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Phytochemical composition, antioxidant, enzyme inhibition, antimicrobial effects, and molecular docking studies of *Centaurea sivasica*

Ümit Yırtıcı[a,*], Aysun Ergene[b], Mehmet Nuri Atalar[c], Şevki Adem[d]

[a] Department of Medical Laboratory, Kirikkale University, TR-71450 Kirikkale, Turkey
[b] Department of Biology, Kirikkale University, TR-71450 Kirikkale, Turkey
[c] Faculty of Health Sciences, Igdir University, TR-76000 Igdir, Turkey
[d] Department of Chemistry, Cankiri Karatekin University, TR-18200 Cankiri, Turkey

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ABSTRACT

In this study, *Centaurea sivasica* Wageitz (Asteraceae) methanol extract was examined regarding phytochemical composition, in vitro antioxidant properties, ability to inhibit tyrosinase, α-amylase, α-glucosidase enzymes, and antimicrobial effects. Also, possible binding and interactions of phytochemicals with enzymes by molecular docking were determined. The extract's phenolic amount was 21.42 mg GAE/g extract, and the extract was determined to be rich in flavonoids (19.73 mg RE/g extract). Due to the results of liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) analysis, the main components of the methanol extract was scutellarin (27843.91 μg/g), quercimeritrin (3629.85 μg/g), chlorogenic acid (2519.68 μg/g) and baicalin (920.49 μg/g). The methanol extract was found to show remarkable activity in all antioxidant activity tests and had a high potential to inhibit the enzymes examined. The extract was radical scavenging on (DPPH and ABTS), reducing power (FRAP and CUPRAC), phosphomolybdenum assays were measured as 2.72, 69.58, 44.78, 141.18, 109.25 mg TE/g extract, respectively. Tyrosinase, α-amylase, and α-glucosidase inhibitory activities were 36.81 mg KE/g extract, 252.60 and 279.40 mg AKE/g extract, respectively. Scutellarin inhibited the tyrosinase enzyme very effectively, and its effect was found as 43.32 μM or 20.38 μg/mL. The extract showed different inhibition zones (16.3, 16.0, 15.0, 15.6 mm) and MIC values (500–1000 μg/mL) on the microorganisms examined (*B. cereus, S. aureus, E. coli, C. albicans*). In molecular docking studies, the most abundant scutellarin in the extract was shown to affect both tyrosinase and α-glucosidase inhibition significantly.

1. Introduction

Plants have been used as a folk medicine to treat a variety of diseases for many years. Therefore, plants are considered an essential target for the discovery of drug candidates because of their bioactive compounds, and they are actively being investigated throughout the world. It was determined that 80% of 122 plant-based drugs were obtained from traditionally used plants (Fabricant and Farnsworth, 2001).

These bioactive compounds or plant metabolites obtained from plants are remarkably effective in eliminating oxidative stress. Oxidative stress occurs due to excessive reactive oxygen species production, which triggers damage to cell structures, including lipids, proteins, and DNA (Gülçin, 2012; Taslimi and Gulçin, 2018). Oxidative stress can also be considered the disruption of homeostasis between reactive oxygen forms and the organism's antioxidative defense system. This damage causes many ailments such as diabetes, cancer, skin aging, inflammation, etc. Plant metabolites with one or more hydroxyl groups (polyphenols) and one or more aromatic rings reduce oxidative stress by preventing the formation of free radicals or neutralizing the radicals with their antioxidant properties. These compounds are safe and more biologically active than synthetic antioxidants (Chiocchio et al., 2018; Khatri et al., 2019).

It has been reported that plant metabolites obtained from plants minimize free radical activity and modulate enzymes involved in aging processes that protect the skin against sunlight. Enzymes such as tyrosinase are essential among these cosmetically intriguing enzymatic targets. Tyrosinase misregulated expression and/or activity causes skin hyperpigmentation (Chiocchio et al., 2018). Accordingly, tyrosinase inhibitors are aspirant skin-whitening factors.

