in Different Tissues of Alcea calvertii (Boiss.) Boiss

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Abstract

The traditionally used plant *Alcea calvertii* (Boiss.) Boiss. (Malvaceae) was extracted by two more methods in addition to those used by the locals, in this study. It was found that ethanol extraction significantly improved the release of total phenolic content of all plant parts compared to extraction by infusion and traditional use. In addition, ethanol appears to be a good solvent for the extracting flavonoids and phenolic contents from *A. calvertii*. However, metal chelating power was found to be higher in the infusion extracts than in the ethanolic extracts. The antibacterial activities of all extracts from the plant parts were also tested. As a result, it has been confirmed in this study that *A. calvertii* is rich in phenolic compounds and flavonoids and has high antioxidant activity with strong metal chelating power, however, the right plant parts must come together with the right extraction method for this effect to occur.

1. Introduction

Plants have an antioxidant effect that neutralizes free radicals because they contain phytochemicals and secondary metabolites. This fact makes plants a potential natural source for the production of new drugs [1]. Flavonoids and phenols are included the most important bioactive compounds in addition to alkaloids, saponins, and tannins in plants [2], [3]. Polyphenols are multiple phenol groups (i.e., aromatic rings with hydroxyl groups) derived from L-phenylalanine [4], and the most important are phenolic acids, containing polymeric structures, such as hydrolyzing tannins, lignans, stilbenes, and flavonoids. The vast number of flavonoids, including flavonols, flavones, isoflavones, flavanones, and anthocyanidins, are also important for their antioxidant and antimicrobial activities [5], [6].

Alcea calvertii (Boiss.) Boiss. (Malvaceae), locally known as hatmi and hiro, has been used in traditional medicine to treat or alleviate various diseases or ailments [7] - [9]. Especially the flowers are boiled with water and used in various ways in Anatolia. Malvaceae family is known for its high antioxidant potential [10], [11]. It is known that many of the plants belonging to the genus *Alcea* L. have ethnomedicinal and biological properties associated

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with antiviral [12], [13], anti-inflammatory, diuretic, astringent, demulcent, febrifugal, circulatory [14], antidiarrheal [15], and antimicrobial activity [16]. Recent studies have shown that *A. calvertii* the flower part or the aerial part of the plant has antioxidant activity [17], [18]. However, the antioxidant properties of four different tissues of *A. calvertii* have not been studied separately. The present study, therefore, aimed to (I) determine the antioxidant capacity of different tissues of *A. calvertii* (Boiss.) Boiss; (II) evaluate the effects of different extraction methods on total phenolic content, total flavonoid content, metal chelation and antibacterial activity in these plant tissues.

2. Material and Method

2.1. Extraction of Plant Sample

Plants used in the study were collected in Catak, Van, Türkiye (38°08'29.3"N 43°10'24.4"E), in July 2020. The GPS location of *A. calvertii* is shown in Figure 1. The specimen was assigned a reference number (F15268) and stored in the VANF Herbarium unit (VANF-20298) of Van Yüzüncü Yil University (Van YYU). The identification of the species was confirmed as *Alcea calvertii* (Boiss.) Boiss. by Van YYU botanist Prof. Dr. Fevzi Ozgokce. Plant specimens were purified from soil and other substances and dried for 6 days at room temperature (RT) at shade. Flowers, seeds, roots, and leaves were separated and cut into small pieces. The plant extract was obtained by three different methods: (I) In the method using ethanol as solvent: 10 g of each plant part was mixed separately with 75 mL of hexane and stirred at 40 °C for 18 hours. After the hexane was filtered, 75 mL of ethanol was added to the extract remaining in the tube and incubated for 48 hours at 4 °C in the dark. The ethanol was removed by evaporation. Then, 75 mL of dH_2O was added to the extract and incubated at 40 °C for 48 hours. After the dH_2O of the mixture was filtered, it was stored horizontally overnight at -80 °C. In order to remove the water from the samples, they were kept in a lyophilizer for 72 hours. The dried samples were stored at -20°C for the subsequent experimental steps. (II) In the infusion method, the prepared plant parts were boiled separately in a closed system. After the obtained extract was kept at -80 °C for at least 48 hours, the water was removed from the samples using the lyophilizer. The extract was stored at -20 °C for further experiments.

