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Antioxidants, enzyme inhibitory activities, and phytochemical profiles of seven medicinal plants grown with organic farming techniques

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Abstract: In this study, the potential antihyperlipidemic, antidiabetic, and antiobesity effects of extracts obtained via infusion techniques from *Artemisia absinthium* L., *Achillea millefolium* L., *Calendula officinalis* L., *Fumaria officinalis* L., *Mentha piperita* L., *Origanum onites* L., and *Urtica dioica* L. grown with organic farming techniques were investigated using enzyme inhibition methods (α-glucosidase, pancreatic cholesterol esterase, pancreatic lipase, and α-amylase). It is important that natural compounds or extracts to be used in the treatment of metabolic problems have antioxidant effects. The antioxidant effects of these species were tested in terms of ABTS and DPPH radical scavenging activity, ferric reducing power, and metal chelating capacity. Extracts (2 mg/mL) of *M. piperita* (91.43 ± 0.90%) and *O. onites* (70.18 ± 2.02%) showed potent inhibitory effects on the α-glucosidase enzyme. Among all species, only *O. onites* extract exerted an inhibitory effect on the cholesterol esterase enzyme close to that of reference compound simvastatin. While all extracts were effective in the tested antioxidant activity methods, it was determined that the *M. piperita* and *O. onites* extracts displayed particularly significantly strong activities in terms of ferric reducing power, ABTS radical scavenging effect, and metal chelating capacity. The total flavonoid and phenolic contents of all extracts were determined. The compositions of the most active extracts were analyzed for phenolic acids and flavonoids using RP-HPLC. The results of RP-HPLC analysis showed that the levels of ellagic acid (0.923 ± 0.000 g/100 g extract) in the *M. piperita* extract and rosmarinic acid (0.813 ± 0.003 g/100 g extract) in the *O. onites* extract were high. As a result, all species grown with organic farming techniques except *U. dioica* were found to have high antioxidant effects. In light of these findings, it was concluded that *M. piperita* and *O. onites* grown with organic farming techniques showed strong inhibitory effects in terms of the tested activities, especially for α-glucosidase and pancreatic cholesterol esterase, and that in vivo studies and activity-guided isolation studies should be conducted on these species in the future.

Key words: Phytochemistry, RP-HPLC, biological activity, organic farming, *Origanum onites*, *Mentha piperita*

1. Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by high blood glucose levels that can result from insufficient insulin secretion or its ineffectiveness, or both. Chronic hyperglycemia can lead to severe damage and dysfunction in various organs and systems, especially the eyes, kidneys, nerves, and cardiovascular and vessel systems (American Diabetes Association, 2014). In preclinical and clinical studies, it has been reported that there is a close link among hyperglycemia, diabetic complications, and oxidative stress. Hyperglycemia may trigger extreme production of reactive oxygen species (ROS) mediated by the electron transport chain of mitochondria (Piconi et al., 2003), and it has been reported that the imbalance between ROS formation rate and antioxidant defense capacity causes DM complications (Ekin et al., 2019). Reducing carbohydrate absorption is one of the options in the treatment of diabetes. Inhibition of enzymes such as α-amylase enzymes and α-glucosidase that play a role in the digestion of carbohydrates causes the digestion of such consumed foods to be delayed, followed by the prolongation of carbohydrate digestion time, resulting in a decrease in the rate of glucose absorption and an increase in postprandial plasma glucose (Bhandri et al., 2008).

Obesity is a globally common metabolic disease characterized by excess adipose tissue and can cause numerous chronic diseases and premature death (Racette et al., 2003). It was reported that at least 2.8 million adults die each year from diseases caused by overweight or obesity (World Health Organization, 2014). Obesity is associated with a wide variety of metabolic problems, including DM and dyslipidemia (Racette et al., 2003). The discovery of drug molecules with digestive and absorption inhibitory effects is a significant target for the treatment of obesity

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(Foster-Schubert and Cummings, 2014). Inhibition of lipase, which is responsible for the digestion and absorption of triglycerides, has been identified as an important methodology for designing potent drugs for the treatment of obesity, as it will result in reduced lipid absorption (Bucholz et al., 2015; Kim et al., 2016). Another target enzyme in the fight against obesity is pancreatic cholesterol esterase. Inhibition of the functions of cholesterol esterase in the absorption of dietary cholesterol has been considered as a potential approach in the treatment of atherosclerosis and hypercholesterolemia (Wei et al., 2014).

In organic farming, chemical pesticides and genetically modified organisms are not used to increase crop yield. In organic farming practices, products are grown in completely natural conditions and the genetics of the product are not changed. Organic farming preserves biodiversity, ensures healthy crops, and prevents water, soil, and air pollution. For these reasons, it is important to popularize the use of organic farming techniques in the cultivation of aromatic and medicinal plants. In addition, the phytochemical contents and biological activities of organic agricultural plants should be investigated and their advantages or disadvantages compared to naturally collected and cultivated plants should be examined.

In the literature, it is stated that some of the plants whose activities were tested in the present study have traditional uses for the aforementioned ailments. For example, it was reported that *Artemisia* species have traditional uses in the treatment of hypertension, DM, and digestive problems in different parts of India (Hassan et al., 2018). It was stated that *Achillea millefolium*, another species belonging to the family Compositae, has traditionally been used in Iran in the treatment of various health problems including hypertension, DM, kidney stones, muscle pain, acne, and bleeding (Rezaei et al., 2020). *Urtica dioica* was reported to be widely used in traditional medicine for the treatment of various ailments including DM, hypertension, and prostate cancer (El Haouari and Rosado, 2019). It is known that *Fumaria officinalis*, a species of the family Papaveraceae, is traditionally used in the treatment of hypertension, DM, and hepatitis in Pakistan (Fatima et al., 2019). Various *Origanum* species of the family Lamiaceae, including *O. onites*, are also traditionally used in the treatment of DM in Anatolia (Ekin et al., 2019).

Natural resources have an important role in research on new drug molecules that are effective against DM, obesity, and lipid metabolism disorders. In this study, the enzyme inhibition (α-amylase, pancreatic lipase, α-glucosidase, and pancreatic cholesterol esterase enzymes) and antioxidant activities (metal chelating capacity, ferric reducing power, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) cation ($ABTS^{\bullet+}$), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity) of extracts obtained from seven medicinal plants from different families (Asteraceae, Urticaceae, Papaveraceae, and Lamiaceae), including *Artemisia absinthium* L., *Mentha piperita* L., *Calendula officinalis* L., *Fumaria officinalis* L., *Origanum onites* L., *Achillea millefolium* L., and *Urtica dioica* L., grown with organic farming techniques were investigated using the infusion method. Total phenolic contents (Folin–Ciocalteu method) and flavonoid contents (aluminum chloride method) of the extracts were measured. Considering the results of in vitro enzyme experiments, the phytochemical contents of the *M. piperita* and *O. onites* extracts, which had higher inhibition values than the other species, were elucidated using reverse phase-high performance liquid chromatography (RP-HPLC).

