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Research Article
Determination of Chemical Composition, Enzyme Inhibitory, Antioxidant Activity and Antibacterial Effect on *Stenotrophomonas* Resistance of *Onobrychis megataphros* Leaf Extract

[1]Salih Muvakit, [2]Eda Elgin Kiliç and [3]Mehmet Özaslan

[1]Department of Biology, Gaziantep University, 27310 Gaziantep, Türkiye
[2]Naci Topçuoglu Vocational School, Gaziantep University, 27310 Gaziantep, Türkiye
[3]Department of Biology, Gaziantep University, 27310 Gaziantep, Türkiye

Abstract

Background and Objective: *Onobrychis* (sainfoin) species are highly valuable forage legumes in terms of nutritional value with beneficial environmental properties. In this study, the chemical composition of the methanol extract obtained from *Onobrychis megataphros* was investigated using qualitative and quantitative methods and its *in vitro* antioxidant, enzyme inhibitory activities and antibacterial effect on multidrug-resistant *Stenotrophomonas maltophilia* were documented. **Materials and Methods:** *Onobrychis megataphros* plant samples were collected in Siverek District of Sanlıurfa-Türkiye in the spring time of 2021-2022. Randomized sampling were applied in the vegetation period of *Onobrychis megataphros*. About 500 g of leaf samples were dried under shade and pulverized. Then the samples were subjected to methanol extraction for 6-8 hrs and finally filtered and evaporated at 40°C to remove the solvent. The qualitative and quantitative parameters were measured. **Results:** Phenolic and flavonoid compounds of the extract were 20.62 and 24.55 mg QE g^{-1}, respectively. Hyperoside and hesperidin were found in the extract among the compounds screened (2580 and 2263 μg g^{-1} extract, respectively). In the phosphomolybdenum assay, the extract showed 2.03 mg mL^{-1} activity. In CUPRAC and FRAP assays, the activity of the extract was 1.76 and 1.45 mg mL^{-1}, respectively. The scavenging activity of the extract on the DPPH radical (6.11 mg mL^{-1}) and ABTS (2.40 mg mL^{-1}). The AChE and α-amylase inhibitory activity of the extract was 1.58 and 1.60 mg mL^{-1}, respectively. The extract showed antibacterial activity against *Stenotrophomonas maltophilia*. The activity level of the extract in the tyrosinase inhibitory activity test was 2.54 mg mL^{-1}. **Conclusion:** *Onobrychis megataphros* extract showed antibacterial effect on antibiotic resistant *Stenotrophomonas maltophilia* bacteria, which is a hospital infection agent and it is a potential plant in the search for active compound.

Key words: *Onobrychis megataphros*, *Stenotrophomonas maltophilia*, antioxidant activity, antibacterial activity

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Corresponding Author: Mehmet Özaslan, Department of Biology, Gaziantep University, 27310 Gaziantep, Türkiye

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The *Onobrychis* genus is represented throughout the world with 162 species and in Türkiye there are 52 species, 27 of which are endemic in Turkiye[1]. The genus *Onobrychis* belongs to Fabaceae family, tribe Hedysareae and subfamily Faboideae. *Onobrychis* Adans. (Fabaceae) species are generally plants with beneficial environmental and agricultural properties and are also very valuable forage legume crops in terms of their nutritional values. Since these species have higher drought tolerance, farmers prefer to cultivate *Onobrychis* species, especially in free-draining soils with deep groundwater and low rainfall. These species are also resistant to many agricultural pests and diseases that are frequently encountered in agricultural areas. *Onobrychis* species are among valuable pollen sources for pollinators, especially in honey production. They have a delicious taste for farm animals and do not cause bloating if consumed excessively. However, the number of *Onobrychis* populations began to decline with the Green Revolution, especially in Northern Europe and the intensification of agricultural activities also supported this situation. Unlike other forage legumes, most *Onobrychis* species are more demanding to grow. While genetic intervention has been carried out in many other cultivars, genetic breeding studies in *Onobrychis* species have been limited. However, interest in these species has started to increase again in recent years and efforts have been made to reproduce them in farm environments. There is, of course, a scientific explanation for this interest. Researchers stated that there are abundant tannins and polyphenol compounds in the leaves of *Onobrychis* species[2,3]. As a result of the detailed literature analysis performed on the entire genus *Onobrychis*, it was determined that the biological activity potential of these species, unfortunately, has not been clarified yet, except for the estrogenic activity of isoflavonoids obtained from *O. ebenoides*[4]. The data in the literature are mostly related to the chemical composition[5-7], pollen morphology[8-11] and phylogenetic characterization[12] of *Onobrychis* species. In this study, the chemical composition of the methanol extract obtained from *O. megataphros* was investigated using qualitative and quantitative methods and it's *in vitro* antioxidant and enzyme inhibitory activities were documented. The data obtained in the present study is an original contribution to the literature and is believed to fill an important gap in the literature regarding the biological activities of these species.