The efficiency of the samples in both extraction methods was calculated using the following equation:

Percentage of Plant Extract (w/w) = (Mass of Dried Extract) / (Total Mass of Sample) x 100 (1)

(III) In the traditional method, the prepared plant parts were boiled separately in a closed system and passed through a filter paper. The resulting liquid fraction was used for the experimental steps.

2.2. Measurement of Total Phenolic Content

Total phenolic content (TPC) in the extracts of plant tissues was determined using the Folin-Ciocalteu method [19]. For each plant part, 20 µL of the extract was mixed with 20 µL of the 25% Folin-Ciocalteu reagent. Then 80 µL of 10% Na_2CO_3 was added and left in the dark at RT for 30 minutes. The absorbance of the samples was measured at 750 nm using a microplate reader. TPC was expressed as mg GAE/ g plant part using the equation derived from a calibration curve of gallic acid (GA). All samples were measured in triplicate.

2.3. Measurement of Total Flavonoid Content

The method to determine the Total flavonoid content (TFC) of tissue extracts was adapted from Zhishen *et al.* [20]. For each plant part, 20 µL of the plant extract was mixed with 80 µl dH_2O and 6 µl of 5% $NaNO_2$. This mixture was incubated for 5 minutes at RT. Then 6 µL $AlCl_3$ was added and incubated for another 6 minutes 40 µL 1M NaOH was added and the total volume was made up to 200 µl with dH_2O. Measurements were made at 415 nm for quercetin and 510 nm for catechin using a microplate reader. TFC was expressed as mg quercetin equivalent (QE)/ g plant part and mg catechin equivalent (CE)/ g plant part using the equation derived from a calibration curve of quercetin and catechin, respectively. All samples were measured in triplicate.