In recent years, efforts to find new agents that can treat diseases, such as type II diabetes, have increased (Khatri et al., 2019; Rashid et al., 2019). Diabetes mellitus is one of the fastest-growing diseases, with approximately 425 million diabetic patients worldwide. Polyphenols, especially flavonoids, phenolic acids, and tannins,

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* Corresponding author.
E-mail address: umityirtici@kku.edu.tr (Ü. Yırtıcı).

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can inhibit α-glucosidase and α-amylase, the essential enzymes responsible for carbohydrate digestion, causing low blood sugar levels (Lin et al., 2016; Moo-Huchin et al., 2015). Therefore, plants can be a supportive source of hypoglycemic agents (Eruygur et al., 2019; Polat Kose et al., 2020; Taslimi et al., 2020; Türkan et al., 2020).

It also has been determined that phenolic compounds have an antibacterial effect by directly killing bacteria resistant to traditional antibiotics, acting synergistically with antibiotics, and weakening bacterial pathogens. It has been shown to prevent antibiotic resistance development, especially in bacteria (Xie et al., 2017). Besides, they inhibit the synthesis of mycotoxins, inactivate fungal enzymatic systems involved in energy production, and synthesize structural components (Christ-Ribeiro et al., 2019). Thus, new and useful plant metabolites can be found against microorganisms that develop resistance to antibiotics.

Centaurea L. is the third largest genus in Turkey. This genus has 255 taxa and 68% endemism (Ozsoy et al., 2015). *Centaurea* species are utilized in conventional medicine for anti-diabetic, antibacterial, anti-malaria, anti-rheumatic, diuretic, antipyretic, neurological dysfunctions, and more (Zengin et al., 2018a). It is also a potential natural source of antioxidants used to prevent and treat diseases induced by reactive oxygen species (Korga et al., 2017). The biological potentials of *Centaurea* species are mostly related to flavonoids and sesquiterpene lactones (Petropoulos et al., 2020).

In this research, the phytochemical composition of methanol extract of *C. sivasica*, its antioxidant qualities, inhibitory activities for various enzymes, and antimicrobial activities are explained. Besides, molecular docking studies were conducted to understand how phytochemicals effect may be caused on the tyrosinase, α-amylase, and α-glucosidase enzyme activities. As there are no studies in the literature concerning the phytochemical composition and bioactivities of *C. sivasica*, the data gave here can be considered the first record.

2. Materials and methods

Chemicals and media used in bioactivity studies were purchased from Sigma-Aldrich (Schnelldorf-Germany). Microorganisms used in antimicrobial studies were obtained from the American Type Culture Collection (ATCC). Ultra-pure water (18 mΩ) was obtained from a Milli-Q water purification system (Millipore Co., Ltd.).

2.1. Collection and extraction of the plant material

C. sivasica was collected in Kayseri-Kocasinan at 1134 m in the Turkey Middle Anatolian region and was previously authenticated. A voucher specimen has been held in the Kırıkkale University Herbarium Anatolicum (ADO).

The aerial parts of the plant were dried in a place with no sunlight and airflow. Then, it was ground in the mill (the mean particle size is 297 microns) and passed to the extraction stage. Ground plant material (100 g) and methanol (1 L) were added to the Erlenmeyer flask. The extraction was continued by maceration for 3 days at room temperature in the dark, and the extract obtained every day was filtered. The solvent was removed in a rotary vacuum evaporator at 40°C. Samples of the extract were stored at 4°C until use. The extract yield was 6.7%. Methanol has been chosen as a suitable solvent in our bioactivity studies because it takes more compounds in plants than other solvents with close polarity to methanol (Chigayo et al., 2016).

2.2. The total phenolic and total flavonoid contents

The total phenolics content (TPC) was studied with the Folin - Ciocalteu method on a 96-well microplate with some modifications (Sarikurkcu et al., 2018). Briefly, 12.5 μL Folin–Ciocalteu reagent (diluted 1:9) was added to 25 μLextract and 187.5 μL ultrapure water in each well of a 96-well microplate. The mixture added 25 μL of 20% (w/v) sodium carbonate. The absorbance was read at 760 nm in a microplate reader. The results were expressed as milligram of gallic acid (standard) equivalents per gram of extract (mg GAE/g extract).