Stenotrophomonas maltophilia is an aerobic, non-fermentative, gram-negative and bacillus-shaped bacterium. It is an opportunistic pathogen that causes serious infections in the hospital, especially in patients hospitalized in intensive care units[13]. *Stenotrophomonas maltophilia* was first isolated from pleural fluid by J.L. Edwards in 1943 and named "*Bacterium bookeri*". In Latin, it means "a malt-loving bacterium that needs very little nutrients for growth"[14]. In nosocomial infections (nosocomial), MRSA (Methicillin resistant *Staphylococcus aureus*), *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* infections have gained importance in recent years. The development of resistance to antibiotics has been observed and the need to find different alternatives with antimicrobial effects other than the antibiotics used routinely has gained importance. For this purpose, the antibacterial activity of *Onobrychis megataphros* on *Stenotrophomonas maltophilia* was investigated.

MATERIALS AND METHODS

Study area: *Onobrychis megataphros* plant used in the study was collected from area (Siverek- Hilvan) in Şanlıurfa between 2021 and 2022 during the vegetation periods. The sample was collected from a separate area of 20 m^2, then the leaves were isolated and weighed, reaching an amount of 500 g.

Plant materials and extract preparation: The aerial parts of *O. megataphros* were laid out on blotting papers in a shaded place and allowed to become dry. Completely dried plant samples were ground into powder. Methanol extraction was performed on powdered plant samples using the Soxhlet device (Gerhardt 412, Germany). Methanol was removed by evaporation in the rotary evaporator (Heidolph Laborota, Germany and stored at +4°C until the beginning of the experiment[15]. All analyses were carried out in the biology laboratory of Gaziantep University.

Total phenolic and flavonoid contents: For determining the total phenolic content, the prepared sample solution (0.25 mL) was mixed with diluted Folin-Ciocalteu reagent (1 mL, 1:9) and shaken strongly. The sample content (0.25 mL) was mixed with diluted Folin-Ciocalteu reagent (1 mL, 1:9) with vigorous shaking to obtain total phenolics. The Na_2CO_3 compound (0.75 mL, 1%) was added after 3 min and incubated for 2 hrs at room temperature. The sample absorbance at 760 nm was then read. Total phenolic content was expressed as gallic acid equivalents. Then 3 min later, 1% Na_2CO_3 solution (0.75 mL) was added and after 2 hrs of incubation (at room temperature), sample absorbance at 760 nm was evaluated. Total phenolic solution was considered as gallic acid equivalents[16].

For total flavonoid content, the sample solution (1 mL) was added to a (2%) aluminium trichloride solution in methanol. Similarly, a blank was prepared by mixing the sample solution (1 mL) with methanol (1 mL) but without $AlCl_3$. After 10 min incubation at room temperature, the absorbance of both the sample and blank was measured at 415 nm. The absorbance of the blank was subtracted from that of the sample. Total flavonoid content was reported as quercetin equivalents.

LC-ECI-MS/MS analysis: The phytochemical analysis of the extract was performed with a previously developed and validated sensitive, rapid, simple and reproducible method using LC–ESI–MS/MS. An Agilent Technologies 1260 Infinity Liquid Chromatography System (hyphenated to 6420 triple quadrupole mass spectrometer, Santa Clara, Canada, USA) was used for quantitative analysis evaluation. Chromatographic separation was performed using a Poroshell (120 EC-C18, 100×4.6 mm ID, 2.7 µm) column. Mobile phase configuration (0.1% formic acid/methanol) was chosen, guided by the better chromatographic resolution of the isomeric compounds. However, the preferred mobile phase configuration also showed higher sensitivity for many phenolic compounds. Finally, the mobile phase was created by combining solvent A (0.1% v/v formic acid solution) with solvent B (methanol). The following parameters were used to create the gradient profile: 0.00 min 2% B eluent, 3.00 min 2% B eluent, 6.00 min 25% B eluent, 10.00 min 50% B eluent, 14.00 min 95% B eluent, 17.00 min 95% B and 17.50 min 2% B eluent. The column temperature was fixed to 25°C. The flow rate was 0.4 mL min^{-1} and the injection volume was 2.0 L[17]. The LC system was connected to the tandem mass spectrometer through an ESI source. The MS's electrospray source functioned in both negative and positive multiple reaction monitoring (MRM) modes. The interface parameters included a capillary voltage of -3.5 kV, a gas temperature of 300°C and a gas flow rate of 11 L min^{-1}. The nebulizer pressure remained constant at 40 psi.