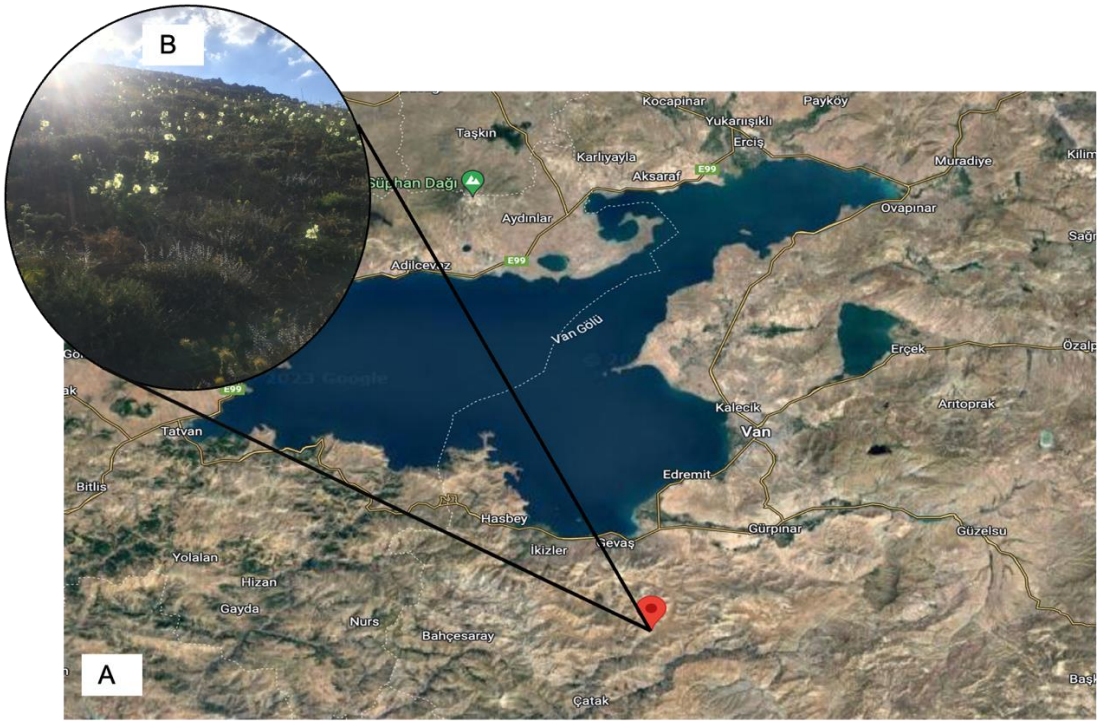

Figure 1. The location where the plant was collected (A) and the natural distribution of *A. calvertii* (Boiss.) Boiss. in the area (B)

2.4. Measurement of Metal Chelating Activity

The metal chelating activity of the plant parts was determined according to a modified method of Dinis *et al.* [21]. For each plant part, 40 µL of the plant extract was mixed with 8 µL of 5 mM ferrozine and 4 µL of 2 mM $FeCl_2$. The total volume was then made up with absolute methanol and incubated for 10 minutes at RT. The absorbance was measured at 562 nm in a microplate reader after 200 µL of the mixture was added in triplicate to 96-well plates.

Percentage inhibition of the EDTA chelating agent and samples was calculated as follows:

$$\% \text{ Inhibition} = (Abs_{blank}-Abs_{sample})/ (Abs_{blank}) \times 100 \quad (2)$$

where Abs_{blank} is the absorbance of the negative control and Abs_{sample} is the absorbance of the sample or reference.

2.5. Determination of Antibacterial Activity

Determination of antibacterial activity of the plant extracts different reference bacterial strains (*Escherichia coli* ATCC 35213, *E. coli* ATCC 35218, *Staphylococcus epidermidis* ATCC 12228, *Bacillus cereus* DSM 22648) were studied. Bacterial cell densities were adjusted with an optical density of 0.5 according to the McFarland standard. Bacterial suspensions were inoculated on Mueller-Hinton agar (MHA). Sterile filter paper disks were impregnated with different concentrations of each tissue. Discs impregnated with dH_2O were used as negative control and Ampicillin (5 µg/ mL) and Kanamycin (5 µg/ mL) were used as positive control. MHA plates were incubated for 24 hours at 37°C. The diameters of inhibition zones were measured in mm. The experiments included three replicates.

2.6. Statistical Analysis

Statistical analyses were performed using GraphPad Prism (v.9.0) software. One-way ANOVA was used to compare more than two groups in the analyses. T-test was used for pairwise comparison of the plant parts. *p* values <.05 were considered significant.

3. Results and Discussion

3.1. Yield of the Ethanolic Extracts and the Infusion Extracts

The mass and percent yield of the ethanolic and the infusion extracts from seed, flower, leaf, and root parts of *A. calvertii* are given in Table 1. In the traditional method, no dry extract was used, but only a liquid extract, as this is the form used by the locals. For this reason, the mass and percentage yield were not calculated in the traditional extraction method used in the study. The dried plant extract in the plant tissues obtained by ethanol extraction ranged from 8.93% to 40.76%; the same value was found between 0.62% and 47.37% for the extracts obtained by infusion method. The highest yields of both extractions were obtained from the seed part of the plant. The yields of leaves in ethanol extraction and infusion method were 13.50% and 22.32%, respectively. The lowest yield was found in the flower part (8.93%) in the ethanolic extracts, while the lowest yield was found in the root (0.62%) in the infusion extracts. However, since the mucosity was too high in the flowers in the infusion method, the yield of the flowers could only be calculated for the ethanol extraction.