This study differs from other studies in the literature in that it evaluates the antioxidant capacity, inhibition of various enzymes, and phytochemical contents of seven different species from various families grown with organic farming.

2. Materials and methods

2.1. Plant material

A. absinthium, *C. officinalis*, *A. millefolium*, *U. dioica*, *F. officinalis*, *M. piperita*, and *O. onites* grown with organic farming methods were obtained in April 2021 from Temmuz Organik Çiftliği in Konya, Türkiye (Beyşehir Road, 3 km Akyokuş Mevki; Certificate No. TR-OT-01-4-İ-197/01). These species were grown in accordance with the Organic Agriculture Law and Regulation of the Turkish Republic and were certified by Nissert (Ankara, Türkiye) as authorized by the Ministry of Agriculture.

2.2. Extraction

For dry plant samples, powdered aerial parts were extracted with hot water. The extracts were then filtered and the process was repeated 3 times. After the obtained filtrates were combined, they were frozen in a deep freezer at –20 °C and dried in a freeze-dryer.

2.3. Chemicals

In RP-HPLC analysis, the solvents used in the extraction procedure and the chemicals used in enzyme and antioxidant activity assays were of high purity and all of these solvents and chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.4. Total phenolic contents

Extracts prepared by the infusion method were incubated with Folin–Ciocalteu reagent at 25 °C for 5 min. Sodium carbonate solution was added to the mixture. The extracts were reincubated at room temperature. The absorbance of the extracts was measured at a wavelength of 735 nm with an ELISA microplate reader (SpectraMax i3x, Molecular Devices, San Jose, CA, USA USA). Phenolic contents of the extracts were calculated as gallic acid equivalent

(GAE) mg/g extract. In this method, a calibration curve was created by using five different concentration values of 0.01, 0.05, 0.25, 0.5, and 1 mg/mL. The study was carried out in triplicate repetitions. The calibration equation was as follows: $y = 3.7855x + 0.1735$, with $r^2 = 0.9931$ (Zongo et al., 2010).

2.5. Total flavonoid contents

Ethanol and then aluminum chloride and sodium acetate solutions were added to the extracts prepared by the infusion technique, and the mixtures were diluted. The absorbances of the mixtures were measured at 415 nm. Results were expressed as quercetin equivalent (QE) mg/g extract. In this method, a calibration curve was created by using five different concentration values of 0.01, 0.05, 0.25, 0.5, and 1 mg/mL. The study was carried out in triplicate repetitions. The calibration equation was as follows: $y = 2.8193x - 0.0996$, with $r^2 = 0.9977$ (Kosalec et al., 2004).

2.6. Antioxidant activity

2.6.1. ABTS radical scavenging activity

ABTS radical was dissolved in distilled water and potassium persulfate solution. This mixture was incubated at 20 °C in the dark. At the beginning of the experiment, ABTS and phosphate buffer solutions were added to the extracts. After vortexing, the absorbance of the mixture was measured at 734 nm. The ABTS radical scavenging activity of the extracts was calculated as follows: (Inhibition %) = $[(Absorbance_{control} - Absorbance_{sample}) / Absorbance_{control}] \times 100$ (Re et al., 1999).

2.6.2. DPPH radical scavenging effect

DPPH solution of 1 mM was added to extracts prepared in appropriate dilutions. The absorbances of the reference compound and extracts were then measured. The DPPH radical scavenging activity of the extracts was calculated as follows: (Inhibition %) = $[(Absorbance_{control} - Absorbance_{sample}) / Absorbance_{control}] \times 100$. Experiments were repeated three times for each sample (Blois, 1958).

2.6.3. Metal chelating capacity

First, $FeCl_2$ solution was added to the extracts and incubated. After this process, after adding ferrozine to the relevant wells, the mixtures were left to incubate. The absorbance values of the extracts were then measured. The metal chelating capacity (%) of the extracts was calculated as follows: $[(Absorbance_{control} - Absorbance_{sample}) / Absorbance_{control}] \times 100$. Experiments were repeated three times for each sample (Dinis et al., 1999).

2.6.4. Ferric reducing power

Extracts and reference compounds were mixed with sodium phosphate buffer solution. $K_3Fe(CN)_6$ solution (1% w/v) was added to the mixture and incubated for 1 h in an incubator at 37 °C. After the incubation process, trichloroacetic acid was added to the mixtures and the absorbance values of these mixtures were calculated. After the first measurement, $FeCl_3$ solution was added to the same samples and the absorbances were calculated again, allowing the difference between absorbance measurements to be determined. Experiments were repeated three times for each sample (Oyaizu, 1986).

2.7. Enzyme inhibition activity

2.7.1. α-Glucosidase enzyme inhibitory activity

The *α*-glucosidase enzyme (4 U/mL) was dissolved in phosphate buffer solution. The extracts and buffer solution were preincubated and then p-nitrophenyl-α-D-glucopyranoside solution (PNG) was added to the mixture in the wells. In microplates, absorbance was measured at 405 nm with the help of an ELISA microplate reader (Orhan et al., 2017).

2.7.2. α-Amylase enzyme inhibitory activity

The α-amylase (0.25 U/mL) and potato starch solution used as a substrate were prepared in phosphate buffer solution. Extracts prepared at different concentrations were mixed with the enzyme solution and incubated and then the substrate solution was added. After this incubation period, 3,5-dinitrosalicylic acid (DNS) color reagent solution was added to the mixtures and the microplates were incubated. The absorbances of the mixtures were measured at 540 nm. In addition, a standard calibration curve for maltose was constructed as follows: $y = 0.6762x - 0.0404$, with $r^2 = 0.9966$. Experiments were repeated three times for each sample (Orhan et al., 2017).

2.7.3. Pancreatic lipase enzyme inhibitory activity

The lipase enzyme used in the experiment was dissolved in 4-morpholino propane sulfonic acid and 1 mM EDTA buffer solution. The enzyme solution and extracts were preincubated in Tris buffer. After this process, 4-nitrophenyl butyrate was added and incubated. The increase in absorption as a result of hydrolysis of 4-nitrophenylbutyrate by pancreatic lipase was measured. Experiments were repeated three times for each sample (Lee et al., 2012).

2.7.4. Pancreatic cholesterol esterase enzyme inhibitory activity

The porcine pancreatic cholesterol enzyme used in the experiment was dissolved in buffer solution containing NaCl. The prepared extracts were added to 50 µL of phosphate buffer solution. After the addition of taurocholic acid (12 mM) and 5 mM 4-nitrophenylbutyrate, the mixture was left to incubate. After incubation, porcine pancreatic cholesterol esterase enzyme was added to the mixture and kinetic measurements was performed (Ngamukote et al., 2011).