Antioxidant activity

Total antioxidant activity: The phosphomolybdenum technique was used to assess the antioxidant total activity of the samples. About 0.2 mL of sample solution was combined with 2 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The sample absorbance at 695 nm was measured after 90 min of incubation at 95°C[18].

Radical scavenging activity

DPPH radical scavenging activity: Sample compound (1 mL) was added to 4 mL of 0.004% methanol DPPH solution for 2,2-Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging. After 30 min of incubation at room temperature and in the dark, the sample absorbance was read at 517 nm[19,20].

Activity of ABTS cation radical scavenging: In summary, $ABTS^{\bullet+}$ radical cation was composed by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and keeping the mixture in the dark for 12-16 hrs at room temperature. Before analysis, the ABTS solution was diluted with methanol to show an absorbance of 0.700±0.02 at 734 nm. The sample compound (1 mL) was added to ABTS solution (2 mL) and mixed. After 7 min of incubation at room temperature, the sample absorbance was read at 734 nm[19].

Activity of metal chelating: Sample solution (2 mL) was added to $FeCl_2$ solution (0.05 mL, 2 mM) in order to chelate metal on Iron ions. The reaction was initiated after addition of 5 mM ferrozine (0.2 mL). However, sample solution (2 mL) was added to $FeCl_2$ solution (0.05 mL, 2 mM) and ferrozine-free water (0.2 mL) for blank preparation. Later, 10 min after incubation, the sample and blank absorbances at room temperature were evaluated at 562 nm[20].

Reducing power

Activity of Cupric Ion Reducing (CUPRAC): For generating the Reducing Activity of Copper Ion (CUPRAC), premixed reaction mixture containing NH4Ac buffer (1 mL, 1 M, pH 7.0), neocuproine (1 mL, 7.5 mM) and $CuCl_2$ (1 mL, 10 mM) sample solution (0.5 mL)) added. Likewise, sample solution (0.5 mL) was added to a reaction mixture (3 mL) without $CuCl_2$ to prepare a blank. Later, after 30 min of incubation at room temperature, the absorbance of the sample and blank was evaluated at 450 nm[21].

Ferric Reducing Antioxidant Power (FRAP): For providing Ferric Reducing Antioxidant Power (FRAP), a premixed FRAP reagent (2 mL) including 2,4,6-Tris(2-Pyridyl)-s-Triazine (TPTZ) (10 mM) in 40 mM HCl, acetate buffer (0.3 M, pH 3.6) and ferric chloride (20 mM) in a ratio of 10:1:1 (v/v/v) was added to sample solution (0.1 mL). Later, after 30 min incubation at room temperature, the sample absorbance was evaluated at 593 nm[19].

Enzyme inhibitory activity

α-Amylase inhibitory activity: The α-amylase inhibitory activity was tested using the Caraway-Somogyi iodine/

potassium iodide (IKI) technique. The sample solution (25 L) was mixed with the α-amylase solution (50 L) in phosphate buffer (6 mM sodium chloride pH 6.9) in a 96-well microplate and incubated at 37°C for 10 min. After pre-incubation, the reaction was started by adding a starch solution (50 L, 0.05%). Similarly, a blank was created by mixing the sample solution with all of the reaction reagents but excluding the enzyme solution (α-amylase). For 10 min at 37°C, the reaction mixture was incubated. The process was stopped by adding HCl (25 L, 1 M). Then 100 L of iodine-potassium iodide solution was added. The absorbance of the sample and the blank were measured at 630 nm. The sample absorbance was subtracted from the blank absorbance[15].

Tyrosine inhibitory activity: Tyrosinase-inhibitory activity was evaluated using a dopachrome method modified with L-DOPA as substrate. The sample solution (25 µL) was mixed with tyrosinase solution (40 µL) and phosphate buffer (100 µL, pH 6.8) in a 96-well microplate and incubated at 25°C for 15 min. The reaction was then started by the addition of L-DOPA (40 µL). Similarly, blanks were prepared by adding sample solution to all reaction reagents that did not contain enzyme (tyrosinase) solution. Sample and blank absorbance were read at 492 nm after 10 min of incubation at 25°C[15].

