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CHARACTERIZATION OF THE ELEMENTAL CONSTITUENTS, PHENOLIC PROFILE, AND ANTIOXIDANT CAPACITY OF CULTIVATED HOREHOUND (*Marrubium vulgare* L.) LEAVES FROM VAN, TÜRKIYE

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Abstract: Horehound (*Marrubium vulgare* L.) is a perennial plant species that originated in the Mediterranean-Central Asian region and has since become globally distributed. *Marrubium vulgare* L. is a plant species that contains a diverse array of bioactive compounds, including alkaloids, sterols, steroids, terpenoids, saponins, flavonoids, tannins, and various phenolic compounds. The flowering aerial parts or leaves of this plant are widely used in traditional medicine throughout Anatolia and other regions for their aromatic, antipyretic, diuretic, and carminative properties, as well as their effectiveness in treating conditions such as colds, coughs, and respiratory disorders. This study aim to explore the total phenolic and flavonoid content, total antioxidant capacity, macro-micro element and heavy metal composition of cultivated *M. vulgare* L. The results of the study revealed that the total flavonoid content was 8.64 mg QE 100 g^{-1}, the total phenolic content was 133.18 mg GAE g^{-1}, and the total antioxidant activity was 53.45 µmol TE g^{-1}. The study also characterized the total ash and dry matter content and mineral composition of the species, including both macronutrients and micronutrients (Ca, K, Mg, Na, Fe, Zn, Cu, Mn, Mo) as well as the presence of heavy metals (Cd, Co, Se, Ni, Pb, As).

Keywords: Aromatic plant, Cultivation, Medicinal plant, Trace elements, Bioactive component

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1. Introduction

Marrubium vulgare L. is a plant species that originated in the region between the Mediterranean Sea and Central Asia, and has become widespread across all continents (Knoss, 1999). The genus name Marrubium is derived from the Hebrew term marrob, signifying 'bitter juice', while the species epithet vulgare connotes 'common' or 'well-known'. In English, the designation 'horehound' originates from the Old English words har and hune, denoting a downy or hairy plant (Aćimović et al., 2020). *M. vulgare* L. is a perennial species that can reach up to 80 cm in height. Its stem exhibits sparse branching and is densely covered in greyish, downy trichomes. The flowering aerial portions of the plant are typically harvested between June and August.

According to a report by the World Health Organization, around 80% of the global population relies on traditional medicine to meet their healthcare needs (Das et al., 2014). The ongoing exploration of novel plant species is accompanied by the continued investigation of those long utilized, with the aim of elucidating their phytochemical profiles and assessing their biological properties and potential applications for human wellbeing (Michalak et al., 2024; Başgel and Erdemoğlu, 2006; Sekeroglu et al., 2008; Tercan et al., 2016). Researchers have directed considerable attention to identifying natural sources of bioactive compounds with promising preventive and therapeutic potential. Medicinal and aromatic plants, as rich reservoirs of biologically active substances, can be leveraged as health-promoting agents in the food, pharmaceutical, and cosmetic sectors (Michalak, 2022). The leaves of *M. vulgare* L. were found to contain a diverse array of bioactive phytochemicals, including alkaloids, sterols, steroids, terpenoids, saponins, flavonoids, catecholic tannins, anthocyanins, and a variety of phenolic compounds (Al-snafi et al., 2021). The flowering aerial parts or leaves of the plant are widely utilized in traditional medicine throughout Anatolia and various other regions for their aromatic, antipyretic, diuretic, and carminative properties, as well as their efficacy in treating conditions such as colds, coughs, and respiratory disorders (Lodhi et al., 2017; Demiroz Akbulut et al., 2023). Numerous prior studies have indicated that *M. vulgare* possesses a higher concentration of phenolic compounds and exhibits greater antioxidant activity compared to other medicinal plants (Matkowski and Piotrowska, 2006; Matkowski et al., 2008; VanderJagt et al., 2002). Phytochemical investigations of *Marrubium vulgare* have identified the presence of phenolic compounds and labdane diterpenes

(Aćimović et al., 2020). Previous research has reported that the phenolic composition of *M. vulgare* is primarily characterized by the presence of phenylethanoids and phenylpropanoids, such as forsythoside B, arenarioside, verbascoside, ballotetroside, and compounds like coumaric, caffeic, and cinnamic acids. Additionally, a range of flavonoids, including apigenin, luteolin, kaempferol, quercetin, chrysoeriol, and their corresponding glycosides, have been identified in samples of this plant species collected from various geographical regions (Boulila et al., 2015; Boudjelal et al., 2012; Wojdyło et al., 2007). Previous studies have also explored the essential oil composition of this plant. The major compounds identified in the essential oils typically include β-bisabolene, germacrene D, β-caryophyllene, bicyclogermacrene, and carvacrol (Demiroz Akbulut et al., 2023).

