

Antioxidant Properties, Total Phenolic Content and LC-MS/MS Analysis of *Mentha Pulegium*, *Lepidium Draba* and *Centaurea Solstitialis*

Adem NECİP^{1*}, Mustafa DURGUN²

ABSTRACT: The *Mentha pulegium*, *Lepidium draba* and *Centaurea solstitialis* have traditionally been used in different cultures for the treatment of various diseases. We investigated the total phenolic content analysis, chemical compositions and antioxidant activities of different solvent extracts such as acetone, methanol and n-hexane obtained from the aerial parts of *Mentha pulegium*, *Lepidium draba* and *Centaurea*. The amount of total phenolic substance was determined as gallic acid equivalent determine. Also, the LC-MS/MS technique was used to determine the phenolic profiles of each extract. Finally, antioxidant activities of three extracts were determined by DPPH and ABTS methods. The highest total phenolic content for acetone, n-hexane and methanol extracts was found as 99 507, 46 305, and 18 227 µg GAE mL⁻¹ in *Centaurea solstitialis* plant, respectively. While the major component rosmarinic acid amount in the acetone extract of *Mentha pulegium* was 128 195 µg analyte g⁻¹ extract, this amount was determined as 780 383 µg analyte g⁻¹ extract in the methanolic extract. The highest DPPH radical scavenging activity was found in the acetone and methanolic extracts of *Mentha pulegium* as 77% and 79%, respectively. ABTS radical scavenging activity was also found to be 98% and 94% for *Mentha pulegium* in acetone and methanol extracts, respectively. The antioxidant capacity of the extracts is related to the total amount of phenolic substances.

Keywords: *Mentha pulegium*, *Lepidium draba*, *Centaurea solstitialis*, antioxidant, LC-MS/MS, total phenolic

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INTRODUCTION

The use of plants for therapeutic purposes dates back to ancient times. The therapeutic properties of plants (root, stem, seed, flower, leaf and fruit) have survived from the past to the present. Thus, scientists accepted traditional medicine treatment and started to use it in modern treatment. As a means of treatment, it is recommended to use natural and herbal products, pure compounds due to their chemical diversity, or standard plant extracts as supplementary food along with modern treatment. In recent years, the demand for natural remedies for the treatment and prevention of diseases has been increasing globally (Petrovska, 2012; Boutemak et al., 2015; Benahmed-Bouhafsoun et al., 2015). In many countries, including Türkiye, the search for alternative medicine has been the focus of attention of scientists. In addition to the treatments used by modern medicine, many patients prefer natural, harmless, inexpensive, herbal remedies with little or no side effects (Begaa et al., 2021).

Lepidium draba, belonging to the *Brassicaceae* family, is commonly known as bleached cress and kinkneer, and has traditionally been used to treat diseases in different cultures (Roughani et al., 2018). *Lepidium draba* can be used fresh or as an ingredient in soups and salads. Secondary metabolites such as flavonoids, phenolics, glucosinolates, and alkaloids in *Lepidium draba* have been reported in previous studies (Frécharde et al., 2002; Senatore et al., 2003; Mahomoodally et al., 2018). Different biological activities of *Lepidium draba* extracts have also been reported (Sharifi-Rad et al., 2015; Kaya et al., 2015; Ouissem et al., 2018; Seebaluck-sandoram et al., 2019; Naser et al., 2019).

Most of the genus *Centaurea* is endemic in Turkey. It commonly contains secondary metabolites such as steroids, flavonoids, fatty acids, volatile compounds, and sesquiterpene lactones in *Centaurea* species. According to the literature, *Centaurea* species are known to contain antioxidant properties (Sham'yanov et al., 1998; Sezik et al. 2001).

Mentha (*Lamiaceae* family), one of the most widely grown spice plants in the world, is used in the food, pharmaceutical industry and traditional medicine treatment due to its chemical diversity (Brahmi et al., 2016; Benomari et al., 2018). It has been reported in studies that extracts from *Mentha pulegium* show antioxidant properties (Stagos et al., 2012; Sarikurkcu et al., 2012; Mozaffari et al., 2012; El Aanachi et al., 2021). It is known that *M. pulegium*, which has been used as a snack and a beverage similar to coffee from past to present, has biological activity due to flavonoid and phenolic contents in its chemical composition (Teixeira et al., 2012).

