RESEARCH ARTICLE

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The biological activity features and mineral element analyses of some *Inula* L. species exhibit natural spread in Mugla (Turkiye)

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Abstract

Medicinal and aromatic plants (MAPs) are rich in nutrients and alternative therapies. Some MAPs become industrial crops that are grown around the world for their nutritional and medicinal properties. The aim of this study was to assess the relationship between mineral nutrient content and antioxidant properties of *Inula viscosa* (*I. viscosa*) and *Inula graveolens* (*I. graveolens*) species found in the Köyceiz region of Muğla province. In this study, the antioxidant activity values of the extracts obtained were found to be the highest in methanol and acetone extracts of *Inula viscosa*. In contrast, the lowest in hexane extracts of *Inula graveolens* species. It was determined that the methanolic extract of *I.viscosa* had the highest 137.1 (µg PE /mg) a and the hexane extract of *I. graveolens* L. had the lowest 22.40 (µg PE /mg) total phenolic content. On the other hand, the mineral content of the species (macro (%): N, P, K, Ca, Mg, and micro (ppm): Fe, Mn, Zn, Cu, B) were also taken into consideration. As a result, it was observed by the analysis that there was a significant interaction between the antioxidant activity values of the species and their mineral nutrition. The antioxidant activities of plants are influenced by a variety of factors. The plant's activity is influenced by a number of variables, including the time of harvest (flowering, seed formation, etc.), extraction technique, solvent polarity, fresh or dry plant material, mineral nutrient content, and method. It is thought to broaden perceptions of these plants beyond their nutritional value by putting the antioxidant effects of the plant on a scientific basis. In this study, *Inula graveolens* L. and *Inula viscosa* L. demonstrated the potential of plant extracts as a readily available source of natural antioxidants, potential food additives, pharmaceuticals, and pharmaceutics.

Keywords: Antioxidant activity, DPPH, *Inula* L., Mineral nutrition, Phenolic content

INTRODUCTION

Plants have been used for treatment for thousands of years and the amount of these plants has been increasing continuously since ancient times. Herbal medicines form a vital part of the culture and traditions of rural communities in developing countries. It is known that in the ages when medicine was not as developed as it is today, people used plants that grow naturally in nature (Berber, et al., 2013). Today, most of the world's population uses plants as pharmaceutical raw materials. Especially in developing countries, 80% of the population meets their health needs from traditional medicinal plants in the first place. Approximately 25% of prescription drugs in developed countries are chemicals of herbal origin (Farnsworth, 1990). In Turkey, as in all countries, plants are considered as food, tea, resin, dye, spice, and phytotherapeutic resources among

the people. The depletion of natural riches and countries' economic problems have made using natural products widespread, no matter how far technology, science, and medicine have advanced. When the World Health Organization (WHO) data are examined, the amount of medicinal plants used for therapeutic purposes is around 20,000. This number is around 500 in Turkey (Çınar, 2012; Arıkan, 2019). Plant-derived secondary metabolites, which have the potential to treat various diseases, are divided into many classes such as flavonoids, phenolic acids, phenolic glycosides, unsaturated lactones, phenylpropanoids, lignins, terpenoids, and steroids. These compounds have many applications in the food, cosmetic and pharmaceutical industries (Banerjee and Bonde, 2011).

Today, although medicinal plants require longer treatment, they are met with great interest since they are more natural than synthetic drugs and do not have many side effects (Silinsin, 2016). In this study, which was carried out considering the aforementioned reasons, *Inula graveolanes* L. and *Inula viscosa* L., the natural species of Muğla province and its districts, were preferred. There are nearly 100 species belonging to the genus *Inula* L. from the *Asteraceae* family, and it is known to spread mainly in Asia, Africa, Europe, and the Mediterranean Region. There are also annual species of members of the genus *Inula* L., known as perennial. There are 27 species in our country, and 7 of them are endemic. Some members of *Inula* L. species are used effectively in traditional medicine worldwide. Numerous biological activities, including anticancer, antibacterial, hepatoprotective, cytotoxic, and anti-inflammatory properties, are associated with the genus *Inula* (*Asteraceae*) (Zhao et al., 2006).