2.8. RP-HPLC analysis of extracts

An HP Agilent 1260 Series LC System and ACE 5 C18 column were used for RP-HPLC analysis (Agilent Technologies, Santa Clara, CA, USA). The device was also equipped with

an HP Agilent 1260 Series autosampler unit. The column temperature was kept constant at 25 °C throughout the analysis. For the quantitative and qualitative analysis of the flavonoids and phenolic acids in the extract, all standard compounds were provided by Sigma-Aldrich. Rates of 5% solvent A (acetonitrile:water:formic acid) and 95% solvent (water:formic acid) were used as mobile phases. Total analysis time was 58 min. Absorbance measurements were carried out at four different wavelengths (260, 280, 320, and 350 nm) with a DAD detector. Dried extracts were dissolved in 25% (v/v) acetonitrile solution. Calibration curves were plotted for ellagic acid, rosmarinic acid, and luteolin (Figures 1–3). These three standard compounds were prepared with a 25% acetonitrile and water mixture at five different concentrations. A calibration curve from the peak areas (y) against ppm values (x) and the amount of compounds in the extracts were calculated (Table 1) (Gök et al., 2021).

2.9. Statistical analysis

Experiments were performed with three replications. The averages of the numerical values were calculated and reported as mean ± standard deviation (SD) in tables. The GraphPad InStat and Microsoft Excel software programs were used in the calculations and differences of $p < 0.05$ in the analysis were evaluated as statistically significant (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

3. Results and discussion

It was determined that *M. piperita* extract had both the highest extraction yield (30.09%, w/w) and the highest total phenolic contents (269.00 ± 9.09 mg GAE/g extract) among the extracts prepared from seven medicinal plants grown with organic farming techniques. On the other hand, the *A. millefolium* extract was found to have the highest total flavonoid contents at 103.43 ± 6.02 mg QE/g extract (Table 2).

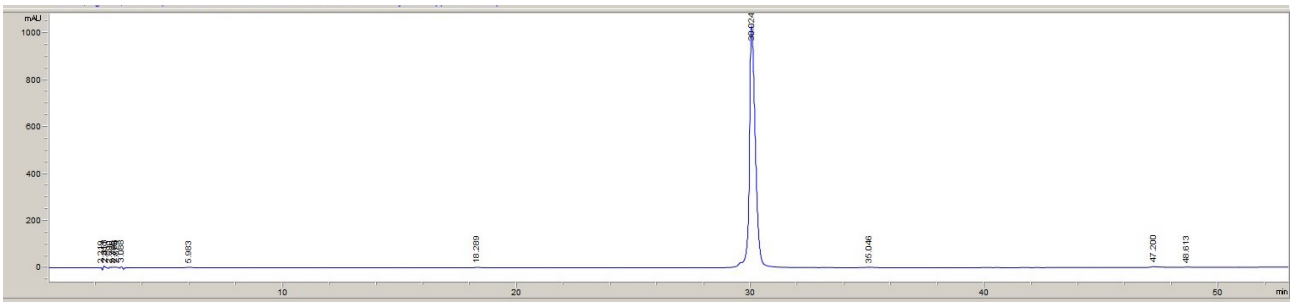

Figure 1. RP-HPLC chromatogram of ellagic acid.

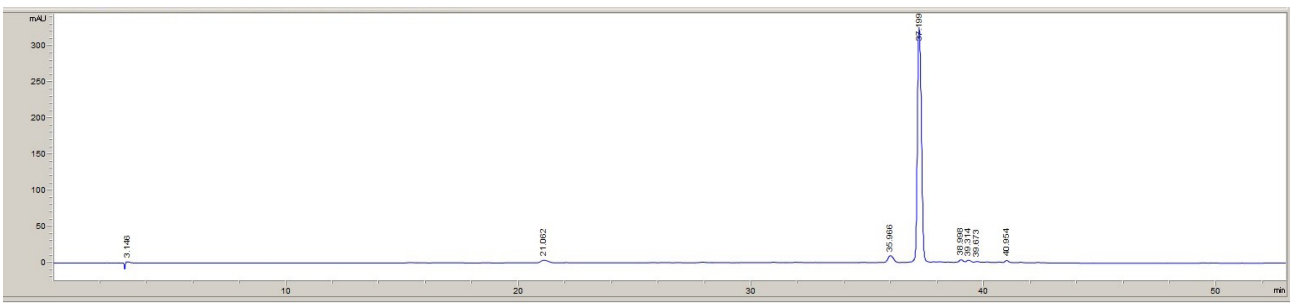

Figure 2. RP-HPLC chromatogram of rosmarinic acid.

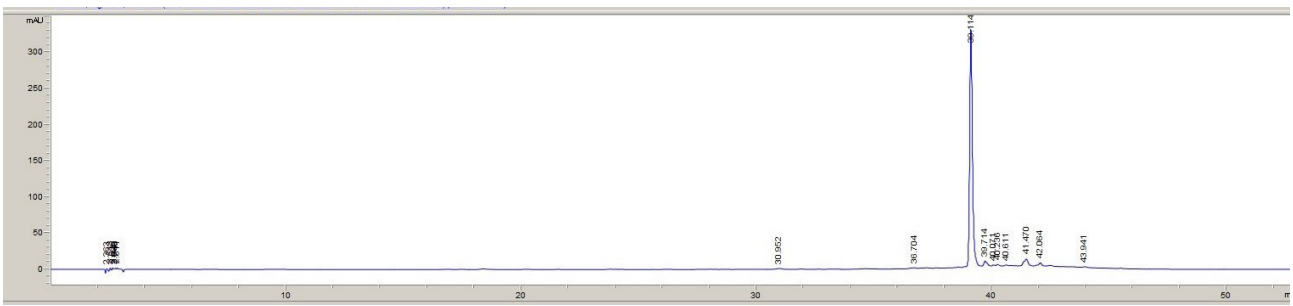

Figure 3. RP-HPLC chromatogram of luteolin.

Table 1. Retention times (Rt), standard curve, and r^2 values of phenolic compounds.

Compounds	Rt (min)	Standard curve	r^2
Ellagic acid	29.60	y = 18.78x – 556.59	0.9984
Rosmarinic acid	37.15	y = 39.255x + 0.2767	1.0000
Luteolin	39.09	y = 27.688x – 42.983	0.9993

Table 2. Results of the yields (w/w) and total phenolic and total flavonoid contents of the extracts.