The discovery and isolation of biologically active compounds from plants, the production of plant-derived drugs and their use as supplements have attracted great interest of scientists. Therefore, in this study, we investigated the total phenolic content analysis, chemical compositions and antioxidant activities of different solvent extracts such as acetone, methanol and n-hexane obtained from the aerial parts of *Mentha pulegium*, *Lepidium draba* and *Centaurea*. The amount of total phenolic substance was determined as gallic acid equivalent determined. Also, the LC-MS/MS technique was used to determine the phenolic profile of each extract. Finally, antioxidant activities of acetone, methanol and n-hexane extracts were determined by ABTS and DPPH methods.

MATERIALS AND METHODS

General Procedure

The aerial parts of plant materials were collected from Şanlıurfa in Türkiye. After the plants were collected, they were dried under suitable conditions. It was pulverized with a laboratory mixer to be used in the analysis of antioxidant and phenolic content. It was maintained at +4 degrees.

Herbal material and extraction procedure

Plant materials were shade-dried for about two weeks and ground into powder with a laboratory mixer. All plants (10.00 g) were extracted separately with acetone, methanol and n-hexane (100 mL) at room temperature for 24 hours. Then, filtration was carried out with ordinary filter paper. All extracts were stored at -20°C until further experiments.

Phenolic component analysis

The phenolic component analysis was carried out by making a few modifications to the method used by Necip and Işık (2019). In order to create the gallic acid calibration graphic, 1 mL of ethyl alcohol was added to 1 mg of gallic acid weighed on a precision balance, so that a standard gallic acid solution was formed. 5, 10, 15, 20 and 25 µl of standard gallic acid solution were taken and prepared in different concentrations and absorbance values were measured at 760 nm. A linear graph was determined by plotting the concentration versus absorbance graph, and the R² value was calculated (Figure 1). Total polyphenols were calculated using a gallic acid calibration graphic at different concentrations. The total amount of phenolic substance was determined as microgram gallic acid equivalent per millilitre (µg GAE mL⁻¹).

The phenolic component analysis was completed by making a few modifications to the method used by Necip and Işık (2019). For experiment, 50 µl of the plant extracts prepared as standard was taken, after adding 1 150 µl of distilled water, 25 µl of Folin-Ciocalteu's reagent was added to each sample and mixed to ensure homogeneity. After 3 minutes, 75 µl of prepared sodium carbonate (Na₂CO₃) was added to the samples and vortexed and kept in the dark at room temperature for two hours. Then, absorbance values were measured by adjusting the wavelength to 760 nm in the UV Spectrophotometer. The equivalent amount of gallic acid corresponding to the measured absorbance value was calculated. Results are given as µg GAE mL⁻¹.

Identification and quantification of phenolic compounds by LC-MS/MS mass spectrometer and chromatograph conditions

Total phenolic content and LC-MS/MS analysis were performed to determine the bioactive components of the plants used in the study. LC-MS/MS analysis was performed by M. Abdullah Yılmaz. The analytical method validation parameters of the LC-MS/MS method are given in determined according to the method developed by Yılmaz (2020). The difference in mass spectrometry and chromatography conditions is that the binary pumps are also model LC-30AD.

DPPH and ABTS radical scavenging activity

The analysis of DPPH and ABTS radical scavenging activity were performed according to the literature method (Necip and Işık 2019; Durgun et al.,2020; Necip et al., 2021).

RESULTS AND DISCUSSION

Phenolic compounds are important in reducing the risk of disease and supporting medical treatment. In order to determine the total phenolic substance, gallic acid calibration graph was drawn by making a few minor changes in the Necip and Işık (2019) method (Figure 1). The R² value was found to be 0.9955 in the absorbance graph of gallic acid read against different concentrations. In this study, extracts of *Mentha pulegium*, *Lepidium draba* and *Centaurea solstitialis* plants were prepared in acetone, n-hexane and methanol solvents, and total phenolic substance amounts are given in Table 1 as µg gallic acid equivalent (GAE) per in mL.

According to the data in the table 1, the total phenolic of *Centaurea solstitialis* in acetone, n-hexane and methanol solvents was found to be 46 305, 18 227 and 99 507 µg GAE mL⁻¹ respectively.

The total phenolic of *Mentha pulegium* in acetone, n-hexane and methanol solvents was found to be 7 389, 2 463 and 13 793 $\mu\text{g GAE mL}^{-1}$, respectively. The total phenolic of *Lepidium draba* in acetone, n-hexane and methanol solvents was found to be 26 601, 30 and 12 808 $\mu\text{g GAE mL}^{-1}$ respectively. According to these results, the highest total phenolic content was found in *Centaurea solstitialis* extract with n-hexane and methanol, *Mentha pulegium* extract with methanol and *Lepidium draba* extract with acetone.