The demand for more efficient ways to deliver life's basics drove the industrial revolution. Biotechnology approaches are currently being employed in agriculture to create new and more efficient food, medicinal, and energy products by manipulating organisms. Ecological factors have been considered in plant cultivation since ancient times. Environmental factors affect medicinal plants more compared to that cultivated plants. This is because the quality of medicinal plants is at least as important as the yield, and even those below the quality limit are not grown regardless of the yield. Moreover, for the safe use of these plants, the sustainability of the study needs to determine the content and amounts of mineral substances and the soil values of the environment where they are grown. In summary, significant interactions were observed between secondary metabolites and mineral nutrition.

MATERIALS AND METHODS

Plant materials and extraction techniques

Through the collection phase of the plants used in the study, the Flora of Turkey (Davis, 1984; 2000) is used for the locality of the plants and the resources related to the previous flora studies within the province of Muğla were scanned so that plats were collected without damaging the ecological balance elements and natural structure. A field study program was prepared by considering factors such as flowering time, spread areas, altitude, and habitat of these species. In light of this information, *Inula viscosa* L. and *Inula graveolens* L. species, which are members of the genus *Inula* L., were collected in October 2020 from the Ağla Highland, located within the borders of Köyceğiz district of Muğla province. Diagnosis of plants was carried out by Dr. Kenan AKBAŞ and Dr. Olcay CEYLAN (Muğla Sıtkı Koçman University, Department of Biology); hence, their diagnosis was prepared following the herbarium and added to the collection.

Extracts of the collected species prepared in different solvents (hexane, acetone, methanol) were appropriately prepared for analysis in order to determine the antioxidant activity studies. In order to determine macro and micro nutrients, plant, and soil samples were taken from each locality under appropriate conditions. After the preliminary stages of mineral analysis were carried out in our laboratories, support was received from Muğla Sıtkı Koçman University Research Laboratories Center for the instrument reading phase. The names of the plant species that are the subject of the study, the localities where they were collected, the date of collection, and the herbarium codes are shown in Table 1 below.

The plants collected and brought from their locations were dried in the shade and at room temperature with little air circulation. The dried plant samples were ground with a blender, and 10 g of each sample was weighed and extracted twice in 175 mL solvent (acetone, hexane, and methanol) for 24 and 48 hours. The mixture formed as a result of the extraction was filtered through filter paper, the solvents were evaporated at 50°C in a rotary evaporator, and the remaining extract was lyophilized and extracted. The remaining extracts were stored at -20 °C until analysis.

Table 1. Collection localities of plant materials

Species Name	Locality	Altitude	Collection Date	Herbarium Code
Inula viscosa L. Aiton	Köyceğiz Ağla Highland 36°59'47"N / 28°42'38"E	178 m	October 2020	O.2116
Inula graveolens L. Desf.	Köyceğiz Ağla Highland 36°59'52"N / 28°42'36"E	217 m	October 2020	O.2115

Antioxidant Activity

The β-carotene-linoleic acid test system developed by Marco (1968) with minor modifications was used to assess lipid peroxidation inhibition activity (Tel-Cayan and Duru, 2019). As previously described, the DPPH activity was measured spectrophotometrically (Blois, 1958). With slight adjustments (Tel et al., 2012), $ABTS^{+}$ activity was calculated, as Re et al. (1999) mentioned. (Tel et al., 2012). Apak et al. (2004)'s method was used to determine the cupric-reducing antioxidant capacity (CUPRAC). To compare the $ABTS^{+}$, DPPH, β-carotene-linoleic acid, and CUPRAC assays, BHA and tocopherol were used as antioxidant standards. The metal chelating activity of plant extracts for Fe^{2+} was measured using spectrophotometry (Decker and Welch, 1990). The reference compound for activity comparison was EDTA. The antioxidant activity results were reported as the 50% inhibitory concentration.

Total phenolic and total flavonoid content analysis

The total amount of phenolic substance of the plant extract, 1mL extract solution, 45mL distilled water, and 1ml Folin-Ciocalteu reagent were placed in test tubes. After 3 minutes, 3 ml of 2% Na_2CO_3 solution was added. The mixture, left to incubate for 2 hours at room temperature, was stirred at regular intervals. The absorbance values of the samples were read at 760 nm (Slinkard and Singleton, 1977).

The total amount of flavonoid substance, 4 mL of distilled water, 1 mL of standard quercetin solutions and plant extract, and 0.3 mL of 5% sodium nitrate were placed in test tubes and incubated for 5 minutes at room temperature conditions. After the incubation, 0.3 mL of 10% aluminum chloride was added and kept in room temperature conditions without light for 5 more minutes. At the end of the incubation period, 2 mL of 4% sodium hydroxide and 2.4 mL of distilled water were added, and the absorbance values were read at 415 nm (Onar, 2015).