Table 1. Mass and percentage yield of dried extract of different tissues of *A. calvertii*

Extract	Plant parts	Mass of dried extract (g)	Percentage yield (%)
Ethanol	Seed	0.92	40.76
	Flower	0.89	8.93
	Leaf	1.35	13.50
	Root	1.27	12.73
Infusion	Seed	0.73	47.37
	Leaf	1.12	22.32
	Root	0.03	0.62

According to Wong and Chye [22], compounds with structures containing hydroxyl and carboxyl groups, such as phenolic acids, lignans, flavonoids, are easily extracted by polar solvents. It is also known that reducing the particle size facilitates solvent extraction [23] and significantly increases the yield due to better extractability [22]. However, Amin *et al.* [24] reported that the extracts were affected by the polarity of the extraction medium, which could selectively determine the type of phenolic groups extracted, whether in the form of glycosides or bound to the cell wall. In this study, yield was calculated for the first time for *A. calvertii* and even for the genus *Alcea*; therefore, no comparative data are available. As a result, there were differences in yield by extraction methods for all plant parts except seed. Ghisalberti [25] interpreted that an alcoholic medium allows a greater amount of endocellular material to be extracted because it damages cell membranes. However, in this study, the yield was higher for the infusion method, which does not contain alcoholic medium, than for the ethanolic extracts for all tissues.

3.2. The Effect of Different Extraction Methods on the Total Phenolic Content

The TPC of different tissues of *A. calvertii* extracted by three methods, as determined by the Folin–Ciocalteu colorimetric method, is shown in Tables 2 and 3. The TPC in the extracts was calculated using the regression equation (y=9.923x; R^2=0.9988) of the calibration curve. The highest amount was found in the ethanol extract of flowers (674.55 mg gallic acid equivalent (GAE)/ g), the lowest amount was found in the traditionally extracted root (4.50 mg GAE/ g) in all measurements (Table 2). Table 3 also shows the comparisons of each plant tissue according to the extraction method. Thus, the TPC of ethanol extraction for leaves (519.55 mg GAE/ g) and roots (190.27 mg GAE/ g) was significantly higher than for other extraction methods. In terms of seeds, both ethanol (260.89 mg GAE/ g) and infusion (296.16 mg GAE/ g) were found to be significantly higher than the traditional method (8.02 mg GAE/ g). Also, the flowers extracted with ethanol (674.55 mg GAE/ g) had a significantly higher TPC than the traditionally extracted flowers (55.75 mg GAE/ g).

It is known that the biologically active compounds in plants are responsible for their antioxidant activity. Phenols are important constituents that acts as radical scavengers due to their hydroxyl groups and thus can directly contribute to the antioxidant activity [22]. In this study, the highest amount of TPC was found in the ethanol extract of flowers (674.55 mg GAE/ g) and the second highest value in the ethanol extraction of leaves (519.55 mg GAE/ g). Based on the extraction methods, the leaves extracted by infusion and traditional methods had higher TPC values than other parts of the plant, with values of 407.08 and 232.98 mg GAE/ g, respectively.

Previous research related to *A. calvertii* found the highest TPC value in all extractions for the water extract of flowers (65.06 mg GAE/ g) [17]; this value is similar to our result of traditional extraction of flowers (55.75 mg GAE/ g). However, our results of ethanol extraction of flowers were much higher (674.55 mg GAE/ g) compared to their ethanolic extract of flowers, which contained only 31.79 mg GAE/ g, because the two ethanol extraction methods differed from each other. According to another study with *A. calvertii* [18], water extraction of the whole upper parts of plant contained lower TPC than methanol extraction, which is similar to our extraction classification; however, their value was very low (64.17 µg GAE/ g).