Cholinesterase (ChE) inhibitory activity: Cholinesterase (ChE) inhibitory activity was measured using the method of Ellman. Sample solution (50 µL) was mixed with DTNB (5,5 -Dithiobis-(2-Nitrobenzoic acid) (125 µL) and AChE solutions (25 µL)) in Tris-HCl buffer (pH 8.0) in a 96-well microplate and incubated for 15 mins at 25°C. Next, the reaction was started by adding acetylthiocholine iodide (ATCI) (BTCI) (25 µL). Similarly, blanks were prepared by adding sample solution to all reaction reagents that did not contain enzyme solutions (AChE). After 10 min of incubation at 25°C, the sample and blank absorbance were read at 405 nm. The absorbance of the blank was subtracted from the sample[15].

Important information about calculations: For enzyme inhibition, radical scavenging and metal chelating assays, the sample concentration reducing the initial concentration by 50% was defined as IC_{50}, whereas EC_{50} values were calculated as the sample concentration that provides 0.500 absorbance for reducing power and phosphomolybdenum experiments and 50% inhibition of the initial concentration for radical capture and metal chelation tests. The extracts biological activities were expressed as mg standard equivalent/g extract and compared to those of the positive controls, which include trolox, Ethylenediaminetetraacetic Acid (EDTA) (disodium salt), galantamine, kojic acid and acarbose.

Antibacterial activity on *Stenotrophomonas maltophilia*: *In vitro*, antibacterial activity against *Stenotrophomonas maltophilia* was determined by disk assay. Five different concentrations of *Onobrychis megataphros* Boiss. (Fabaceae) extract (512, 256, 128, 64 and 32 µg mL^{-1}) were used and determined by diffusion method according to CLSI. Sterile 6 mm diameter blank discs were prepared by impregnating 20 µL of liquid extract (dissolved in DMSO) CFU mL^{-1} (McFarland Turbidity OD = 0.5) *Stenotrophomonas maltophilia* bacteria adjusted according to optical density were inoculated homogeneously on Mueller-Hinton agar medium using a cotton swab. The extract containing discs and control discs were then placed on the agar surface using sterilized forceps. The Petri dishes were then incubated at 37°C for 24 hrs. After 24 hrs, bacterial growth inhibition zone diameters formed on the plates were measured. Dimethyl sulfoxide saturated paper disks and antibiotic disks were used as controls to check the possible activity of the solvent[22].

Statistical analysis: All tests were performed in triplicate to ensure data were statistically significant and results were expressed as mean and standard deviation (Mean±SD). Statistical significance between data was determined using Tukey's honestly significant difference *post hoc* test and ANOVA (One-way Analysis of Variance) test with a = 0.05. Statistical calculations were made using the SPSS v.22.0 program.

RESULTS AND DISCUSSION

Chemical composition: Both qualitative (spectrophotometric) and quantitative (chromatographic) methods were used to determine the chemical composition of the methanol extract obtained from the aerial parts of *O. megataphros*. The total phenolic content was 20.62±0.15 mg GAE g^{-1} extract and the total flavonoid content was 24.55±0.22 mg QE g^{-1} extract. The results of the chromatographic analyses were given in Table 1, respectively. According to the data in Table 1, the total amount of phenolic and flavonoid compounds in the extract was determined as 20.62 mg GAE g^{-1} extract and 24.55 mg QE g^{-1} extract, respectively. Considering the data in Table 2, where the LC–ESI–MS/MS analysis results are presented, it was determined that hyperoside and hesperidin were found as the abundant compounds in the extract among

Table 1: Concentrations of selected phenolic compounds in the methanol extract of *Onobrychis megataphros*

RT	Compound	Concentration (μg g^{-1} extract)
8.7829	Gallic acid	25.8±0.8
10.5569	Protocatechuic acid	73.1±0.4
10.8157	Pyrocatechol	13.5±1.0
10.8352	3,4-Dihydroxyphenylacetic acid	5.24±0.19
11.2863	(+)-Catechin	Not detected
11.7605	Chlorogenic acid	43.6±0.3
11.9714	2,5-Dihydroxybenzoic acid	6.37±0.15
12.0643	4-Hydroxybenzoic acid	42.9±0.1
12.2281	(-)-Epicatechin	9.65±0.04
12.6006	Caffeic acid	7.37±0.14
12.6904	Syringic acid	11.4±0.8
12.8289	3-Hydroxybenzoic acid	3.97±0.32
13.0044	Vanillin	7.25±0.32
13.4183	Verbascoside	0.21±0.01
13.6707	Taxifolin	Not detected
13.7506	p-Coumaric acid	18.5±0.1
13.8236	Sinapic acid	5.20±0.19
13.8837	Ferulic acid	39.9±0.7
14.2311	Luteolin 7-glucoside	29.8±0.3
14.4193	2-Hydroxycinnamic acid	Not detected
14.4289	Hesperidin	2263±2
14.4634	Hyperoside	2580±5
14.4915	Rosmarinic acid	5.84±0.14
14.7066	Apigenin 7-glucoside	93.6±1.8
14.9445	Pinoresinol	12.2±0.3
15.0470	Eriodictyol	Not detected
15.5459	Quercetin	2.58±0.04
15.7763	Luteolin	Not detected
16.0729	Kaempferol	3.34±0.11
16.2364	Apigenin	Not detected