In a study by Taştan et al., *C. iberica*, *C. urvillei* ssp. *hayekiana*, and *C. urvillei* ssp. (Elazığ, Türkiye). The total phenolic substance amounts in the methanol extract of *Centaurea* species are 182 460, 40 2080 and 24 3910 $\mu\text{g GAE g}^{-1}$ dry matter respectively (Taştan et al., 2022). In the study conducted by Alper et al., they found the total amount of phenolic substance in the ethanolic extract of *Centaurea solstitialis* as 52 310 $\mu\text{g GAE g}^{-1}$ extract (Alper et al., 2021).

In the study of Younes et al., both the leaf part and the root part of *Lepidium draba* were examined. Dichloromethane, water and ethyl alcohol were used as solvents. Total phenolic content was found to be 19 620, 57 790 and 41 820 $\mu\text{g GAE g}^{-1}$ herb for the leaf part, and 12 230, 30 410 and 19 310 $\mu\text{g GAE/g}$ herb for the stem part, respectively (Younes et al., 2015). In the study by Eruygur et al., different parts of *Lepidium draba* (flower, stem, root and leaf) were used as ethanol, methanol and aqueous solvent. Total phenolic substance amounts are 40 040, 34 160 and 64 320 $\mu\text{g GAE g}^{-1}$ plant for the flower part, 13 820, 12 860 and 20 360 $\mu\text{g GAE g}^{-1}$ plant for stem, 17 990, 20 670 and 29 840 $\mu\text{g GAE g}^{-1}$ plant for root part and for the leaf part, these values were found as 21 890, 24 920, and 21 420 $\mu\text{g GAE g}^{-1}$ plant, respectively (Eruygur et al., 2022).

In the study conducted by Mohammed Messaoudi et al., they found the total amount of phenolic substance in the methanolic extract of *Mentha pulegium* as 18 770 $\mu\text{g GAE g}^{-1}$ extract (Messaoudi et al., 2021).

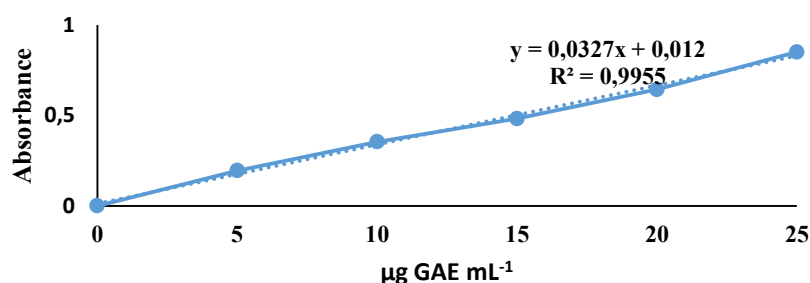


Figure 1. Gallic acid calibration graphic

Table 1. Total amount of phenolic substance

| Solvent | Plant | Total amount of phenolic substance ($\mu\text{g GAE mL}^{-1}$) |
|----------|-------------------------------|--|
| Acetone | <i>Mentha pulegium</i> | 7389 |
| | <i>Lepidium draba</i> | 26 601 |
| | <i>Centaurea solstitialis</i> | 46 305 |
| n-hexane | <i>Mentha pulegium</i> | 2 463 |
| | <i>Lepidium draba</i> | 30 |
| | <i>Centaurea solstitialis</i> | 18 227 |
| Methanol | <i>Mentha pulegium</i> | 13 793 |
| | <i>Lepidium draba</i> | 12 808 |
| | <i>Centaurea solstitialis</i> | 99 507 |

Free radical scavenging activity studies to determine the antioxidant capacity of plants, DPPH and ABTS were performed according to the literature method (Necip and Işık 2019; Durgun et al., 2020; Necip et al., 2021). The percent scavenging activity results of ABTS and DPPH at different

concentrations are given in Figures 2 and 3. The highest DPPH radical scavenging activity was found in the acetone and methanolic extracts of *Mentha pulegium* as 77% and 79%, respectively.