The seed and root parts of all three extractions had lower TPC compared to the flowers and leaves. On the other hand, opposite results were found for the TPC of seeds in other species of the genus *Alcea*. For example, although methanol extraction showed the highest value for seed extracts compared to flower and leaf extracts of *A. hyrcana* Grossh [26], the values were lower than the result of this study. Moreover, the amount of total phenolics in all our seed extracts varied from 8.02 to 296.16 mg GAE/ g, while the other study [10] found higher values of as 149.01 and 412.23 mg GAE/ g in both water and methanol extracts, respectively. The discrepancy in the values of the same plant parts might be due to the different origin of the samples, harvest period, storage time, the difference in species and different extraction methods. Polar components such as phenolic compounds in plants can be easily extracted with a polar solvent such as water [22], [27]. Moreover, according to Guler [28], these molecules have strong antioxidant capacities as they can stop the formation of free radical chain reactions in the presence of hydroxyl groups that act as reducing agents.

3.3. The Effect of Different Extraction Methods on the Total Flavonoid Content

Two flavonoids, catechin and quercetin, were used as standards to determine the TFC in different tissues of *A. calvertii*. The TFC in the plant extracts was calculated from the regression equations (y= 0.0013x; R^2= 0.9996 for quercetin, y=0,0011x; R^2= 0.9995 for catechin) of the calibration curves. TFC values differed significantly for almost all plant parts studied (Table 2) and for each extraction method (Table 3). When considering QE, the highest values in TFC were found for the flowers (1784.52 mg QE/ g) of ethanolic extract, and the lowest in the traditionally extracted roots (26.50 mg QE/ g). Considering the values of CE, the TFC value was highest in the roots extracted with ethanol (1237.78 mg CE/ g), followed by the flowers extracted with ethanol (1061.10 CE/ g). The lowest TFC value was found in the traditionally extracted roots (16.14 mg CE/ g). In addition, significant differences were found in the TFC values of the different parts of the plant. Thus, the ethanolic extracts showed the highest values for leaf and root parts, while the traditional method gave the lowest values. The TFC content of flower was significantly higher in ethanol extraction (1784.52 mg QE/ g; 1061.10 mg CE/ g) than in traditional extraction (446.36 mg QE/ g; 187.22 mg CE/ g).

Flavonoids have radical-scavenging activity due to their hydroxyl group acting as proton donor [29]. The TFC values in *A. calvertii* differ depending on the extraction method. This could be because the flavonoids have different structural types. In addition, there are numerous bioactive compounds with different activities in the extracts [29]. In accordance with the previous study with *A. calvertii* [17], the highest TFC value for CE among the different extractions was found for the methanol extraction of the flowers while in our study, the highest value was obtained from the ethanol extraction of flowers. Moreover, this value was about 1000 times higher than the value obtained in their study. Zakizadeh *et al.* [26] reported that methanol extraction of leaves of *A. hyrcana* had a higher TFC value than extraction of seeds and flowers; these results were similar for hot water-based extractions (infusion and traditional extraction methods) in this study, whereas, in contrast, flowers had the highest TFC values in ethanolic extracts. Previous studies have shown that there is a linear relationship between antioxidant activity and phenolic and flavonoid content [30], [31], and the results of this study confirm this statement. The antioxidant activity of the extracts is affected by the variety of phytochemical content [32]. In the present study, the extracts from the different parts of *A. calvertii* exhibited high antioxidant activity. In particular, the ethanolic extracts were the most active fraction in almost all tissues (Table 3). The literature also reports that the polarity of ethanol is lower than that of water [33]. Furthermore, some flavonoids, such as O-methylated flavonoids, are considered to be less polar compounds compared to unmethylated flavonoids [34]. Moreover, some tissues in this study had comparatively low TPC but comparatively high TFC, for example the ethanolic extract of the root as 190.27 mg GAE/ g versus the ethanolic extract of the root as 871.43 mg QE/ g and 1237.78 mg CE/ g. These

results suggest that the main antioxidant components could be flavonoids in some tissues, while other phenolic compounds are in other tissues. Moreover, synergistic effects among bioactive compounds could influence the high level of antioxidant activity [35]. Our results were also confirmed by previous studies with other species of the genus *Alcea* [36] and some medicinal plants which that exhibited antioxidant activities with different levels of phenolic and flavonoid compounds [37], [38].