Table 2: Antioxidant activities of the methanol extract of *Onobrychis megataphros*

Assays	*O. megataphros*	Trolox	EDTA
Phosphomolybdenum (EC_{50}: mg mL^{-1})	2.03±0.07	0.49±0.04	
CUPRAC reducing (EC_{50}: mg mL^{-1})	1.76±0.03	0.11±0.001	
FRAP reducing (EC_{50}: mg mL^{-1})	1.45±0.01	0.04±0.002	
DPPH scavenging (IC_{50}: mg mL^{-1})	6.11±0.33	0.06±0.001	
ABTS scavenging (IC_{50}: mg mL^{-1})	2.40±0.02	0.11±0.001	
Ferrous ion chelating (IC_{50}: mg mL^{-1})	2.12±0.02		0.03±0.001
Phosphomolybdenum (mg TEs/g extract)	242±8		
CUPRAC reducing (mg TEs/g extract)	62.6±1.2		
FRAP reducing (mg TEs/g extract)	30.4±0.2		
DPPH scavenging (mg TEs/g extract)	9.34±0.51		
ABTS scavenging (mg TEs/g extract)	45.8±0.4		
Ferrous ion chelating (mg EDTAEs/g extract)	15.6±0.2		

TEs and EDTAEs mean trolox and ethylenediaminetetraacetic acid (disodium salt) equivalents, respectively, Values indicated by the same superscripts are not different from the honestly significant difference after Tukey's *post hoc* test at 5% significance level and ±: Standard deviation

the compounds screened (2580 and 2263 μg g^{-1} extract, respectively) (Fig. 1 and 2). On the other hand, it was found that the extract did not contain (+)-catechin, taxifolin, 2-hydroxycinnamic acid, eriodictyol, luteolin and apigenin.

As stated in the introduction section of the present study, there is no study in the literature on the chemical composition of *O. megataphros*. However, it is useful to take a look at the chemical composition of other *Onobrychis* species to reflect the general characteristics of the genus. Literature data show that studies generally focus on *O. viciifolia*. In a study carried out to determine the phytochemical composition of *O. viciifolia*, it was reported that arbutin (17.7 mg g^{-1} of dry weight) and catechin (3.5 mg g^{-1} of dry weight) are abundant in the petioles of the plant and rutin (19.9 mg g^{-1} of dry weight) in the leaves[6]. In another study on the same species, the presence of two new hydroxycinnamic acid esters named methyl 6-O-p-trans-coumaroyl-β-D-glucopyranoside and methyl 6-O-p-cis-coumaroyl-β-D-glucopyranoside and a new