Extracts	Yield (w/w)	Total phenolic contents, mg GAE/g extract ± SD	Total flavonoid contents, mg QE/g extract ± SD
A. absinthium	25.97	85.85 ± 3.55	40.77 ± 6.96
C. officinalis	25.60	24.17 ± 9.29	56.73 ± 5.50
A. millefolium	18.26	127.59 ± 6.54	103.43 ± 6.02
U. dioica	19.60	15.45 ± 3.36	37.57 ± 4.11
F. officinalis	18.41	48.21 ± 7.19	53.18 ± 1.95
M. piperita	30.09	269.00 ± 9.09	72.57 ± 1.00
O. onites	5.62	216.92 ± 9.54	60.75 ± 1.60

Four different methods were used to estimate the antioxidant activities of the extracts. In the method with which ABTS radical scavenging activity was evaluated, the *A. absinthium* extract showed the strongest effect among all extracts with an inhibition value of 99.92 ± 0.13% at a concentration of 2 mg/mL, and this inhibition value was found to be higher than that of gallic acid (98.10 ± 0.80%). Findings of DPPH activity revealed that the percent inhibition values of the extracts were inversely related to their concentrations. It was determined that the most potent inhibitor of DPPH radicals was *A. millefolium* extract (83.95 ± 0.97%) at a concentration of 1 mg/mL. Reference compound ascorbic acid inhibited DPPH radicals at the same concentration with a value of 90.72 ± 0.63%. Interestingly, the *U. dioica* extract showed almost no DPPH activity at all studied concentrations. The metal chelating capacity of the extracts increased in a concentration-dependent manner and most of the extracts had 100.00 ± 0.00% inhibition values at a concentration of 2 mg/mL. These inhibition values were higher than that of reference compound EDTA for all concentrations. In the ferric reducing power assay, *O. onites* (3.475 ± 0.010) and *M. piperita* (3.418 ± 0.020) extracts displayed particularly significant activities, found to be as strong as quercetin (3.540 ± 0.020). It was determined that the ferric reducing powers of *C. officinalis*, *U. dioica*, and F. *officinalis* extracts were extremely weak compared to the other tested extracts (Table 3).

Four different enzymes (α-amylase, pancreatic cholesterol esterase, α-glucosidase, and pancreatic lipase) were chosen to test the enzyme-inhibiting effects of the extracts prepared by the infusion technique. The *M. piperita* (91.43 ± 0.90%) and *O. onites* (70.18 ± 2.02%) extracts exerted strong inhibitory activities against the *α*-glucosidase enzyme at a concentration of 2 mg/mL. Similarly, while most of the extracts tested in the *α*-amylase enzyme inhibition method, where their potentials for antidiabetic effects were estimated, did not display any inhibitory activity, the *O. onites* extract (24.90 ± 1.40%) displayed the highest inhibitory activity at 1 mg/mL concentration. Except for *O. onites* (26.16 ± 6.74% at 1 mg/mL), no extracts exerted inhibitory effects against the pancreatic lipase enzyme, while reference compound simvastatin had an inhibition rate of 69.54 ± 4.19% at a concentration of 1 mg/mL. The *O. onites* extract (46.43 ± 1.01%) exerted a potent inhibitory effect against the cholesterol esterase enzyme comparable to that of reference compound simvastatin (53.18 ± 3.36%) (Table 4).

According to the enzyme inhibitory activity results, it was concluded that the most effective extracts were those of *M. piperita* and *O. onites*, and the phytochemical contents of these particular extracts were elucidated using RP-HPLC. Quantitative and qualitative analysis of the extracts was conducted for some phenolic acids and flavonoids. Based on the RP-HPLC analysis, the *M. piperita* extract contained ellagic acid (0.923 ± 0.000 g/100 g dry extract) and rosmarinic acid (Figures 4 and 5), while the *O. onites* extract contained both of those compounds and luteolin (0.185 ± 0.001 g/100 g dry extract) (Figures 6–8). The quantitative analysis findings showed that the *M.*

Table 3. ABTS and DPPH radical scavenging activity, metal chelating capacity, and ferric reducing power assay results of the extracts.

Extract	Concentration (mg/mL)	Antioxidant activity assays			
		ABTS radical scavenging effect inhibition, % ± SD	DPPH radical scavenging activity inhibition, % ± SD	Metal chelating capacity inhibition, % ± SD	Ferric reducing power, absorbance ± SD
A. absinthium	0.5	20.21 ± 2.10**	80.28 ± 1.02***	64.37 ± 4.33***	0.839 ± 0.030**
	1	31.91 ± 3.30***	74.20 ± 1.36***	64.93 ± 9.66***	1.489 ± 0.010***
	2	99.92 ± 0.13***	61.08 ± 10.50***	100.00 ± 0.00***	2.825 ± 0.110***
C. officinalis	0.5	14.56 ± 3.23*	78.57 ± 1.23***	64.10 ± 1.03***	0.486 ± 0.030**
	1	34.69 ± 0.91**	79.39 ± 1.00***	70.35 ± 1.54***	0.864 ± 0.030**
	2	34.09 ± 0.79**	78.28 ± 9.47***	100.00±0.00***	1.652 ± 0.050***
A. millefolium	0.5	18.85 ± 0.86**	82.42 ± 1.75***	23.78 ± 1.07**	0.934 ± 0.050***
	1	38.84 ± 1.83**	83.95 ± 0.97***	37.87 ± 2.38**	1.722 ± 0.000***
	2	68.40 ± 0.94***	69.31 ± 9.76**	55.10 ± 1.65***	2.902 ± 0.140***
U. dioica	0.5	13.5 ± 1.20***	4.74 ± 2.22***	16.49 ± 5.40*	0.345 ± 0.040*
	1	20.66 ± 0.57**	-	88.82 ± 2.10***	0.700 ± 0.080**
	2	35.97 ± 1.77**	-	100.00 ± 0.00***	1.445 ± 0.090***
F. officinalis	0.5	22.47 ± 1.88**	78.06 ± 1.77***	17.23 ± 1.86**	0.523 ± 0.020***
	1	28.51 ± 0.23**	77.85 ± 0.90***	68.23 ± 6.75***	1.000 ± 0.010***
	2	48.27 ± 0.91***	57.43 ± 2.63***	92.92 ± 7.70***	1.866 ± 0.060***
M. piperita	0.5	41.93 ± 0.57***	77.42 ± 0.85***	27.17 ± 5.08*	1.660 ± 0.140***
	1	73.53 ± 1.18***	74.31 ± 1.34***	69.47 ± 4.63***	2.851 ± 0.100***
	2	99.25 ± 0.47***	55.70 ± 3.44***	100.00 ± 0.00***	3.418 ± 0.020***
O. onites	0.5	33.48 ± 1.92**	83.23 ± 0.80***	-	1.598 ± 0.110***
	1	57.32 ± 0.35***	80.92 ± 0.38***	78.20 ± 6.51***	2.754 ± 0.060***
	2	93.74 ± 3.00***	65.39 ± 8.96***	100.00 ± 0.00***	3.475 ± 0.010***
References	GA/AA/ EDTA/QE 0.5	99.55 ± 1.05^{a***}	89.43 ± 3.10^{b***}	99.85 ± 0.39^{c***}	3.251 ± 0.390^{d***}
	GA/AA/ EDTA/QE 1	98.97 ± 0.26^{a***}	90.72 ± 0.63^{b***}	99.80 ± 0.23^{c***}	3.565 ± 0.020^{d***}
	GA/AA/ EDTA/QE 2	98.08 ± 0.78^{a***}	90.35 ± 0.88^{b***}	95.75 ± 0.61^{c***}	3.540 ± 0.020^{d***}

-: No activity. SD: Standard deviation. *: $p < 0.05$. **: $p < 0.01$. ***: $p < 0.001$. GA: ^{a}Gallic acid. AA: ^{b}Ascorbic acid.
^{c}EDTA: Ethylenediaminetetraacetic acid.

piperita extract contained a high amount of ellagic acid, while the *O. onites* extract was richer in rosmarinic acid (0.813 ± 0.003 g/100 g dry extract) (Table 5).