In this study, DPPH scavenging activity was found to be 77% and 79% for *Mentha pulegium* and 13% and 69% for *Lepidium draba* in acetone and methanol extracts respectively (Figure 2). In a study, ethanolic and aqueous extracts of both leaves and stems of *Lepidium draba* were obtained. The DPPH radical scavenging activity in the extract obtained from the leaf parts was found to be 86.85% and 92.48%, respectively. In the ethanolic extract obtained by using the stem parts, this value was found to be 90.61% by Younes et al., (2015). Eruygur et al., (2022) who examined the methanolic, ethanolic and aqueous extracts of the flower, stem, root and leaf parts of *Lepidium draba*, determined the DPPH radical scavenging activities as IC_{50} were found 1.57, 2.02 and 1.27 mg mL⁻¹ for the flower, 6.51, 9.04 and 11.14 mg mL⁻¹ for stem, 8.30, 6.14 and 2.39 mg mL⁻¹ for the root portion and 3.11, 3.12 and 5.27 mg mL⁻¹ for the leaves respectively. Ouissem et al. (2018) reported the DPPH radical scavenging activity as IC_{50} of 305.69 µg mL⁻¹ for the ethyl acetate extract of *Lepidium draba*. In a study by Gülçin et al., DPPH radical scavenging IC_{50} values of *Mentha pulegium* were found to be 16.92 µg/mL for methanolic extract and 18.52 µg mL⁻¹ for aqueous extract (Gülçin et al., 2020). Taştan et al., (2022) reported DPPH radical scavenging activities in methanolic extract of three different *Centaurea* species as IC_{50} of 55.82, 17.18 and 36.71 µg mL⁻¹, respectively. In a study by Alper et al., (2021) DPPH radical scavenging IC_{50} values of *Centaurea solstitialis* were found to be 55.04 mg trolox equivalents g⁻¹ for ethanolic extract.

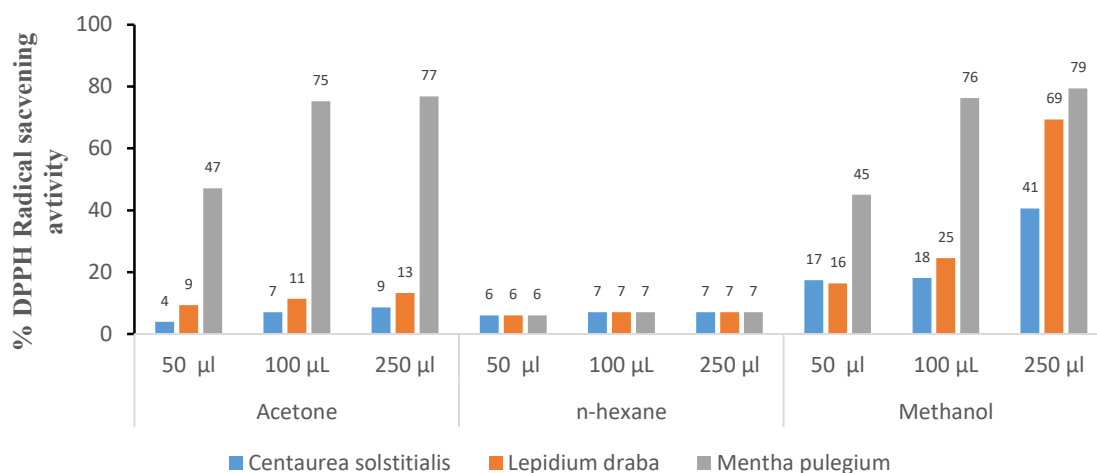


Figure 2. DPPH radical scavenging activity

In this study, ABTS scavenging activity was found to be 97%, 26% and 94% for *Centaurea solstitialis*, 98%, 76% and 94% for *Mentha pulegium* and 98%, 41% and 94% for *Lepidium draba* in acetone, n-hexane and methanol extracts respectively (Figure 3). Eruygur et al., (2022) who examined the methanolic, ethanolic and aqueous extracts of the flower, stem, root and leaf parts of *Lepidium draba*, determined the ABTS radical scavenging activities as IC_{50} were found 0.51, 0.45 and 0.30 mg mL⁻¹ for the flower, 0.62, 0.68 and 0.74 mg mL⁻¹ for stem, 0.07, 0.56 and 0.43 mg mL⁻¹ for the root portion and 0.48, 0.87 and 0.44 mg mL⁻¹ for the leaves respectively. In a study by Gülçin et al., (2020) ABTS radical scavenging IC_{50} values of *Mentha pulegium* were found as 7.92 µg mL⁻¹ for methanolic extract and 9.37 µg mL⁻¹ for aqueous extract. In a study by Taştan et al., (2022) ABTS radical scavenging IC_{50} values for three different *Centaurea* species were found as 50.13, 98.43 and 75.04 µg mL⁻¹ for methanolic extract. In a study by Ouissem et al., (2018) ABTS radical scavenging IC_{50} values for *Lepidium draba* was found 39.42 µg mL⁻¹ for ethyl acetate extract.