Analysis of mineral elements

Soil properties and elemental analysis methods

Analyzes applied to soil samples; using the texture hydrometer test, lime; based on calcimetric, organic matter (Walkley and Black, 1934) method, available Zn, Fe, Mn, and Cu; DTPA (diethylene-triamine-penta-acetic-acid) method (Lindsay and Norvell, 1978), available K, Ca and Mg content (Thomas, 1982), available Na content (Knudsen et al., 1982) were found in the extracts obtained with 1 N neutral ammonium acetate solution in atomic absorption spectrophotometer. In addition, the content of Water-Soluble Phosphorus was read calorimetrically in the spectrophotometer using the Bingham (1982) method.

Plant nutrient content analysis

According to Kacar (1992), nutrient element analysis of plant extracts was determined. P, K, Ca, Mg, and Na in the macro element class; Fe, Cu, Mn, Zn, and B contents, which are in the microelement group, were measured in the ICP-AES device at the specific wavelength of each element. The Kjeldahl method was used to determine total nitrogen in plant leaf samples. The data obtained are shown in % and ppm according to the dry matter principle.

Data analysis

Each bioassay measurement and absorbance was performed in triplicate. The results were recorded as the means ± standard error (SE) of the mean for three parallel measurements. MINITAB 16 was used for statistical analysis, and the ANOVA (variance analysis) procedure was used to determine significant differences between means, with $p<0.05$ considered significant.

RESULTS AND DISCUSSION

In this study, *Inula graveolens* (L.) DESF. and *Inula viscosa* (L.) AITON, which are plants belonging to the genus *Inula* L. of the *Asteraceae* family, were used in various solvents extraction with the β-carotene-linoleic acid system, DPPH method, ABTS method, CUPRAC method, and Metal chelation; hence, total antioxidant activity levels determined by its capacity and total phenolic and flavonoid substance contents were determined. In addition, the nutritional status and elemental contents of these plants with high medicinal value and the soil properties of the environment where they grow are also discussed in comparison with the analyzes conducted.

Total antioxidant activity of plants

The total antioxidant activity results determined by the methanol, acetone, and hexane extracts of *Inula graveolens* L. and *Inula viscosa* L. plants and the β-carotene-linoleic acid system, DPPH method, ABTS method, CUPRAC method, and metal chelating capacity are presented in Table 2.

According to the antioxidant activity results of the extracts of *Inula graveolens* L. and *Inula viscosa* L. plants, which are the subject of the study, determined by the β-carotene-linoleic acid system, *Inula viscosa* methanol extract showed the strongest antioxidant activity compared to the other tested extracts, with an IC50 value of 10.33 (µg/mL). The polarities of the solvents can be shown as the reason why the extracts obtained from the same plants with different solvents show very different antioxidant activities from each other. These various antioxidant activities of the extracts can be attributed to their effective hydrogen-donating abilities and free radical scavenging (Hayouni et al., 2007). Bayraktar (2019) determined the antioxidant activities of n-hexane and methanol extracts of *Inula graveolens* and *Inula viscosa* species and standard antioxidants with the β-carotene-linoleic acid system and reported that antioxidant activities increased with the increase in the

Table 2. Total antioxidant activity[a]