Mahmoudi et al. (2009) determined the total flavonoid and phenolic contents of the methanol extract of the aerial parts of *A. absinthium* collected from Iran and likewise evaluated the antioxidant capacity of the extract by DPPH activity, metal chelating capacity, and ferric reducing power methods. The total phenolic content of the extract was found to be 194.9 ± 9.7 mg GAE/g extract and the total flavonoid content was 12.4 ± 0.6 mg QE/g extract. The IC_{50} values of the extract and ascorbic acid for DPPH activity were calculated as 612.00 ± 30.60 mg/mL and 1.26 ± 0.11 mg/mL, respectively. It was reported that the extract had ferric reducing power at concentrations ranging from 50 to 800 µg/mL, and the IC_{50} value for its metal chelating capacity was 419.00 ± 20.95 µg/mL.

Ercetin et al. (2012) examined the total flavonoid and phenolic contents and antioxidant potentials of the aqueous extracts of leaves and flowers of *C. officinalis* grown in Bartın, Türkiye, and found that the total flavonoid and total phenolic amounts of the leaf extract were higher

Table 4. Effects of the extracts on **α**-glucosidase, α-amylase, pancreatic lipase, and pancreatic cholesterol esterase enzymes.

Extract	Concentration (mg/mL)	Inhibition % ± SD			
		α-Glucosidase	α-Amylase	Pancreatic lipase	Pancreatic cholesterol esterase
A. absinthium	0.5	1.91 ± 0.03^{ns}	-	-	22.50 ± 7.29***
	1	7.59 ± 1.13*	-	-	23.79 ± 4.56***
	2	17.20 ± 3.04*	-	-	30.88 ± 1.65***
C. officinalis	0.5	5.81 ± 2.08*	-	-	15.19 ± 0.73***
	1	12.35 ± 1.15**	9.13 ± 2.87*	-	32.48 ± 1.46***
	2	3.17 ± 2.05^{ns}	-	-	24.97 ± 4.52***
A. millefolium	0.5	9.36 ± 5.38*	16.35 ± 0.52**	-	16.74 ± 3.12***
	1	15.90 ± 1.63**	-	-	15.87 ± 1.60**
	2	22.50 ± 1.97**	12.34 ± 5.36**	-	23.71 ± 1.83***
U. dioica	0.5	10.32 ± 2.78**	22.33 ± 1.28***	-	18.02 ± 2.84***
	1	10.29 ± 1.29**	12.47 ± 2.90**	-	18.52 ± 0.09***
	2	8.93 ± 1.29**	12.07 ± 3.72**	-	12.94 ± 2.28*
F. officinalis	0.5	6.29 ± 2.69**	15.33 ± 2.18**	-	18.87 ± 0.78***
	1	10.81 ± 0.13**	14.16 ± 2.27*	-	20.83 ± 1.39***
	2	12.14 ± 0.30**	16.16 ± 1.51**	-	23.50 ± 2.22***
M. piperita	0.5	47.56 ± 5.01***	-	-	26.34 ± 1.95***
	1	76.32 ± 2.62***	-	-	22.53 ± 1.93***
	2	91.43 ± 0.90***	-	-	17.36 ± 3.03***
O. onites	0.5	25.45 ± 4.26***	18.53 ± 3.39**	-	34.19 ± 0.42***
	1	46.86 ± 4.90***	24.90 ± 1.40***	26.16 ± 6.74***	36.43 ± 3.40***
	2	70.18 ± 2.02***	21.88 ± 4.65***	10.54 ± 4.40**	46.43 ± 1.01***
References	ACA/OR/SIM 0.5	98.88 ± 0.21^{a***}	94.85 ± 0.60^{a***}	52.16 ± 0.00^{b***}	47.88 ± 5.11^{c***}
	ACA/OR/SIM 1	99.47 ± 0.13^{a***}	98.38 ± 0.50^{a***}	69.54 ± 4.19^{b***}	52.21 ± 0.12^{c***}
	ACA/OR/SIM 2	99.35 ± 0.22^{a***}	95.24 ± 2.60^{a***}	62.55 ± 1.76^{b***}	53.18 ± 3.36^{c***}

-: No activity. SD: Standard deviation. ^{ns}: Not statistically significant. *: $p < 0.05$. **: $p < 0.01$. ***: $p < 0.001$. ^{a}ACA: Acarbose. ^{b}OR: Orlistat. ^{c}SIM: Simvastatin.

than those of the flower extract. In the ferric reducing power test, it was found that the absorbance value for the leaf extract at a concentration of 1 mg/mL was 0.286 ± 0.01, while it was 0.086 ± 0.01 for the flower extract at the same concentration. It was determined that both extracts had almost no DPPH activity. When the metal chelating capacity of the aqueous extracts was evaluated, the leaf extract showed the highest activity at a concentration of 1 mg/mL with an inhibition rate of 39.80 ± 2.30%, and this value was calculated as 76.71 ± 1.25% for reference compound EDTA at the same concentration.

Keser et al. (2013) determined the total flavonoid and phenolic contents of the aqueous and ethanolic extracts of leaves, flowers, and seeds of *A. millefolium* grown in Muş Province, Türkiye. It was found that the leaf aqueous extract (20.25 mg pyrocatechol equivalent/g extract and 1797.00 µg naringin equivalent/g extract, respectively) had the highest total flavonoid and phenolic contents.

Pourmorad et al. (2006) calculated the total flavonoid and total phenolic contents of methanol extracts obtained from all parts of various plants growing in Iran, including *U. dioica*, and evaluated the antioxidant potentials of the extracts by DPPH radical scavenging method. The total flavonoid and phenolic contents of the extracts were calculated as 24.10 ± 1.00 mg GAE/g extract and 43.30 ± 0.37 mg QE/g extract, respectively. At a concentration of 4 mg/mL, the DPPH radical scavenging activity of the extract was 70.8 ± 1.0%, while quercetin at a concentration of 0.025 mg/mL caused 95.60 ± 0.40% inhibition.