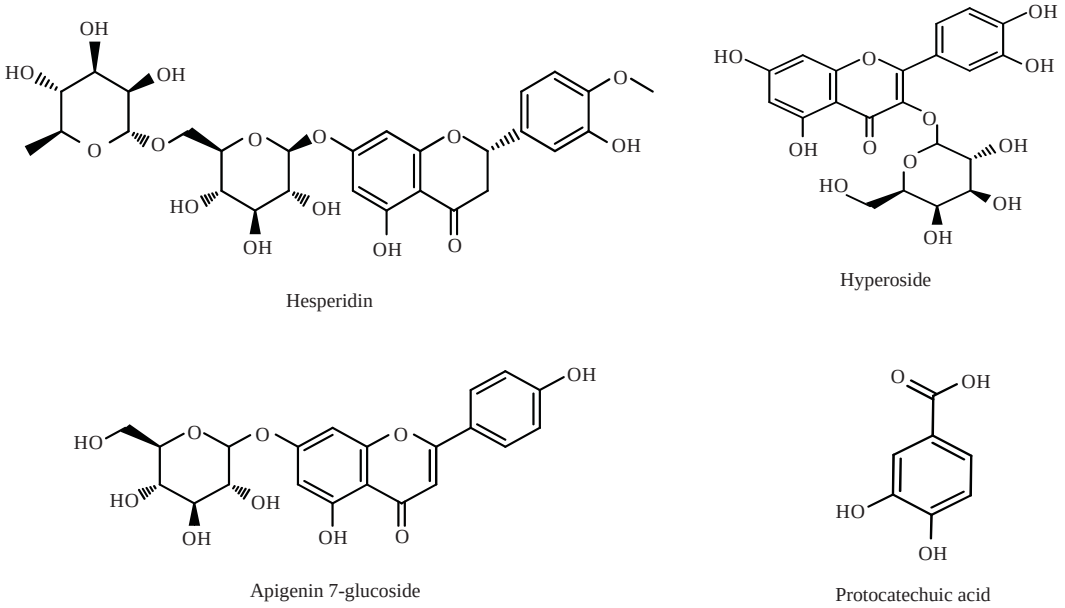

Fig. 1: Structures of the main phenolic compounds identified in the methanol extract of *Onobrychis megataphros*

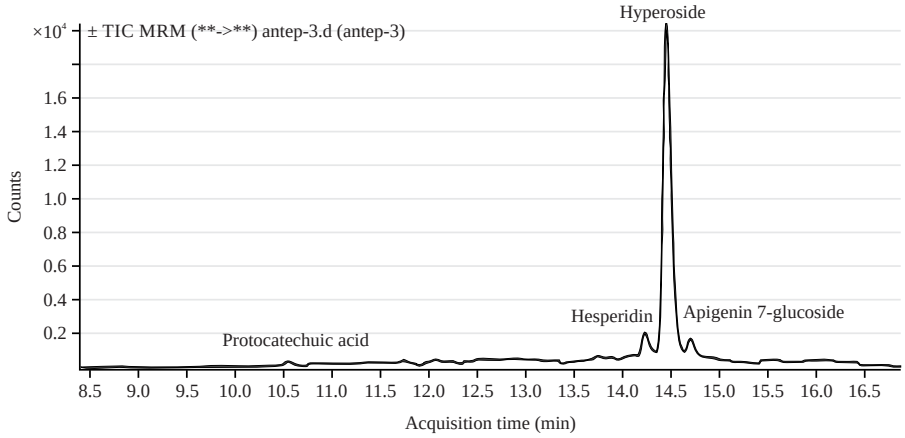

Fig. 2: LC-ESI-MS/MS chromatograms of the methanol extract from *Onobrychis megataphros*

flavonoid named chrysoeriol-4-O-(6"-O-acetyl)-β-D-glucopyranoside were reported by Halabalaki *et al.*[4]. In some other studies, it has been suggested that *O. viciifolia* is a significant natural source in terms of condensed tannins[15-17] proanthocyanidin pigments[7]. However, the chemical composition of other species of the genus *Onobrychis*, including *O. megataphros*, which is addressed in the current study, remains an important unclarified gap for the scientific community. Although the chemical composition data shown in the present study are original for the literature, it is thought that examining other species of the genus *Onobrychis* in terms of phytochemicals will also make important contributions to the field.

Antioxidant activity: The antioxidant activity results of the methanol extract obtained from the aerial parts of *O. megataphros* by six different test systems were given in Table 2. While EDTA was used as a positive control in the ferrous ion chelation test, trolox was used as the positive control agent in other tests. Test results are given in both EC_{50} (mg mL^{-1}) and positive control equivalents. In phosphomolybdenum test, the methanol extract showed 2.03 mg mL^{-1} activity. It was determined that the activity potential of the extract was too close to each other in each parameter of the reducing power assays (CUPRAC and FRAP) (1.76 and 1.45 mg mL^{-1}, respectively). The radical scavenging activity of the extract was tested on two different free radicals. According to the results in the Table 2, it was understood that

Table 3: Enzyme inhibition activity of the methanol extract of *Onobrychis megataphros*

Assays	*O. megataphros*	Galantamine	Kojic acid	Acarbose
AChE inhibition (IC_{50}: mg mL^{-1})	1.58±0.04	0.003±0.0001		
Tyrosinase inhibition (IC_{50}: mg mL^{-1})	2.54±0.22		0.08±0.001	
α-Amylase inhibition (IC_{50}: mg mL^{-1})	1.60±0.01			0.79± 0.01
AChE inhibition (mg GALAEs/g extract)	1.7±0.04			
Tyrosinase inhibition (mg KAEs/g extract)	31.9±2.8			
α-Amylase inhibition (mg ACEs/g extract)	490±1			