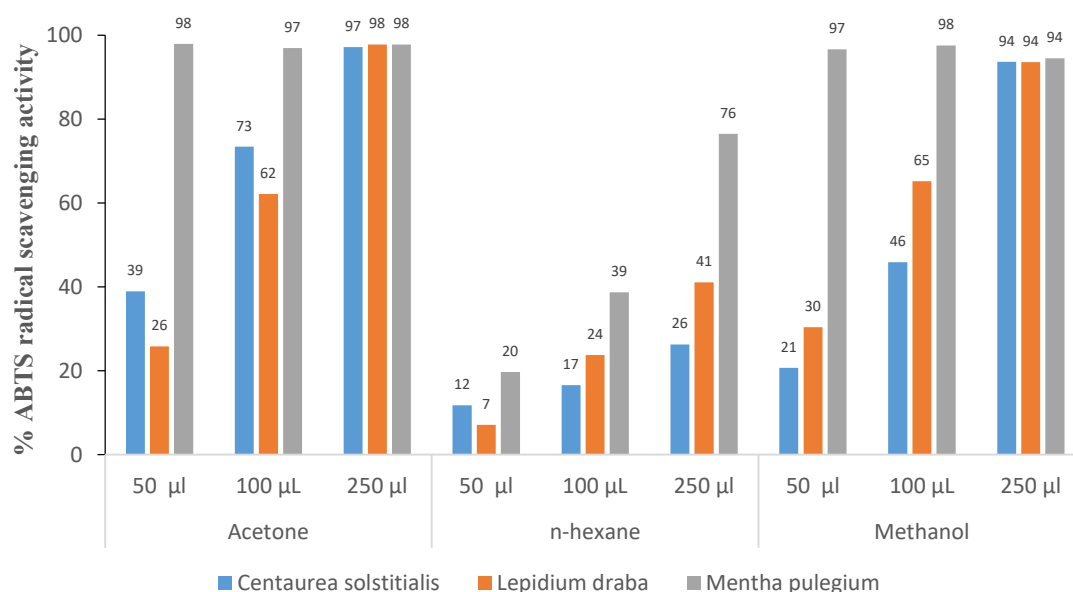


Figure 3. ABTS radical scavenging activity

Herein, LC-MS/MS analyses of acetone and methanol extracts for the quantification and identification of 53 phenolic compounds and three non-phenolic organic acids (ferulic acid-D3-IS, quercetin-D3-IS and rutin-D3-IS) were performed as outlined in the references (Yılmaz, 2020). Quercetin D3, rutin D3, and ferulic acid D3 were used to increase the reliability of the results. LC-MS/MS chromatograms of standard compounds are given in Figure 4.