Antioxidant Activity								
	Period	Plant Species	Solvent	β-Carotene-Linoleic acid	DPPH·	ABTS·+	CUPRAC	Metal Chelating
				IC_{50} (µg/mL)	IC_{50} (µg/mL)	IC_{50} (µg/mL)	$A_{0.50}$ (µg/mL)	IC_{50} (µg/mL)
Extracts	24 hours	*Inula graveolens*	Acetone	23.61±0.82	30.71±0.41	25.43±0.65	23.66±0.47	280.3±1.35
	24 hours	*Inula graveolens*	Hexane	205.6±1.45	401.2±0.62	183.7±1.40	331.3±0.31	487.5±1.08
	24 hours	*Inula graveolens*	Methanol	19.51±0.31	25.65±0.75	20.55±0.53	17.61±0.18	205.6±1.85
	24 hours	*Inula viscosa*	Acetone	**13.54±0.48**	**28.88±0.35**	**12.34±0.28**	**16.47±0.63**	**184.2±1.96**
	24 hours	*Inula viscosa*	Hexane	195.3±1.89	355.3±1.73	168.3±1.05	291.8±1.21	365.1±1.90
	24 hours	*Inula viscosa*	Methanol	**11.51±0.25**	**19.81±0.36**	**10.25±0.14**	**12.14±0.27**	**154.1±1.44**
	48 hours	*Inula graveolens*	Acetone	21.47±0.18	28.51±0.50	21.38±0.38	23.80±0.51	282.5±1.65
	48 hours	*Inula graveolens*	Hexane	207.8±1.75	390.8±1.95	175.3±0.90	297.6±1.46	459.2±1.36
	48 hours	*Inula graveolens*	Methanol	18.75±0.56	21.33±0.23	17.85±0.42	15.83±0.17	194.3±0.97
	48 hours	*Inula viscosa*	Acetone	**13.41±0.20**	**26.51±0.61**	**12.05±0.21**	**12.85±0.36**	**177.5±1.25**
	48 hours	*Inula viscosa*	Hexane	196.7±1.29	362.4±0.90	155.8±1.70	258.9±0.86	361.0±1.86
	48 hours	*Inula viscosa*	Methanol	**10.33±0.80**	**17.63±0.34**	**9.58±0.22**	**11.93±0.19**	**139.2±1.37**
Std	BHA		α-Tocopherol	2.20±0.05	38.00±0.40	35.50±0.21	63.50±0.80	NT[b]
	EDTA			1.41±0.02	19.85±0.35	12.80±0.15	25.30±0.50	NT[b]
				NT[b]	NT[b]	NT[b]	NT[b]	5.80±0.50

a Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).
b NT: not tested.

concentrations of both plant extracts. In addition, *Crocus mathewii* and *Crocus biflorus* Mill. subsp. *isauricus* plants' different solvents in the study on the total antioxidant activities determined by the β-carotene-linoleic acid method displayed the highest antioxidant activity (83.1±1.75%) in *Crocus biflorus* Mill. subsp. *isauricus*' ethanolic extract of the underground part, the lowest antioxidant activity (27.05±0.93%) was observed in the extract of the above-ground parts of the *Crocus mathewii* species obtained with the same solvent (Yıldıztekin, 2015).

In the analysis using the DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging activity determination method, the highest free radical scavenging activity value (24 h) of *Inula viscosa* was determined in the methanolic extract (19.81±0.36%). In contrast, the lowest free radical scavenging activity was determined in the lowest concentration of *Inula graveolens* hexane solvent (401.2±0.62). While the highest free radical scavenging activity was detected in the methanolic extract (17.63±0.34) of *Inula viscosa* species (48 h), the lowest free radical scavenging activity was measured in the lowest concentration of *Inula graveolens* hexane solvent (390.8±1.95%). For both periods (24 and 48 h), similar results were found in the same solutions (Table 2). The low IC50 value indicates that 50% of the free radical is removed in the DPPH free radical scavenging activity and that the antioxidant capacity is strong. The free radical scavenging activity of the extracts depends on the ability of the antioxidant compounds in the extract to give their hydrogen and the structural conformation of the compound (Fukumoto and Mazza, 2000). The DPPH radical, dark purple, gives the highest absorption at a wavelength of 517 nm. With the addition of an antioxidant to the DPPH solution, absorbance decreases, and the radical changes from purple to yellow with the effect of antioxidants (Huang et al., 2005). According to the method above, the highest scavenging value was determined in *Saponaria kotschyi* water (89.93±1.30) and the lowest in *Saponaria pumilio* water (5.17±2.39%) extract (Saraç, 2019).

ABTS activity, on the other hand, according to the BHA

standard, *Inula viscosa* methanol extract showed the strongest antioxidant activity compared to the other tested extracts, with IC50 values of 9.58 and 10.25 µg/mL, respectively, when both periods were taken into account. Following this, it was determined that *Inula graveolens* acetone extracts showed high activity with IC50 values of 12.05 and 12.34 µg/mL, respectively. In addition, acetone and methanolic extracts of *I.graveolens* and *I.viscosa* plants were determined to show high activities when the results of 24-hour and 48-hour data analysis were examined; however, stronger results were obtained through the activities of BHA and α-Tocopherol where the methanol extract used as standard antioxidants (Table 2). ABTS activity according to BHA standard, *Inula viscosa* methanol extract was determined to have the strongest antioxidant activity compared to other extracts tested when both periods were considered (Table 3). Erbil et al. (2018) found that the methanol extract of the fruit and leaf parts of the *Arum maculatum* plant was 54.8 ± 0.32% in the fruit and 41.9 ± 1.79% in the leaf. In the study by *Thymus cariensis* where Essential oil, hexane, acetone, and methanol extracts and the cation removal activity of the standards were determined using $ABTS^{+}$ cation, acetone, and methanol extracts reported high activities, he underlined that especially the methanol extract achieved higher results at the concentrations of 100 µg/ml, 200 µg/ml, and 400 µg/ml than the activities of BHA and α-Tocopherol, which they used as standard antioxidants, at the same concentrations (Küçükaydın, 2014). The determination of the highest values in the methanolic extract in both literature data showed parallelism with our study.