the scavenging activity of the extract on the DPPH radical (6.11 mg mL^{-1}) was approximately three times stronger than its activity on ABTS (2.40 mg mL^{-1}). The extract showed an activity of 2.12 mg mL^{-1} in the ferrous ion chelating test. In none of the tests the extract showed as high activity as the positive control agents. As far as our literature survey could ascertain, there is no study in the literature on the antioxidant activity of *O. megataphros*. However, It is useful to take a look at some literature data on the contribution of the major compounds in the extract to the antioxidant activity. According to many researchers, hyperoside is a flavonoid glycoside with proven antioxidant activity[23]. According to Piao *et al.*[23], hyperoside obtained by HPLC method from *Ligularia fischeri* has been reported to have high antioxidant activity potential. In a study investigating the antioxidant activity of hyperoxide on oxidative stress induced by hydrogen peroxide, carbon tetrachloride and cadmium in *Saccharomyces cerevisiae*, it was reported that the compound in question increased cell viability, decreased the level of lipid peroxidation and the amount of intracellular reactive oxygen species (ROS)[24]. In another study carried out by He *et al.*[25], it has been reported that hyperoside protects against cerebral ischemia-reperfusion injury by attenuating oxidative stress, inflammation and apoptosis in rats. Of course, it is possible to increase the number of literature data on the antioxidant activity of hyperoside. However, it would be useful to look at the literature data on the antioxidant activity potential of hesperidin. In a study by Choi *et al.*[26], the antioxidant activity potential of hesperidin and hesperidin glucoside was compared and it was reported that these two compounds had similar and significant radical scavenging activity. Known to have antioxidant activity on hypertensive animal models[27], hesperidin has been reported to have preventive effects on hypertension and cerebral thrombosis in spontaneously hypertensive rats prone to stroke due to this feature[28]. Additionally, in rats with radiation-induced brain injury, hesperidin has been reported to increase plasma and tissue malondialdehyde levels and total antioxidant status in all tested oxidative stress parameters[29]. It is also possible to increase the number of literature data on this compound[30-34]. As can be seen from the literature data above, hyperoside and hesperidin, which are found in high amounts in the extract, have high antioxidant activity potential. Based on these data, it is thought that these compounds contribute significantly to the antioxidant activity of *O. megataphros* in the current study.

Enzyme inhibitory activity: Acetylcholinesterase (AChE), tyrosinase and α-amylase inhibitory activity tests were performed, respectively, in order to investigate the anti-Alzheimer, skin whitening and anti-diabetic activities of the methanol extract obtained from *O. megataphros* and the results were given in Table 3. As with antioxidant activity test systems, results are given in both IC_{50} (mg mL^{-1}) and positive control equivalents. Galantamine, kojic acid and acarbose were used as positive control agents in enzyme inhibitor activity tests, respectively. Although the extract did not show as high activity as positive control agents in any of the activity tests, it showed remarkable activity especially in AChE and α-amylase inhibitory activity tests (1.58 and 1.60 mg mL^{-1}, respectively). The activity level of the extract in the tyrosinase inhibitory activity test was determined as 2.54 mg mL^{-1}.

Since biological activity-guided fractionation or a similar test system cannot be applied, it is of course not possible to detect the phytochemical responsible for enzyme inhibitory activity with current possibilities. However, documenting the literature data on the enzyme inhibitory activity potential of the major compounds, hyperoside and hesperidin, may give an idea about the contribution of these compounds to the activity. In a molecular docking study by Shen *et al.*[35], hyperoside was reported to exhibit significant α-amylase inhibitory activity (IC_{50}: 0.491 mg mL^{-1}). The polyphenol profile and enzyme inhibitory activities of *Astragalus macrocephalus* subsp. *Finitimus* and stated that together with hyperoside, some other phytochemicals exhibited tyrosinase inhibitory activity at IC_{50} values ranging from 1.02-1.41 mg mL^{-1} by Sarikurkcu and Zengin[36]. The study carried out by Jung *et al.*[37] supports the literature data given above. In this study performed on hyperoside, one of the main components of *Nelumbo nucifera*, it was reported that the compound in question showed tyrosinase inhibitory activity and suppressed melanin biosynthesis in alpha-melanocyte-

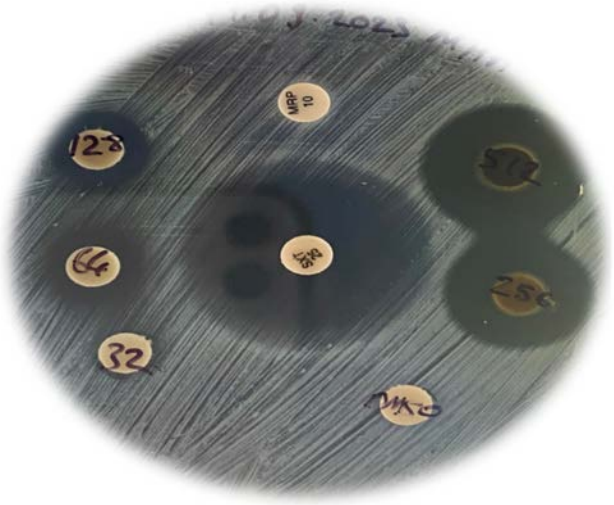

Fig. 3: Inhibition diameter zone of *Onobrychis megataphros* and concentrations

Table 4: Antibacterial activity of the extract of *O. megataphros*

	µg mL^{-1}	Inhibition zone
Onobrychis megataphros extract	512	22
	256	16
	128	14
	64	10
	32	5
Antibiotics		
SXT		30
MRP		0
Dimethylsulfoxide saturated paper disks		0