Table 2. TPC and TFC of three different extracts from the seeds, flowers, roots, and leaves of *A. calvertii*

Extract	Plant parts	TPC (mg GAE/g)	TFC (mg QE/ g)	TFC (mg CE/ g)
Ethanol	Seed	260.89 ^{a} ± 3.32	333.33 ^{a} ± 26.94	677.77 ^{a,b} ± 141.42
	Flower	674.55 ^{b} ± 6.36	1784.52 ^{b} ± 5.05	1061.10 ^{b} ± 7.86
	Leaf	519.55 ^{c} ± 12.84	1000.00 ^{c} ± 26.93	725.00 ^{a} ± 35.36
	Root	190.27 ^{d} ± 1.06	871.43 ^{c} ± 60.61	1237.78 ^{b} ± 144.57
Infusion	Seed	296.16 ^{a} ± 18.80	517.86 ^{a} ± 21.88	336.11 ^{a} ± 3.92
	Flower	-	-	-
	Leaf	407.08 ^{b} ± 12.40	895.24 ^{b} ± 26.93	416.66 ^{a} ± 39.29
	Root	38.78 ^{c} ± 0.05	160.71 ^{c} ± 1.68	161.11 ^{b} ± 0
Traditional	Seed	8.02 ^{a} ± 0.02	-	834.37 ^{a} ± 35.95
	Flower	55.75 ^{b} ± 2.01	446.36 ^{a} ± 1.51	187.22 ^{b} ± 9.42
	Leaf	232.98 ^{c} ± 23.70	845.56 ^{b} ± 1.04	366.83 ^{c} ± 5.56
	Root	4.50 ^{d} ± 0.05	26.50 ^{c} ± 6.07	16.14 ^{d} ± 0.21

All values are expressed as mean ± standard error of the mean (SEM) (n=3). Means with different superscripts indicate a significantly difference ($p < .05$) among different plant tissues extracted with the same method

Table 3. TPC and TFC of seed, flower, root, and leaf of *A. calvertii* for each extraction method

Plant parts	Extract	TPC (mg GAE/g)	TFC (mg QE/g)	TFC (mg CE/g)
Seed	Ethanol	260.89 ^{a} ± 3.32	333.33 ^{a} ± 26.94	677.77 ^{a,b} ± 141.42
	Infusion	296.16 ^{a} ± 18.80	517.86 ^{b} ± 21.88	336.11 ^{b} ± 3.92
	Traditional	8.02 ^{b} ± 0.02	-	834.37 ^{a} ± 35.95
Flower	Ethanol	674.55 ^{a} ± 6.36	1784.52 ^{a} ± 5.05	1061.10 ^{a} ± 7.86
	Traditional	55.75 ^{b} ± 2.01	446.36 ^{b} ± 1.51	187.22 ^{b} ± 9.42
Leaf	Ethanol	519.55 ^{a} ± 12.84	1000.00 ^{a} ± 26.93	725.00 ^{a} ± 35.36

	Infusion	407.08 ^{b} ± 12.40	895.24 ^{a,b} ± 26.93	416.66 ^{b} ± 39.29
	Traditional	232.98 ^{c} ± 23.70	845.56 ^{b} ± 1.04	366.83 ^{b} ± 5.56
	Ethanol	190.27 ^{a} ± 1.06	871.43 ^{a} ± 60.61	1237.78 ^{a} ± 144.57
Root	Infusion	38.78 ^{b} ± 0.05	160.71 ^{b} ± 1.68	161.11 ^{b} ± 0
	Traditional	4.50 ^{c} ± 0.05	26.50 ^{c} ± 6.07	16.14 ^{c} ± 0.21

All values are expressed as mean ± SEM (n=3). Means with different superscripts indicate a significant difference (p < .05) in the same plant tissue extracted by different methods