According to the CUPRAC analysis results, when the 24-hour and 48-hour data were examined, it was determined that the acetone and methanolic extracts of *I. graveolens* and *I.viscosa* plants showed high activity. However, especially BHA and α-Tocopherol activities where the methanol extract used as standard antioxidants displayed stronger results (Table 2.) The chelating capacity of Fe^{2+} ions of the extracts obtained from the species was calculated as the EDTA standard (mg EDTA/g) and the results are given in Table 2. As a result of the examination, while the highest activity (24 and 48 h) was observed in *I. viscosa* methanol extract, it was determined that the acetone extracts of the same plant species also had high activity. On the other hand, the lowest activity values were determined in *I. graveolens* hexane extract. The low absorbance values indicate that the metal ions are chelated before the ferrozine bonds; the metal chelating activity is high. Considering the 24-hour and 48-hour data according to the CUPRAC method, acetone and methanolic extracts of *I. graveolens* and *I. viscosa* plants were determined to show high activities (Table 2). Antioxidant activities of standards and methanolic extracts calculated using different solvents (essential oil, hexane, methanol) in *Inula graveolens* and *Inula viscosa* species at 100 µg/mL concentration (1.85% and 2.17%, respectively) displayed higher activity compared to BHA at 100 µg/mL concentration (1.70%). (Bayraktar, 2019). According to Table 2, the highest metal chelating activity (24 and 48 h) was observed in *I.viscosa* methanol and acetone extract. The low absorbance values indicate that the metal ions are chelated before the ferrozine bonds; that is, the metal chelating activity is high. The extracts' metal chelating activities differed depending on the solvent used. These findings are consistent with a previous report published in the literature (Kaska et al., 2019; Uysal et al., 2016).

Total phenolic and flavonoid contents

Phenolic compounds are highly soluble in polar solvents (Zhou and Yu, 2004), and ethanol is one of the best solvents for polyphenolic compounds that is also safe for human consumption (Shi et al., 2005; Naidoo et al., 2016). The total amount of phenolic substance was compared between species depending on the methanol solvent, the highest was determined in *Inula viscosa* species (48 h) (137.1), the lowest in *Inula graveolens* species (24 h) (118.7 (µg PE /mg)[a]. When the total amount of phenolic substance was evaluated depending on the acetone solvent, the highest was determined in *Inula viscosa* (48 h) (98.57), the lowest in *Inula graveolens* species (24 h) (90.58 (µg PE /mg)[a]. When the total amount of phenolic substance was evaluated depending on the hexane solvent, the highest was determined in *Inula viscosa* (48 h) (31.05), the lowest in *Inula graveolens* species (24 h) (22.40 (µg PE /mg)[a] (Table 3). In this context, it was concluded that the total amount of phenolic substances obtained in the species is related to both the type of solvents and the residence time. It is thought that there is a linear relationship between phenolic substances and antioxidant activity. Salim et al. (2017), extracts were prepared from the whole plant, leaf, stem, and flower parts of the plant (*Inula viscosa* L.) collected from Palestine using ethanol and methanol solvent, and the total phenolic content was examined. The results showed that methanolic extracts of all plant parts had higher total phenolic content and antioxidant activity than ethanolic extracts. However, the data presented in reference (Rhimi et al., 2017) showed that Tunisian-collected *Inula viscosa* methanol extracts had a higher value of total phenolic content (123.07 1.69 mg GAE/g extract). In addition, an EtOAc *Inula viscosa* sample from Morocco's Sefrou region was found to have a high total phenolic content value (274.4 6.94 mg GAE/g DW) (Chahmi et al., 2015).