Edziri et al. (2020) tested the antioxidant potential of a methanolic extract prepared from aerial parts of *F. officinalis* collected from Tunisia using ABTS and DPPH

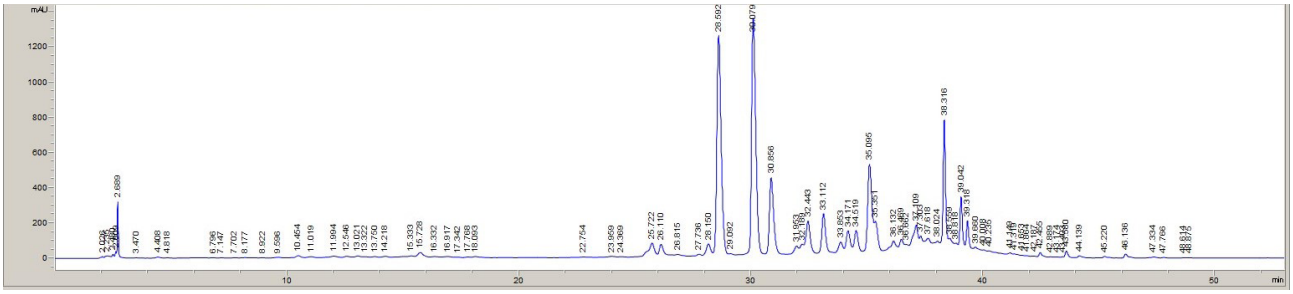

Figure 4. RP-HPLC chromatogram of *M. piperita* extract at 260 nm.

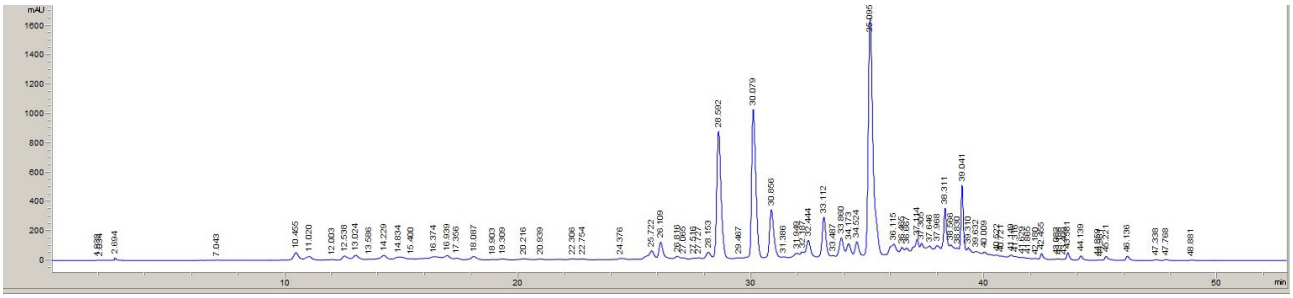

Figure 5. RP-HPLC chromatogram of *M. piperita* extract 320 nm.

Table 5. Ellagic acid, rosmarinic acid, and luteolin contents of *M. piperita* and *O. onites* extracts.

Samples	Phenolic compounds (g/100 g dry extract)		
	Ellagic acid	Rosmarinic acid	Luteolin
M. piperita	0.923 ± 0.000	0.214 ± 0.001	-
O. onites	0.206 ± 0.002	0.813 ± 0.003	0.185 ± 0.001

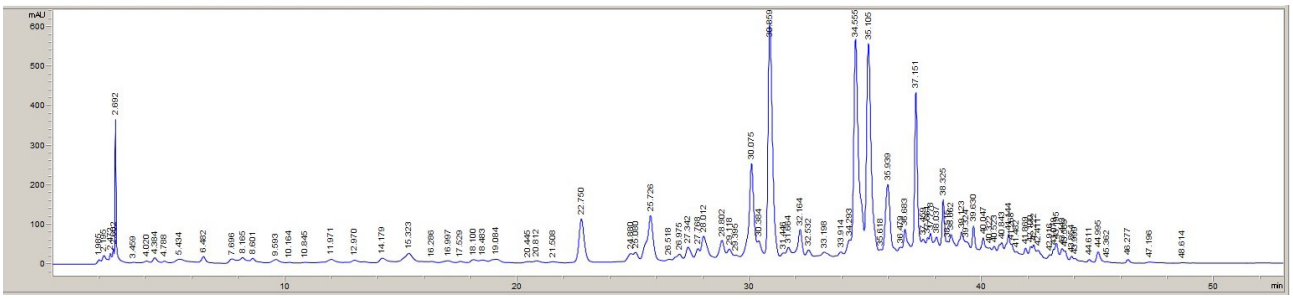

Figure 6. RP-HPLC chromatogram of *O. onites* extract at 260 nm.

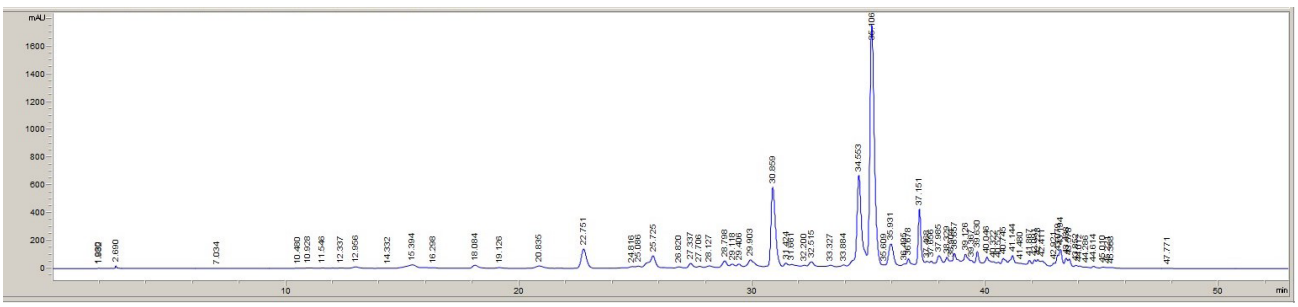

Figure 7. RP-HPLC chromatogram of *O. onites* extract at 320 nm.

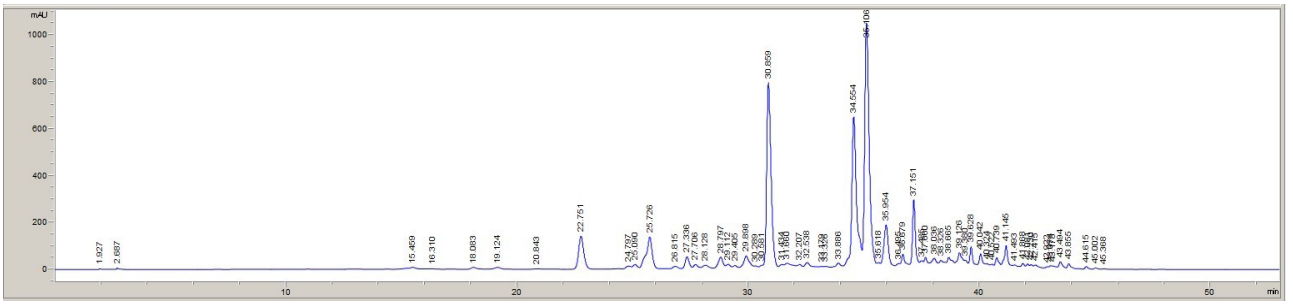

Figure 8. RP-HPLC chromatogram of *O. onites* extract at 350 nm.

radical scavenging methods. The extract scavenged DPPH radicals with an IC_{50} value of 2.5 ± 0.57 µg/mL and ABTS radicals with an IC_{50} value of 7.39 ± 0.12 µg/mL. The total phenolic content of the methanol extract was calculated as 23.87 ± 1.02 mg GAE/g extract and the total flavonoid content was 46.54 ± 3.12 mg QE/g extract.