3.4. The Effect of Different Extraction Methods on Metal Chelating Power

The experiment was designed to determine whether the chelating activity of iron (II) (Fe^{2+}) is due to the chelation of this metal ion with ferrozine to form a red coloured complex. In the presence of other chelating agents, the formation of the iron (II)-ferrozine complex was impaired and the red colour of the complexes was reduced. Therefore, measuring the percent inhibition allows us to estimate the chelating power of iron [39]. Figure 2 shows the metal chelating power of the two extraction methods for tissues of *A. calvertii*. When comparing the ethanol extracts of the plant tissues, the leaves extract had the best chelating effect (52.20% at 0.5 mg/mL and 42.82% at 1 mg/mL), followed by the seed extract (44.57% at 0.5 mg/mL and 41.93% at 1 mg/mL), the root extract (39.39% at 0.5 mg/mL and 40.90% at 1 mg/mL), and finally, the flower extract (38.79% at 0.5 mg/mL). Similar results were obtained for the infusion extracts of the three parts studied. The leaf extract (57.27% at 0.5 mg/mL and 54.55% at 1 mg/mL) had the highest antioxidant activity, followed by the root extract (55.15% at 0.5 mg/mL and 53.03% at 1 mg/mL), and the seed extract (45.45% at 0.5 mg/mL and 43.64% at 1 mg/mL). However, at the same concentrations, the chelating effect of the synthetic metal chelator EDTA were recorded as 90.1% iron chelation percentage at 0.5 mg/mL and 91.46% at 1 mg/mL.

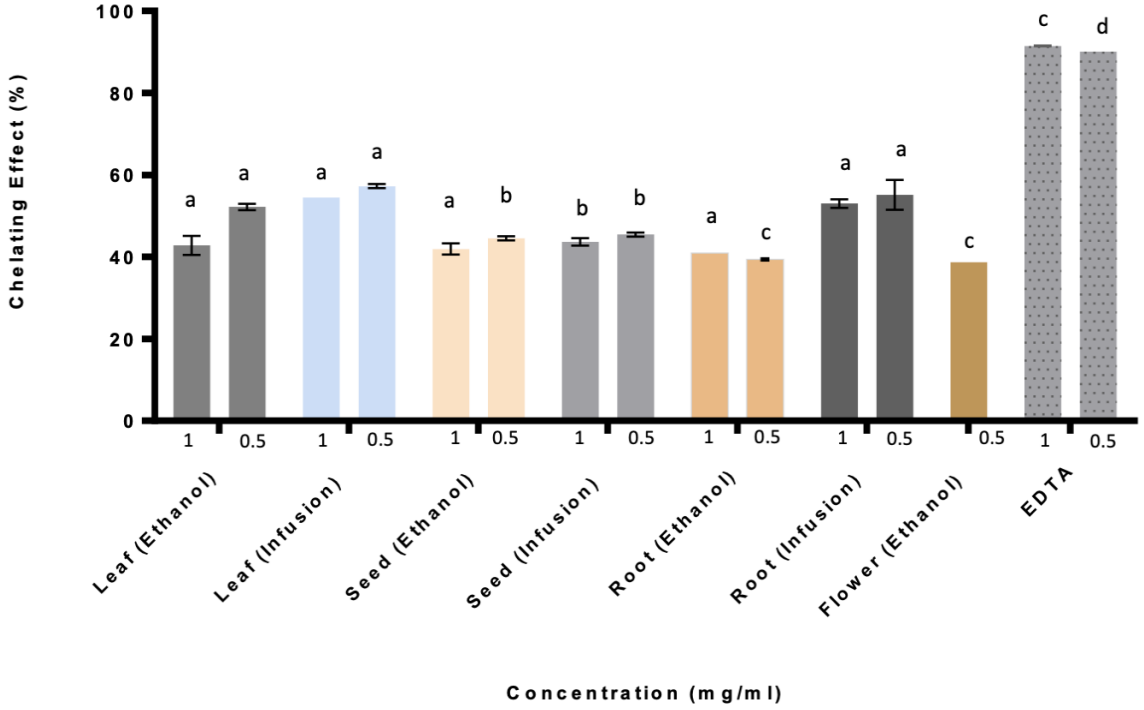

Figure 2. Metal chelating effect of ethanolic extracts and infusion extracts of the leaves, seeds, roots and flowers of *A. calvertii*. Each value is the mean ± SEM (n=3). Different letters indicate a significantly difference (p< .05) among different plant tissues extracted by the same method at the same concentration.