When the total amount of flavonoid substance was analyzed depending on the methanol solvent, the highest in *Inula viscosa* (48 h) (95.78 (µg QEs /mg)[a], the lowest in *Inula graveolens* (24 h) (88.54 (µg QEs /mg))[a] has been determined. When evaluated depending on the acetone solvent, the highest was determined in *Inula viscosa* species (48 h) (80.12), the lowest in *Inula graveolens* species (24 h) (65.51 (µg QE /g)[a]. When looking at the hexane solvent, the highest was determined in

Table 3. Total phenolic content of plant extracts

Extracts	Period	Plant Extract	Solvent	Total phenolic amount	Total flavonoid amount
				µg PEs/mg extract[a]	µg QEs/mg extract[b]
	24 hours	*Inula graveolens*	Acetone	90.58±0.07	65.51±0.11
	24 hours	*Inula graveolens*	Hexane	22.40±0.05	13.25±0.05
	24 hours	*Inula graveolens*	Methanol	118.7±0.15	88.54±0.07
	24 hours	*Inula viscosa*	Acetone	98.35±0.10	79.65±0.15
	24 hours	*Inula viscosa*	Hexane	30.45±0.09	16.50±0.01
	24 hours	*Inula viscosa*	Methanol	133.8±0.12	93.50±0.08
	48 hours	***Inula graveolens***	**Acetone**	91.33±0.10	68.57±0.07
	48 hours	***Inula graveolens***	**Hexane**	22.85±0.06	13.41±0.04
	48 hours	***Inula graveolens***	**Methanol**	126.7±0.13	90.21±0.20
	48 hours	***Inula viscosa***	**Acetone**	98.57±0.12	80.12±0.18
	48 hours	***Inula viscosa***	**Hexane**	31.05±0.09	16.55±0.03
	48 hours	***Inula viscosa***	**Methanol**	137.1±0.04	95.78±0.05

aPEs: pyrocatechol equivalent; bQEs: quercetin equivalent

Inula viscosa species (48 h) (16.55), the lowest in *Inula graveolens* species (24 h) (13.25 (µg QE /g)[a] (Table 3). It has been determined in literature studies that flavonoids can prevent damage caused by free radicals in various ways (Panche et al., 2016). Gökbulut (2011) conducted pharmacognostic studies on some *Inula* species belonging to the *Asteraceae* family and naturally spread in our country.

According to the results of the study, it was determined that the species were rich in terpenic compounds and flavonoid amounts. Flower, leaf, and root parts of *Inula* species were examined separately, DPPH and ABTS in vitro tests, and antioxidant activity tests were performed from aqueous, methanol, and ethyl acetate extracts. He shared that activity levels, especially flavonoids, are higher in *Inula viscosa* than in other species. All this information supports our work.

Mineral nutrition contents

Determination of soil characteristics of species

Soil factor comes to mind when considering parameters such as productivity and quality in crop production. In some conditions, the excess or deficiency of one or more of the mineral elements negatively affects the intake of other elements. This situation affects product yield and quality negatively. Recently, the issue of determining the nutrient content of plants that are rich in medicinal and aromatic aspects, especially in our country, has started to attract attention. In light of this information, determining the nutritional status of the areas where the researched species spread and the content of macro and micronutrients in terms of their safe consumption when necessary were determined.

Considering the soil characteristics of the localities where the species grow; While the soil of *Inula graveolens* was low in salt and lime, clay textured, and rich in organic matter, the soil of *Inula viscosa* was found to be salt-free, clay loamy, slightly alkaline, and the organic matter content was moderate. The N content of the soil sample where *Inula graveolens* and *Inula viscosa* species were spread was determined as 0.15%. When the macro element contents of the localities of the species that are the subject of the study are examined, it was determined respectively, the P content (1-2 ppm); K (6.59-71.42ppm); Ca (656.12-7356.9 ppm); Mg (15.76-166.81 ppm) and Na (4-25.93 ppm). On the other hand, when the soil microelement contents were examined; it was determined respectively, Fe (2.76-34.63 ppm), Mn (39.58-12.28 ppm), Zn (0.22-0.15 ppm), Cu (1.1-0.37 ppm). The N content of the two soil samples is also 0.15%. (Table 4). The desired N level in soils has been reported as 0.11-0.15% (Chapman, 1973). The soil N level obtained in our study is in the reference range and is considered to be at an appropriate level.