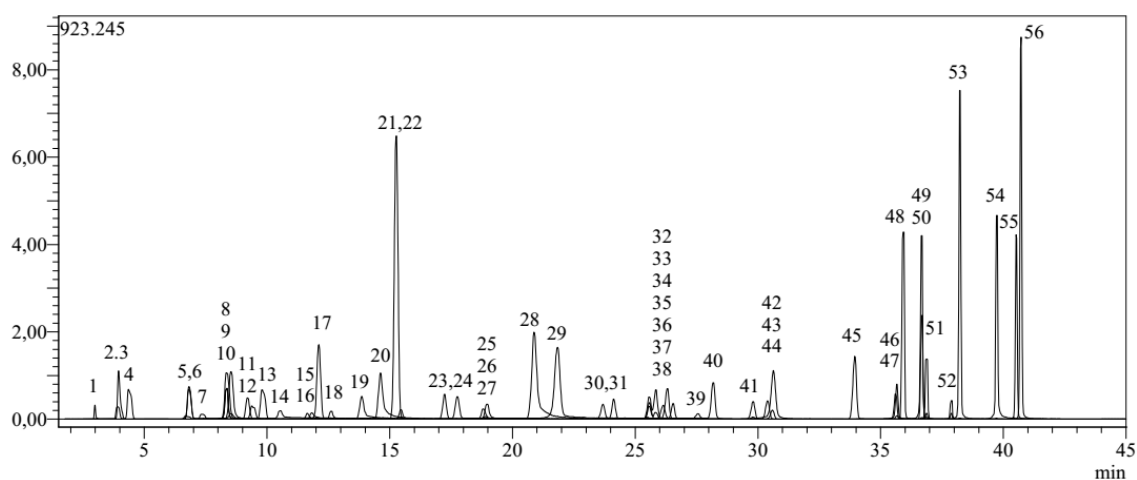


Figure 4. LC-MS/MS chromatograms standard mix

According to LC-MS/MS results, methanolic extract is very rich in phenolic components. The main phenolic components in the extract are quinic acid, protocatechic acid, chlorogenic acid, protocatechic aldehyde, 4-OH benzoic acid, caffeic acid, *p*-coumaric acid, cyanoside, rutin, isocercitrin, hesperidin, rosmarinic acid, cosmosin, astragaloside and acacetin. The mg amounts of these phenolics per gram were found to be 44.129, 0.491, 16.994, 0.377, 1.338, 1.591, 0.146, 0.805, 0.861, 0.795, 40.123, 780.383, 0.854, 0.800 and 3.031, respectively (Table 2).

In the acetone extract of *Centaurea solstitialis* quinic acid, protocatechic acid, chlorogenic acid, 4-OH benzoic acid, protocatechuic aldehyde, vanillin, caffeic acid, *p*-coumaric acid, coumarin, salicylic acid, apigenin, astragaloside, naringenin, luteolin, chrysin and acacetin components were found. The substance amounts of the phenolic components are given in Table 2 as mg/g. The highest phenolic quinic acid and 4-OH benzoic acid were found 0.265 mg g⁻¹ and 0.331 mg g⁻¹, respectively. In the

methanolic extract of *Centaurea solstitialis* quinic acid, fumaric acid, gallic acid, protocatechic acid, gentisic acid, chlorogenic acid, protocatechuic aldehyde, 4-OH benzoic acid, caffeic acid, vanillin, *p*-coumaric acid, ferulic acid, coumarin, salicylic acid, cyanoside, cosmosiin, astragalol, naringenin, luteolin, apigenin, chrysin and acacetin components were found. The substance amounts of the phenolic components are given in Table 2 as mg g^{-1} . The highest phenolics quinic acid, chlorogenic acid and 4-OH benzoic acid were found to be 44.029, 16.994 mg g^{-1} and 1.338 mg g^{-1} , respectively.

According to the LC-MS/MS results for *Lepidium draba*, the highest detected phenolic compounds for acetone solvent were found hesperidin and acacetin as 0.408 and 0.136 mg g^{-1} extract, respectively. The quinic acid, hesperidin and rosmarinic acid were found 14.438, 3.966 and 27.173 mg g^{-1} extract, respectively for methanol solvent. Other components detected and their amounts are given in Table 2.

According to the LC-MS/MS results for *Mentha pulegium*, the phenolic compounds with the highest amount were found hesperidin (5.863 mg g^{-1}), Rosmarinic acid (128.195 mg g^{-1}) and Acacetin (3.031 mg g^{-1}) for acetone solvent, *o*-Coumaric acid (40.123 mg g^{-1}) and Rosmarinic acid (780.383 mg g^{-1}) for the methanol solvent. Other components detected and their amounts are given in Table 2. Considering all the results it can be said that the highest phenolic substance of the methanolic extract of *Mentha pulegium*, contains rosmarinic acid (780.383 mg g^{-1}), hesperidin (40.123 mg g^{-1}), and the acetone extract of *Mentha pulegium* contains rosmarinic acid (128.195 mg g^{-1}). Considering both solvents and extracts together, the dominant phenolics are protocatechic acid (0.024 - 0.491 mg g^{-1}), protocatechin aldehyde (0.009 - 0.377 mg g^{-1}), caffeic acid (0.013 - 1.591 mg g^{-1}), astragalol (0.025 - 0.674 mg g^{-1}), naringenin (0.010 - 0.066 mg g^{-1}), chrysin (0.009 - 0.025 mg g^{-1}) and acacetin (0.030 - 3.031 mg g^{-1}).