Farnad et al. (2014) prepared extracts from the leaves of *M. piperita* collected from Azerbaijan with a mixture of methanol and ethanol (1:1) and methanol alone and determined that the methanol extract had the highest total phenolic content (3.57 ± 0.26 g GAE/g extract) while the methanol:ethanol extract had the highest total flavonoid content (3.33 ± 0.12 g QE/g extract). The extract in which methanol and ethanol were equally used had the highest DPPH activity with an inhibition rate of 82.82 ± 2.57% at a concentration of 20 mg/mL. Gallic acid showed inhibitory activity of 93.00 ± 0.45% at the same concentration. It was determined that the highest ferric reducing power (184.22 ± 14.10 µmol/100 g) was exerted by the extract in which methanol and ethanol were used in equal proportions.

Mahomoodally et al. (2019) evaluated both the antioxidant potential and the total flavonoid and phenolic contents of aqueous and methanol extracts obtained from the aerial parts of *O. onites* collected from Muğla, Türkiye. While the aqueous extract had the highest total phenolic content at 111.41 ± 1.83 mg GAE/g extract, the methanol extract had the highest total flavonoid content at 37.46 ± 0.79 mg QE/g extract. In that study, antioxidant potential, ABTS and DPPH activity, metal chelating capacity, and ferric reducing power potential were also examined, and the activity of the aqueous extract was found to be more potent than that of the methanol extract for all methods. Ekin et al. (2019) assessed the total phenolic content of 80% ethanol extracts obtained from aerial parts of *O. onites* samples collected from Isparta, Türkiye, and concluded that the total phenolic content was 112.37 ± 8.02 mg GAE/g extract, which is lower than the value obtained in our study for *O. onites* grown by organic agriculture (216.92 ± 9.54 mg GAE/g extract). Thus, based on the findings of previous studies, it can be predicted that the increased total phenolic content obtained in *O. onites* in this study may have been caused by organic farming techniques.

Ramirez et al. (2012) evaluated the inhibitory effects of 60% ethanolic extracts of some plants used in the treatment of DM in Mexico on lipase and α-glucosidase enzymes and reported that *A. absinthium* extract at a concentration of 1 mg/mL inhibited α-glucosidase by 67.70 ± 3.70%, while the extract at a concentration of 0.25 mg/mL inhibited the lipase enzyme by 25.20 ± 2.14%. Zengin et al. (2017b) evaluated the α-amylase and α-glucosidase enzyme inhibitory activities of an ethanol extract prepared from *A. absinthium* samples bought in a market in Konya, Türkiye, and the α-glucosidase inhibition of the extract was calculated as 0.40 ± 0.06 mmol acarbose equivalent/g extract while the α-amylase inhibition of the extract was calculated as 1.41 ± 0.02 mmol acarbose equivalent/g extract.

Nickavar and Yousefian (2011) examined the α-amylase activity of extracts prepared with 70% ethanol from *C. officinalis* samples collected from different regions and purchased from markets in Iran. Their findings showed that the extracts exerted weak inhibition (11.13 ± 0.31%) at 2.304 mg/mL. Hernández-Saavedra et al. (2016) examined the pancreatic lipase activity of an extract prepared by the infusion technique from *C. officinalis* samples purchased from a local market and concluded that the IC_{50} value of the *C. officinalis* infusion against the pancreatic lipase enzyme was ~15.0 mg/mL. Olennikov et al. (2017) evaluated the antidiabetic activity of compounds isolated from the n-butanol fraction of *C. officinalis*. The isolated haploperoside A showed the highest inhibitory activity against the α-glucosidase enzyme with an IC_{50} value of 85.06 ± 2.38 µM. On the other hand, neoisobaisseoside displayed the highest inhibitory activity against the α-amylase enzyme with an IC_{50} value of 92.51 ± 2.59 µM.

Ramirez et al. (2012) examined the inhibitory effects of 60% ethanolic extracts prepared from various parts of some plants used in the traditional treatment of DM in Mexico against lipase and α-glucosidase enzymes. They reported that the *A. millefolium* extract inhibited α-glucosidase by 52.30 ± 4.10% (concentration of 1 mg/mL) and the same extract inhibited lipase by 11.50 ± 2.67% (concentration of 0.25 mg/mL). Zengin et al. (2017a) estimated the

α-amylase and α-glucosidase inhibitory activities of ethyl acetate, aqueous, and methanol extracts of the aerial parts of *A. millefolium* collected from Afyon, Türkiye. The *A. millefolium* aqueous extract inhibited α-glucosidase with a value of 8.87 ± 0.07 mmol acarbose equivalent/g extract and α-amylase with a value of 0.15 ± 0.01 mmol acarbose equivalent/g extract.

Gholamhoseinian et al. (2008) examined the α-glucosidase inhibitory activity of methanolic and aqueous extracts of the aerial parts of *U. dioica* collected from Iran and reported that the inhibition rate of the aqueous extract on the α-glucosidase enzyme was 4.00 ± 0.30%. Nickavar and Yousefian (2011) concluded that 70% ethanolic extracts of *U. dioica* samples collected from different regions and purchased from Iranian markets inhibited the α-amylase enzyme with an IC_{50} value of 1.89 mg/mL. Evaluating the lipase inhibitory activity of aqueous extracts of various plant mixtures, including *U. dioica*, collected from Ukraine, Savych and Marchyshyn (2021) found that the mixture containing 20% *U. dioica* at a concentration of 1000 µg/mL inhibited that enzyme by 65.38 ± 2.85%. They concluded that herbal mixtures could be effective for the prevention and treatment of obesity and type 2 diabetes.

Gholamhoseinian et al. (2008) determined that methanolic and aqueous extracts of the aerial part of *F. parvifolia* collected from Iran weakly (3.0 ± 0.3%) inhibited the α-glucosidase enzyme. In the same study, they evaluated the α-glucosidase activity of aqueous and methanolic extracts obtained from aerial parts of *M. piperita* collected from Iran. Their findings showed that the aqueous extract strongly (90.00 ± 2.00% at 5 mg/mL) inhibited the α-glucosidase enzyme. The inhibitory effects of extracts obtained by infusion method from *M. piperita* samples grown under drought stress on α-glucosidase and lipase enzymes were studied by Figueroa-Pérez et al. (2014). It was found that the extracts of the samples grown at 65% soil moisture inhibited the α-glucosidase enzyme (8.10 ± 1.10%). Samples grown at 24% soil moisture inhibited the α-amylase enzyme by 12.50 ± 0.10%, while samples grown at 12% soil moisture inhibited the lipase enzyme by 55.10 ± 6.50%.