The plant-available phosphorus (P) concentration of soil samples of all the species in question was determined to be between 1-2 ppm (Table 4). Olsen et al. (1954) determined as a result of their study that the P limit values should be between 7.1 and 25.0 ppm. In this case, it was determined that the % phosphorus content of both soil samples was at deficient levels. The available Potassium (K) concentration of the soil samples made in the research area has been determined to be between 6.59 – 71.42 ppm. The desired limit values in Cottenie (1980) soils should be between 201 - 250 ppm. However, all of the soil samples were found to be at deficient levels in terms of K (Table 4). The amount of available Calcium (Ca) in the soil

Table 4. Results of the species' soil analysis

Parameters		Soil sample from *Inula viscosa* locality	Soil sample from *Inula graveolens* locality
Structure (%)	**Loamy**	-	39
	Clay Loamy	53	-
pH		7.76	7.07
EC (dS/m)		0.83	0.41
Lime (%)		15.7	0.45
Organic Matter (%)		2.95	3.05
Macro Element (%)	**Nitrogen (N)**	0.15	0.15
Macro Elements (ppm)	**Phosphorus (P)**	2	1
	Potassium (K)	71.42	6.59
	Calcium (Ca)	7356.9	656.12
	Magnesium (Mg)	166.81	15.76
	Sodium (Na)	25.93	4
Micro Elements (ppm)	**Iron (Fe)**	34.63	2.76
	Manganese (Mn)	12.28	39.58
	Zinc (Zn)	0.15	0.22
	Copper (Cu)	0.37	1.1

sample of *Inula viscosa* L. type collected in the research area is very high; Magnesium (Mg) content was found to be sufficient. The Ca and Mg levels of *Inula graveolens* L. species determined in the soil were determined at very low values (Cottenie., 1980). However, the reference range of recommended microelements in soils is Cu (>0.2 ppm), Mn (15-20 ppm), Fe (6-10 ppm), and Zn (0.8-2.5 ppm) (Lindsay and Norwell, 1978). When the soil microelement contents were evaluated in general terms by looking at the reference intervals, it was determined that *Inula graveolens* L. species was at higher levels than *I.viscosa* species (Table 4). Although microelements are mineral elements that have various biochemical functions in living organisms and are vital for human health, they can have harmful effects when taken in high concentrations (Gürel, 2014). In the study conducted in Fethiye-Babadag, *Crocus mathewii,* and *Crocus biflorus* subsp. *isauricus* endemic species' soil nutrient content was investigated, macro elements were determined as respectively; N content (0.18-0.2%); P (5-2.33 ppm); K (403-213 ppm); Ca (3497-2972 ppm); Mg (124-138 ppm) and Na (25.83-21.43 ppm) and micronutrient contents were measured as respectively Fe (37.05-47.48 ppm), Mn (111-122 ppm), Zn (2.45-4.07 ppm), Cu (3.11-3.85 ppm) and B (1.12-0.9 ppm) (Yıldıztekin, 2015). The study in question showed a similar quality to our research, although there were differences in some parameters.

Determination of plant nutrient content of species

In order to determine the nutritional status of *Asteraceae* family members *Inula viscosa* L. and *Inula graveolens* L. species in their environment, which are naturally spread within the borders of Muğla province, their mineral content was compared with the analyzes made and examined (Table 5). In this context, the nitrogen content was determined as 0.28% in *Inula graveolens* species and 0.21% in *Inula viscosa* species. In addition, other macro element contents measured in leaves of *Inula graveolens* and *Inula viscosa* species and respectively were found as; P: 0.09% - 0.22%; K: 1.39%-1.2%; Ca: 0.35%-1.81% and Mg: 0.14%-0.43%. When the nitrogen content of *I.graveolens* and *I.viscosa* species was examined, it was determined that the values were 0.21-0.28%. In general, it is recommended that the N ratio in the structure of plants should be in the range of 1.5-5% (Kacar and Katkat, 2007). According to the reference range, the plant N content was determined at very low values. In addition, when the other macro element contents were measured in the leaves of *Inula graveolens* and *Inula viscosa* species, it was determined that the P and K contents were at low levels compared to the limit values reported by Kacar and Katkat (2007). When the leaf Ca and Mg contents were examined, it was found to be sufficient in *Inula viscosa* species and low in *Inula graveolens* species (Table 4). From some literature studies on *Asteraceae* family members: *Achillea millefolium* L. K: 13869.1±15.95mg/100gr Mg: 339.62±3.40mg/100gr Fe: 73.317±0.84 mg/100gr Ca: 794.82±9.528 mg/100gr; *Cichorium intybus* L. K: 124.7±2.9 mg/100gr Mg: 485.15±2.96 mg/100gr Fe: 29.256±0.34 mg/100gr Ca: 1130.66±13.56 mg/100 gr data were obtained (Ashirova et al., 2021).