In the study of Gülçin et al. (2020), the phenolic compounds in the methanolic extract of *Mentha pulegium* were found gallic acid, epicatechin, ascorbic acid, apigenin, caffeic acid, chlorogenic acid, ellagic acid, salvigenin, fumaric acid, pyrogallol, luteolin, isorhamnetin, quercetin, 3, 6-dimethylether, kaempferol, 3-O-rutinoside, and rutin (1.04, 8.45, 6.51, 8.89, 6.81, 27.42, 9.75, 5.97, 41.15, 2.04, 6.27, 4.22, 63.29, 209.02, and 15.49 mg kg^{-1} , respectively) have reported that. In the extraction of *Lepidium draba* with ethyl acetate by Ouissem et al., (2018) the phenolic contents of chlorogenic acid, caffeic acid, rutin, hesperidin, rosmarinic acid, 4-OH benzoic acid, *p*-coumaric acid and salicylic acid are as 2.50, 819.90, 10.80, 7.11, 4 040.00, 3 880.50, 0.90 and 24.20 mg kg^{-1} plants respectively. In our study, unlike the study of Ouissem et al., quinic acid, protocatechuic acid, chlorogenic acid, protocatechuic aldehyde, coumarin, cyanoside, isoquercitrin, hesperidin, cosmosiin, astragalol and naringenin were found 14,438, 0.063, 0.06, 0.02, 0.00.795, 0.055, 3.966, 0.028, 0.674 and 0.01 mg g^{-1} extract respectively.

In the study by Alper et al., (2021) phenolic components in the ethanolic extract of *Centaurea solstitialis* gallic acid, 4-hydroxybenzoic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid and naringin were found as 92.59, 2 077.33, 52.16, 24 078.00, 41.00, 127.21 and 120.62 $\mu\text{g g}^{-1}$ extract respectively. In our study, unlike the study of Alper et al., quinic acid, fumaric acid, protocatechuic acid, chlorogenic acid, protocatechuic aldehyde, vanillin, ferulic acid, coumarin, salicylic acid, cyanoside, cosmosiin, astragalol, luteolin, apigenin, chrysin and acacetin were found as 44.029, 0.267, 0.491, 16.994, 0.024, 0.048, 0.151, 0.05, 0.036, 0.805, 0.854, 0.8, 0.003, 0.157, 0.025 and 0.086 mg g^{-1} extract respectively.

In our study, the presence of phenolic components in acetone and methanol extracts increases antioxidant activity. Such studies in the literature have shown results that support us.

Table 2. Quantitative Results (mg analyte/g extract)