This study aimed to comprehensively characterize the total antioxidant capacity, phenolic and flavonoid profiles, nitrogen balance index, chlorophyll, flavonol, anthocyanin, nutrient elements, and heavy metal content of *M. vulgare*.

2. Materials and Methods

2.1. Plant Material

The plant material investigated in this study consisted of the *M. vulgare* L. species, cultivated at the Medicinal and Aromatic Plants Garden of Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Field Crops. The plant specimens were identified and verified at the species level, and then cultivated in the Medicinal and Aromatic Plants Garden to assess their adaptation capabilities. Samples were collected during the plant's full flowering stage at the year of 2024. The harvesting was conducted between 9:00 and 10:00 in the morning.

2.2. Determination of Ash, Dry Matter, Heavy Metal and Nutrient Contents

Nutritional parameters, including total ash, dry matter, select heavy metals, and minerals, were analyzed in different plant parts. An electric muffle furnace set to 550 ºC was employed for the total ash determination. The dry matter content of the samples was determined by drying the samples for 24 hours at 105°C in an oven. The mineral composition of the plant samples was analyzed as follows: the dried samples were first ashed in a furnace using hydrochloric acid and nitric acid (Kacar and Inal, 2008). Subsequently, distilled water was added to the samples in a volumetric flask. All analyses were conducted in triplicate, and standard materials were utilized for the chemical analyses. Atomic Absorption Spectrometry was employed to quantify the contents of K, Ca, Mg, Na and Fe. Additionally, ICP-OES was used to determine the levels of other micronutrients and heavy metals, including Cu, Zn, Ni, Mn, As, Cd, Co, Cr, and Pb.

2.3. Total Antioxidant, Total Phenolic and Total Flavonoid Content

The total phenolic compound content was determined using the method described by Obanda and colleagues (Obanda et al., 1997). Additionally, the antioxidant activity was assessed based on the Antioxidant Power ((FRAP) (iron (III) antioxidant power reduction) method, with absorbance readings taken at 593 nm, and the results reported as µmol Trolox equivalent g^{-1} (Lutz et al., 2011). The total flavonoid content was measured with some modifications to the procedure developed by Quettier-Deleu et al. (2000). The total flavonoid amount was quantified at 415 nm and calculated as mg quercetin equivalent per 100 g of dry matter, using a calibration curve prepared with standard quercetin.

2.4. Bioactive Properties

Nitrogen balance index (NBI), chlorophyll and Anthocyanin content in leaves were measured non-destructively and in real time on the leaf with the Dualex scientific+ (FORCE-A, Orsay, France) device.

2.5. Statical Analyses

All analyses carried out in the study were performed in three repetitions and standard deviations were determined.

3. Results and Discussion

The phenolic composition, antioxidant capacity, and bioactive constituents of *M. vulgare* are detailed in Table 1. The total ash content was determined to be 16.131%, and the dry matter content of the leaves was 34.004%. The total flavonoid content of the species was determined to be 8.64 mg QE per 100 g, the total phenolic content was 133.188 mg GAE per g, and the total antioxidant activity was 53.45 µmol TE per g. The nitrogen balance index, chlorophyll content, and anthocyanin content of the plant were determined to be 21.33, 31.83, and 0.03 dualex index, respectively. Previous research reported that *M. vulgare* exhibited a total phenolic content of 56.76 mg GAE/g dry weight, a flavonoid content of 81.21 mg RE/g dry weight, and an antioxidant activity of 101.82 mg As.AE/g dry weight (Amessis-Ouchemoukh et al., 2014). In another study, the total phenolic content of *M. vulgare* was determined to be 26.8±0.01 mg gallic acid equivalents per gram, whereas the total flavonoid content was 0.61 ±0.05 mg catechin equivalents per milliliter (Chedia et al., 2014). The study examined eight distinct populations of *M. vulgare* and found that the total phenolic content ranged from 20.80 to 44.65 mg GAE/g DW, while the flavonoid content varied between 8.91 and 37.48 mg RE/g DW (Guedri Mkaddem et al., 2022). The results indicate that the total phenolic and flavonoid content, as well as the overall antioxidant activity, are substantially impacted by multiple factors, encompassing environmental conditions, harvesting procedures, and drying techniques (Bautista et al., 2016). In contrast to many other medicinal plants, *M. vulgare* presents a more abundant source of phenolic compounds (Nwozo et al., 2023).