Halliwell [40] had explained that iron stimulates lipid peroxidation through the Fenton reaction and accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals, which can abstract hydrogen and maintain the chain reaction of lipid peroxidation. According to the results, the chelating capacity of Fe^{2+} varied significantly among different plant parts. In addition, it was determined that infusion extraction was more effective than ethanol extraction for iron chelating capacities, especially in the leaf and root parts. However, Figure 2 shows that both extracts from all parts of *A. calvertii* exhibited pronounced iron binding capacity, suggesting that their effect as peroxidation protectants might be related to their iron binding capacity. It was also observed the increase in activity was proportional to the respective concentration in all extracts and all tissues. In a previous study with the whole upper parts of *A. calvertii*, in contrast to this study, a higher metal chelating activity was observed in the methanolic extract than in the water extract [18]. Moreover, methanolic extracts of leaves and flowers of *A. hyrcana* have been shown to have higher reducing power than the seeds [26]; however, in this study the ethanolic extracts of leaves were found to be higher than those of seeds and flowers. This may be due to the fact that the content of biologically active compounds differs even among species within the same genus, and that climatic and geographic structural characteristics of the soil affect these contents. Yuan *et al.* [41] reported that compounds with structures containing two or more of the functional groups -OH, -SH, -COOH, $-PO_3H_2$, C=O, $-NR_2$, -S and –O are suitable for metal chelation. Thus according to Bhandari and Kawabata [42], molecules containing organic acids and polyphenols can increase the chelating ability of the plant. Moreover, they prevent the formation of free radicals by chelating pro-oxidant metal ions such as iron and copper.

3.5. The Effect of Different Extraction Methods on the Antibacterial Activity

The antibacterial activity of seed, flower, leaf, and root parts of three different extracts obtained at different concentrations was tested by the disc diffusion method. However, no inhibitory activity was detected against the four tested bacteria (*E. coli* ATCC 35213, *E. coli* ATCC 35218, *S. epidermidis* ATCC 12228, *B. cereus* DSM 22648). Although the antibacterial effect has been described as weak or moderate in the literature in *A. calvertii* [16], [17] and other studies with other species of the genus *Alcea* [31], [43], there was no evidence of antibacterial effect in this study. The reason for the lack of effect may be due to the differences in the strains and species tested.

4. Conclusion and Suggestions

This study showed interesting results and indicated that the traditional use of *A. calvertii* has antioxidant potential. However, it was also emphasized that the extraction methods are important to fully determine the exact antioxidant capacity of the plant. In view of these experiments, it was concluded that the different tissues of the plant have different potential for TPC, TFC, and metal chelating power. The results showed that the ethanol extraction had higher efficacy on TPC and TFC in almost all parts of the plant compared to hot water-based extractions, while hot-water based extractions had higher efficacy only in the seeds. The infusion extraction was also found to be more effective than the ethanol extraction in terms of yield and metal chelating ability in the plant parts studied. Therefore, *A. calvertii* is considered as a potential source of natural antioxidants. However, it is important to choose an appropriate solvent and methodology variables to obtain the valuable result. In addition, the purified constituents could be even more effective in terms of their pharmacological abilities.

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Contributions of the authors

Fevzi Ozgokce and Ayse Gozde Bicek collected plant materials and identified, Deniz Irtem Kartal and Ayse

Gozde Bicek designed extraction and antioxidant experiments. Tugba Ozaktas and Ayse Gozde Bicek conducted antibacterial experiments and wrote the manuscript. Tugba Ozaktas proofread the manuscript. Finally, all authors read and approved the manuscript.

Conflict of Interest Statement

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics.

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