| | | Acetone | | | Methanol | | |
|----|---------------------------|-------------------------------|-----------------------|------------------------|-------------------------------|-----------------------|------------------------|
| | | <i>Centaurea solstitialis</i> | <i>Lepidium draba</i> | <i>Mentha pulegium</i> | <i>Centaurea solstitialis</i> | <i>Lepidium draba</i> | <i>Mentha pulegium</i> |
| 1 | Quinic acid | 0.265 | - | 0.157 | 44.029 | 14.438 | 4.785 |
| 2 | Fumaric acid | - | - | - | 0.267 | - | - |
| 3 | Aconitic acid | - | - | - | - | - | - |
| 4 | Gallic acid | - | - | - | 0.118 | - | 0.018 |
| 5 | Epigallocatechin | - | - | - | - | - | - |
| 6 | Protocatechuic acid | 0.058 | 0.024 | 0.343 | 0.491 | 0.063 | 0.315 |
| 7 | Catechin | - | - | - | - | - | - |
| 8 | Gentisic acid | - | - | - | 0.02 | - | - |
| 9 | Chlorogenic acid | 0.163 | - | 0.046 | 16.994 | 0.062 | 1.144 |
| 10 | Protocatechuic aldehyde | 0.009 | 0.01 | 0.377 | 0.024 | 0.022 | 0.205 |
| 11 | Tannic acid | - | - | - | - | - | - |
| 12 | Epigallocatechin gallate | - | - | - | - | - | - |
| 13 | 1,5-dicaffeoylquinic acid | - | - | - | - | - | - |
| 14 | 4-OH Benzoic acid | 0.331 | - | - | 1.338 | 0.219 | - |
| 15 | Epicatechin | - | - | - | - | - | - |
| 16 | Vanilic acid | - | - | - | - | - | - |
| 17 | Caffeic acid | 0.014 | 0.013 | 0.794 | 0.335 | 0.102 | 1.591 |
| 18 | Syringic acid | - | - | - | - | - | - |
| 19 | Vanillin | 0.035 | - | 0.033 | 0.048 | - | - |
| 20 | Syringic aldehyde | - | - | - | - | - | - |
| 21 | Daidzin | - | - | - | - | - | - |
| 22 | Epicatechin gallate | - | - | - | - | - | - |
| 23 | Piceid | - | - | - | - | - | 0.033 |
| 24 | <i>p</i> -Coumaric acid | 0.038 | - | 0.077 | 0.146 | 0.131 | 0.179 |
| 25 | Ferulic acid-D3-IS | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. |
| 26 | Ferulic acid | - | - | - | 0.151 | - | - |
| 27 | Sinapic acid | - | - | - | - | - | - |
| 28 | Coumarin | 0.05 | - | - | 0.044 | 0.019 | 0.031 |
| 29 | Salicylic acid | 0.007 | - | 0.009 | 0.036 | 0.009 | 0.016 |
| 30 | Cyanoside | - | - | 0.127 | 0.805 | 0.055 | 0.209 |
| 31 | Miquelianin | - | - | - | - | - | - |
| 32 | Rutin-D3-IS | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. |
| 33 | Rutin | - | - | 0.285 | - | 0.03 | 0.861 |
| 34 | isoquercitrin | - | 0.018 | 0.255 | - | 0.795 | 0.546 |
| 35 | Hesperidin | - | 0.408 | 5.863 | - | 3.966 | 40.123 |
| 36 | <i>O</i> -Coumaric acid | - | - | - | - | - | - |
| 37 | Genistin | - | - | - | - | - | - |
| 38 | Rosmarinic acid | - | 0.083 | 128.195 | - | 27.173 | 780.383 |
| 39 | Ellagic acid | - | - | - | - | - | - |
| 40 | Cosmosiin | - | 0.011 | 0.158 | 0.854 | 0.028 | 0.319 |
| 41 | Quercitrin | - | - | - | - | - | - |
| 42 | Astragalın | 0.025 | 0.03 | 0.033 | 0.8 | 0.674 | 0.052 |
| 43 | Nicotiflorin | - | - | - | - | - | - |
| 44 | Fisetin | - | - | - | - | - | - |
| 45 | Daidzein | - | - | - | - | - | - |
| 46 | Quercetin-D3-IS | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. |
| 47 | Quercetin | - | - | - | - | - | - |
| 48 | Naringenin | 0.018 | 0.01 | 0.066 | 0.018 | 0.008 | 0.019 |
| 49 | Hesperetin | - | 0.005 | 0.059 | - | 0.01 | 0.091 |
| 50 | Luteolin | 0.003 | - | 0.022 | 0.05 | - | 0.013 |
| 51 | Genistein | - | - | - | - | - | - |
| 52 | Kaempferol | - | - | - | - | - | - |
| 53 | Apigenin | 0.061 | 0.005 | 0.018 | 0.157 | - | 0.01 |
| 54 | Amentoflavone | - | - | - | - | - | - |
| 55 | Chrysin | 0.025 | 0.02 | 0.012 | 0.015 | 0.009 | 0.009 |
| 56 | Acacetin | 0.086 | 0.136 | 3.031 | 0.052 | 0.072 | 0.617 |

- : Not detected. N.A.: Not applicable and IS: Internal standard

CONCLUSION

Herein, extracts of the aerial parts of *Mentha pulegium*, *Lepidium draba* and *Centaurea solstitialis* plants collected from Şanlıurfa in three different solvents (acetone, n-hexane and methanol) were prepared. Total phenolic content analysis of these extracts, phenolic content analysis using LC-MS/MS technique and free radical scavenging activities were investigated. According to these results, the extract with the highest total phenolic content is the methanolic plant extract of *Centaurea Solstitialis* (99 507 mg GAE mL⁻¹). In the phenolic content analyzes using LC-MS/MS technique, rosmarinic acid (780 383 µg analyte g⁻¹) was found in the methanolic extract of *Mentha pulegium* the most. The highest DPPH radical scavenging activity was found in the methanolic extracts of *Mentha pulegium* as 79%. ABTS radical scavenging activity was found to be 97% for *Centaurea solstitialis*, 98% for *Mentha pulegium* and 98% for *Lepidium draba* in acetone extracts. The antioxidant capacity of the extracts is related to the total amount of phenolic substances. It has been determined that acetone and methanol extracts are effective in both DPPH and ABTS radical scavenging activity.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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