Table 1. Bioactive properties and phenolic profile of *M. vulgare*

Properties	Mean ± S.D.
Total Ash (%)	16.131 ± 0.86
Dry matter (%)	34.004 ± 1.22
Total Flavonoid Content (mg QE 100 g^{-1})	8.64 ± 1.30
Total Antioxidant Activity (µmol TE g^{-1})	53.45 ± 3.61
Total Phenolic Content (mg GAE g^{-1})	133.188 ± 2.67
Nitrogen Balance Index (NBI)	21.333 ± 11.66
Chlorophyll	31.833 ± 10.64
Anthocyanin	0.03 ± 0.01

QE= quercetin equivalent, TE= trolox equivalent, GAE= gallic acid equivalent.

The macro and micronutrient composition, as well as the heavy metal content, of the *M. vulgare* species are presented in Table 2. The leaf samples of species contained the following macronutrients in substantial quantities: calcium at 12.796 g kg^{-1}, potassium at 30.855 g kg^{-1}, magnesium at 2.826 g kg^{-1}, and sodium at 26.373 g kg^{-1}. A previous study examining *M. vulgare* samples from 5 different locations reported calcium levels ranging from 38.72 to 55.76 g kg^{-1}, potassium levels between 8.4 and 112.0 g kg^{-1}, and magnesium levels in the 7.67 to 128.4 g kg^{-1} range (Rezgui et al., 2021). In another study, the researchers reported potassium and magnesium concentrations of 91.27 mg kg^{-1} and 0.64 mg g^{-1}, respectively (Maiti et al., 2016). The micronutrient composition of the species was characterized, with iron measured at 555.30 mg kg^{-1}, manganese at 49.072 mg kg^{-1}, copper at 9.756 mg kg^{-1}, zinc at 31.464 mg kg^{-1}, and molybdenum at 0.610 mg kg^{-1}. Earlier studies have reported a wide range of micronutrient concentrations in *M. vulgare*, including iron levels between 208-2.848 mg kg^{-1}, zinc levels between 531-1.148 mg kg^{-1}, copper levels between 18-55 mg kg^{-1}, and manganese levels between 5-33 mg kg^{-1} (Rezgui et al., 2021). Additionally, other research has reported copper at 25.14 mg kg^{-1}, iron at 374.78 mg kg^{-1}, and zinc at 46.79 mg kg^{-1} (Maiti et al., 2016), while another study revealed the zinc level of the species to be 74.49 mg kg^{-1} and the copper level to be 19.44 mg kg^{-1} (Ahmed and Kebi, 2023).

Table 2. Mineral and Heavy metal content of *M. vulgare*

Elements	Mean±S.D.
Ca (g kg^{-1})	12.796 ±0.49
K (g kg^{-1})	30.855 ± 1.04
Mg (g kg^{-1})	2.826 ± 0.23
Na (g kg^{-1})	26.373 ± 0.13
Fe (mg kg^{-1})	555.300 ± 52.89
Mn (mg kg^{-1})	49.072 ± 2.07
Cu (mg kg^{-1})	9.756 ± 0.26
Zn (mg kg^{-1})	31.464 ± 0.38
Mo (mg kg^{-1})	0.610 ± 0.08
Co (mg kg^{-1})	0.162 ± 0.02
Cd (mg kg^{-1})	nd
As (mg kg^{-1})	1.297 ± 0.35
Ni (mg kg^{-1})	nd
Pb (mg kg^{-1})	0.582 ± 0.16
Se (mg kg^{-1})	nd

SD= Standart deviation, nd= not detected.

The heavy metal composition of *M. vulgare* species is detailed in Table 2. The study found cobalt at 0.162 mg kg^{-1}, arsenic at 1.297 mg kg^{-1}, and lead at 0.582 mg kg^{-1}. However, cadmium, nickel, and selenium were not detected in the leaves of plant. Previous research reported the heavy metal content of the species, with cadmium levels ranging from 2.0 to 12.0 mg kg^{-1} and lead levels between 0.80 and 11.0 mg kg^{-1}. Cobalt and nickel were also detected, with concentrations of 13.0 and 123.0 mg kg^{-1}, respectively (Shallari et al., 19998; Rezgui et al., 2021; Ahmed and Kebi, 2023).

4. Conclusion

The findings demonstrate that the cultivated *Marrubium vulgare* is a rich source of phenolic compounds and displays substantial antioxidant capacity. Moreover, the plant is characterized by a diverse array of macro- and micro-nutrients. *Marrubium vulgare* may serve as a valuable wellspring of natural bioactive molecules with potent preventive and therapeutic properties, particularly for treating conditions like colds, coughs, respiratory disorders, and skin problems. The cultivation and investigation of this species hold considerable potential for applications in the medical, pharmaceutical, cosmetic, and beverage sectors. Additionally, further studies on the concentrations of active ingredients under different cultivation techniques could yield valuable insights to fully harness the species potential.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	L.N.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declare no conflict of interest.

Ethical Consideration

Since no studies involving humans or animals were conducted, ethical committee approval was not required for this study.

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