Ekin et al. (2019) investigated the effects of an 80% ethanol extract obtained from the aerial parts of *O. onites* samples collected from Isparta, Türkiye, on various enzymes. It was shown that the extract at a concentration of 2 mg/mL exerted inhibition of 77.39 ± 0.76% against the α-glucosidase enzyme, 3.16 ± 0.99% against the α-amylase enzyme, and 20.72 ± 2.08% against the pancreatic lipase enzyme. These researchers stated that various species of the family Lamiaceae, including *O. onites*, show significant inhibitory activities and can be used as possible sources for the discovery of drug molecules with α-glucosidase inhibitory effects.

Mahomoodally et al. (2019) evaluated the effects of extracts prepared from the aerial parts of *O. onites* collected from Muğla, Türkiye, on α-amylase and α-glucosidase enzymes. They reported that the aqueous extract did not show activity against the α-glucosidase enzyme. On the other hand, it had an inhibitory effect against the α-amylase enzyme (0.18 ± 0.01 mmol acarbose equivalent/g extract).

Figueroa-Pérez et al. (2014) analyzed the changes in the metabolite contents of *M. piperita* samples grown under different drought stress levels by HPLC. As a result of phytochemical analyses using HPLC, the amount of rosmarinic acid was determined to be 51.6 ± 2.3 ng/µL in the *M. piperita* extracts used as the control, while the amount of rosmarinic acid increased to 78.10 ± 3.20 ng/µL in samples grown at 35% soil moisture. Luteolin was undetectable in the extracts used as the control, while the amount of luteolin was found to be 1.60 ± 0.00 ng/µL in *M. piperita* samples grown at 24% soil moisture.

Using the HPLC-PDA method, Dorman et al. (2009) determined that an aqueous extract of *M. piperita* leaf contained rosmarinic acid at 12.00 ± 0.20 mg/g extract but did not contain luteolin. Adham (2015) used the HPLC technique to determine the rosmarinic acid amounts of *M. piperita*, *M. longifolia*, and *Ocimum basilicum* and concluded that *M. piperita*, *M. longifolia*, and *O. basilicum* contained 0.143%, 0.208%, and 0.306% rosmarinic acid, respectively. Mahomoodally et al. (2019) analyzed the phytochemical content of an aqueous extract of the aerial parts of *O. onites* collected from Muğla, Türkiye, using the HPLC-MS/MS technique and found that the aqueous extract contained the rosmarinic acid-*O*-glucoside isomer. Ozkan et al. (2010) investigated the effect of harvest time on changes in the phenolic composition of *O. onites*. Extracts were obtained from aerial parts of *O. onites* samples collected at different harvest times with a mixture of ethanol, water, acetone, and acetic acid. Their results indicated that the extracts of the samples obtained from the harvest in July were rich in rosmarinic acid (2948.70 g/kg plant) and the extracts of the samples obtained from the harvest in August were rich in luteolin (325.20 g/kg plant).

This is the first study to estimate the inhibitory effects of extracts obtained from these species grown with organic farming techniques on enzymes that play key roles in carbohydrate and lipid metabolism. The RP-HPLC analysis findings for *M. piperita* and *O. onites* extracts, which showed the strongest enzyme inhibitory activities in this study, revealed that both plants contained both rosmarinic acid and ellagic acid, and the *O. onites* extract also contained luteolin. Our literature survey suggested that ellagic acid was detected for the first time in these two species in our study, and this situation may have been

caused by organic farming practices. In the literature, rosmarinic acid is described as a potent inhibitor of enzymes that digest carbohydrates and can increase insulin sensitivity. Rosmarinic acid is a compound with extraordinary pharmacological properties for the control of hyperglycemia (Ngo et al., 2018). The in vivo and in vitro antidiabetic effects of ellagic acid have been confirmed by previous studies (You et al., 2012). Unlike *M. piperita*, the *O. onites* extract inhibited the α-amylase enzyme, and it was thought that the effect of this extract might have been due to the presence of luteolin, which was reported to inhibit this enzyme in previous studies (Kim et al., 2000). Among the studied extracts, the *O. onites* extract was the only one with an inhibitory effect against the pancreatic lipase enzyme, which is effective in controlling obesity. On the other hand, the inhibitory effect of the *O. onites* extract against the pancreatic cholesterol esterase enzyme was close to that of reference compound simvastatin, which is an important finding for this species.

In this study, extracts were prepared using the infusion technique for *A. absinthium*, *C. officinalis*, *A. millefolium*, *U. dioica*, *F. officinalis*, *M. piperita*, and *O. onites* grown with organic farming methods, and the total phenolic and flavonoid contents and antioxidant effects of these extracts were determined. In our study, *M. piperita* had the highest total phenolic content and *A. millefolium* had the highest total flavonoid content. Compared to previous scientific studies of these species, the differences in the total flavonoid and phenolic contents obtained in the present study might have been caused by the harvest time, soil and climate characteristics, and, most importantly, organic farming management practices. When the antioxidant activities of the extracts were evaluated, it was determined that the antioxidant effects of the species differed according to the methods. When the results of previous studies of the antioxidant effects of cultured *M. piperita* and wild *O. onites* samples were evaluated, it was concluded that the antioxidant effects of these species would increase if they were grown with organic farming methods. The present study revealed that *M. piperita* and *O. onites* grown with organic farming practices can show strong antioxidant effects when consumed as simple herbal teas prepared by infusion method.

4. Conclusion

Organic agriculture aims to control agricultural activities in order to protect human and animal health. For this reason, it is aimed to protect underground water resources, to prevent the deterioration of biological diversity and soil structure, and to decrease the use of synthetic pesticides and fertilizers while improving agricultural production. In this study, the antioxidant activities of important medicinal plants grown with organic farming techniques and their activities against the enzymes involved in lipid and carbohydrate metabolism were evaluated. In general, it was determined that all studied species except *U. dioica* had high antioxidant effects. It was thought that the strong inhibitory effects of *M. piperita* and *O. onites* infusions against the **α**-glucosidase enzyme may have been due to their contents of rosmarinic acid and ellagic acid. In conclusion, the promising antioxidant activity (ABTS radical scavenging, metal chelating capacity, and ferric reducing power) and **α**-glucosidase inhibition of *M. piperita* and *O. onites* infusions may be related to their high total phenolic and flavonoid contents. On the other hand, the *O. onites* infusion inhibited the pancreatic cholesterol esterase and pancreatic lipase enzymes. Considering the other quantitative analysis results achieved for *M. piperita* to date, it can be predicted that the high amount of rosmarinic acid found in this species may be due to the application of organic farming techniques. Likewise, it can be thought that ellagic acid, which was detected for the first time in these two species in our study, may have occurred as a result of organic farming practices. Overall, the infusions prepared from *O. onites* and *M. piperita* grown with organic farming techniques showed stronger antioxidant and **α**-glucosidase enzyme inhibition activities compared to the results of previous studies on wild or cultured samples. These findings suggest that these two plant species can be used as important sources for the isolation of new drug molecules or for the development of medicinal herbal products, both in lowering blood sugar levels and in fighting against oxidative stress caused by diabetes. The design of activity-guided isolation and in vivo activity studies of these two plants should be the target of future research.

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