The microelement changes of the species are shown in Table 5. When the results were evaluated, leaf Fe content was found to be 1.09 ppm in *Inula graveolens* species and 910.06 ppm in *Inula viscosa*. In addition,

Table 5. Macro and microelement contents of leaves of *Inula viscosa* and *Inula graveolens* species

Mineral Nutrients		*Inula viscosa* L.	*Inula graveolens* L.
Macro Elements (%)	Nitrogen (N)	0.21	0.28
	Phosphorus (P)	0.22	0.09
	Potassium (K)	1.2	1.39
	Calcium (Ca)	1.81	0.35
	Magnesium (Mg)	0.43	0.14
Micro Elements (ppm)	Iron (Fe)	910.06	1.09
	Manganese (Mn)	125.72	2.92
	Zinc (Zn)	56.16	1.51
	Copper (Cu)	11.03	1.46
	Boron (B)	53.87	33.21

when the amounts of other microelements in the leaf are examined for *Inula graveolens* and *Inula viscosa* species, the following results are found respectively; Mn: 2.92-125.72 ppm; Zn: 1.51-56.16 ppm; Cu: 1.46-11.03 ppm and B: 33.21- 53.87 ppm. When the leaf Fe content of *Inula graveolens* species, which is the subject of the study, was examined (1.09 ppm), it was found to be relatively low compared to the reference values. In comparison, relatively high values were found in *Inula viscosa* (910.06 ppm) species (Table 5).

When the leaf Fe content (1.09 ppm) of the *I. graveolens* species, which is the subject of the study, was examined, it was found to be relatively low compared to the reference values (Kacar and Katkat, 2007), while relatively high values were determined for *I. viscosa* (910.06 ppm). We may explain the situation in question with the difference in the Fe content of the soil where the species spread. That is, while the soil Fe content of *I. graveolens* species was determined as 2.76 ppm, the soil Fe content of *I. viscosa* species was found to be 34.63 ppm which is approximately 13 times of 2.76.

As a result of studies on some *Asteraceae* family members, *Achillea millefolium* L. Zn: 2.487±0.029 mg/100 gr; Cu: 0.161±0.019 mg/100 g; Cichorium intybus L. Cu: 0.140±0.016 mg/100 g; Zn: 2.996±0.034 mg/100 g; *Chamomilla recutita* (L.) Rausch. Zn: 2.440±0.028 mg/100 g; Cu: 0.343±0.039 mg/100 g (Ashirova et al., 2021). The literature review about some other parameters that are the subject of our study is as follows; Baydar and Erdal (2004) examined the leaf Fe content of İzmir thyme and reported that they measured values in the range of 47.25-97.50 ppm, Meraler (2010) looked at the leaf Mn content of mahlep plant and determined the lowest as 8 ppm and the highest as 36 ppm. As a result, when the leaf macro and micronutrient data of *I. graveolens* and *I. viscosa* species, which constitute the material of our study, were examined, it was found that there were values that were contrary or in accordance with the literature information in general.

CONCLUSION

The current study shows that the antioxidant substance amounts and mineral substance contents of the extracts prepared in methanol, hexane, and acetone solvents of *Inula graveolens* L. and *Inula viscosa* L. species belonging to the genus *Inula* L. from the *Asteraceae* family, which naturally spread in Muğla province, were analyzed. Many factors affect the antioxidant activity and phenolic content in plants; the period the plant was collected, the solvent used, the waiting duration, the method used, etc., affect the antioxidant capacity of the plant. In light of the data obtained in this study, it is suggested that the extracts obtained with different solvents can be used in easily accessible natural antioxidant sources, food supplements, food additives, pharmacology, and pharmacy, but additional studies on the subject are recommended.

Moreover, the macro and microelement contents of the species were examined, and it was determined that *Inula viscosa* L. had higher values than *Inula graveolens* L. in terms of both micro and macronutrients. This situation is thought to be due to topographical features and environmental factors. However, since it is known that the number of microelements and heavy metals must be at a limited value, and it is known that they can have toxic effects on plants and humans when exceeded, thus, the studies need to be detailed. Finally, it is our sincere hope that the findings of this study will serve as a foundation for future research to capitalize on the natural antioxidant substances found in the extracts of these two *Asteraceae* plants.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

Authors do not declare any conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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