and SXT: Trimethoprim/sulfamethoxazole and MRP: Meropenem

stimulating hormone-stimulated B16F10 melanoma cells. On the other hand, there are reports in the literature that hyperoside may have cholinesterase inhibitory activity. Jouini *et al.*[38] suggested that as a result of molecular docking analysis, hyperoside could bind to the active site of BuChE and suppress the catalytic activity of the enzyme.

In addition to hyperoside, it can be argued that hesperidin is also quite capable of enzyme inhibitory activity. Khan *et al.*[39] reported that hesperidin showed significant binding to α-amylase. Further evidence that the compound in question has enzyme inhibitory activity was reported by Zhang *et al.*[40]. In the aforementioned study, it was determined that hesperidin had tyrosinase diphenolase activity (16.08 mM) and its inhibition mechanism was competitive. There is also data on the cholinesterase inhibitory activity potential of this compound in the literature. In a study conducted by Arumugam *et al.*[41], it was reported that the AChE inhibitory activity of the extract obtained from *Sophora alopecuroides* var. alopecuroides was 0.97 mg mL^{-1}, one of the main components of the extract was hesperidin and this compound may contribute to the activity. In addition, Sarria *et al.*[42] reported that hesperidin complexed with copper (II) and zinc (II) exhibited more selective cholinesterase inhibitory activity. The literature data detailed above support the hypothesis that both hyperoside and hesperidin may contribute significantly to the enzyme inhibitory activity of the extract.

Antibacterial effect on multidrug-resistant *Stenotrophomonas maltophilia*: Antibiotic resistance of *S. maltophilia*, used in this study, were given in Table 4. The results obtained showed that all extracts had inhibitory effect on *Stenotrophomonas maltophilia* bacteria and it was seen in Fig. 3 that the zone diameter increases with increasing concentration. The highest zone diameter was 512 µg mL^{-1} and the lowest zone diameter was 32 µg mL^{-1}. In the resistance of antibiotics (SXTand MRP) against bacteria, it was determined that SXT was effective and MPR was not effective. It was determined that SXT antibiotic had the highest effect since its zone diameter was the widest (30 mm). This study

provides evidence that the plant is a potentially rich source of multi-resistant antibacterial agents against *S. maltophilia*. Thus, *O. megataphros* extracts may be a potential alternative antimicrobial agent for *S. maltophilia*.

In this study, the chemical composition, antioxidant and enzyme inhibitory activity of the methanol extract obtained from the aerial parts of *O. megataphros* were investigated. As can be understood from the literature data discussed in detail above, the main components of the extract, hyperoside and hesperidin, are important compounds in terms of both antioxidant and enzyme inhibitory activities and are thought to contribute to the activities in question. However, it is recommended to use a biological activity-guided fractionation or an equivalent test system to detect other components (if any) responsible for the activity.

CONCLUSION

Stenotrophomonas maltophilia has been frequently reported as a nosocomial infection agent in recent years. This bacterium, which is highly resistant due to its structural characteristics, has become increasingly resistant with the use of antibiotics. In case of inadequacy of existing antibiotics, it is very important to find new compounds effective against this agent. In this study, the antibacterial activity of methanol extract obtained from *Onobrychis megataphros* plant on *Stenotrophomonas maltophilia* was determined. Our study provides important data that will contribute to the development of drugs that can be used against infections caused by this bacterium by purifying the active compounds of *Onobrychis megataphros*.

SIGNIFICANCE STATEMENT

In recent years, indiscriminate use of antibiotics all over the world and drug resistance are closely related. This situation has encouraged scientists to search for new antimicrobial compounds from different sources. Since plants constitute a source from which new antimicrobial chemotherapeutic substances can be obtained, research has been particularly focused on medicinal plants. Although there is literature information that different *Onobrychis* species are used in the treatment of various diseases among humans, it is the first in terms of investigating the antioxidant, antibacterial, chemical composition and enzyme Inhibitory of *O. megataphros* plant and bringing it to the literature.

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