The total flavonoids content (TFC) was assessed using the aluminum chloride colorimetric method with some modifications (Sarikurkcu et al., 2018). The extract was mixed with methanol (25 μL), 5% $NaNO_2$ (10 μL), and ultrapure water (100 μL) in each well of a 96-well microplate. The mixture was added 10% aluminum chloride (15 μL). Five minutes later, 100 μL 1 M NaOH and 50 μL ultrapure water were added, and absorbance was measured at 510 nm. A standard curve was plotted using rutin, and results were expressed as milligram of rutin equivalent (RE) per gram of extract (mg RE/g extract).

2.3. LC-ESI-MS/MS analysis

2.3.1. Preparation of C. sivasica and standard solutions

All the standards were dissolved in methanol to develop the method of 57 phenolic compounds, and stock solution at a concentration of 1 mg/ml of the compounds was prepared. The stock solutions were diluted to a concentration of 8 μg/mL, but scutellarin, quercetin-3-galactoside, quercetin-3-β-D-glucoside, isoquercitrin, kaempferol-3-glucoside, baicalin, baicalein, 7-hydroxyflavone, 6-hydroxyflavone, and flavon were prepared at 0.8 μg/ml concentration with 50% methanol in grade water. Nine calibration levels were prepared by serial dilution of the standard mixture containing 57 phenolic compounds to plot the calibration curve with 50% methanol in grade water. At least five concentration points were determined within different concentrations.

The dry extract solution of 10 mg *C. sivasica* was prepared at a concentration of 2 ml in methanol, and the solution was diluted to 2 mg/ml with 50% methanol in grade water. Subsequently, the solution was filtered through 0.45 μm filters and transferred into vials prior to LC–MS/MS analysis.

2.3.2. LC-ESI–MS/MS instrumentation conditions

An Agilent Technologies 1260 Infinity II liquid chromatography System combined with a 6460 Triple Quad mass spectrometer was used for quantitative and quantitative analysis of 57 phytochemical compounds. Poroshell 120 EC-C18 (100 mm $\times$ 4.6 mm I.D., 2.7 μm) column was used for the chromatographic separation of the compounds. Mobile phase flow rate, column temperature conditions, and different mobile phases additives such as formic acid, ammonium acetate, and acetic acid were applied together with acetonitrile, purified water, and methanol mobile phases the ideal separation and ionization of the compounds. Thus, in chromatographic separation, mobile phases of 0.1% formic acid and 5 mM ammonium formate in water A mobile phase and 0.1% formic acid and 5 mM ammonium formate in methanol B mobile phase were used. Also, using a flow rate of 0.4 mL/min, a gradient program of 15% for 1–12 min, 50% for 12–30 min, 90% for 30–32 min, and 10% for 32–35 min was applied in the B mobile phase, respectively. The column temperature was maintained at 40°C, and the injection volume was 4.0 μL (Yilmaz, 2020).

An electrospray ionization (ESI) source operating in both negative and positive ionization modes was used to determine the mass to ion ratio (m/z) of the compounds. The ESI Source parameters were set at capillary voltage to 4000 V, nebulizing gas (N_2) flow to 11 L/min, nebulizer pressure to 15 psi, and gas temperature to 300°C to ensure ideal ionization of all compounds and achieve the ideal peak intensity. The precursor and product ions, collision energies, and fragmentor voltage of each compound were determined for quantitative measurement as multiple reaction monitoring (MRM).

2.3.3. Method validation

In this part of the study, validation of the LC–MS/MS method for the analytical measurement of phytochemical compounds was performed. MRM conditions such as ion transition, collision energies, and fragmentor voltage for each compound were determined. Validation studies for quantitative analytical methods are typically determined by the parameters of linearity, limits of detection and quantification (LOD/LOQ), repeatability, and recovery (Eurachem/Citac, 2012; Yilmaz, 2020). The stock solutions of compounds were diluted to the lowest limit of detection (LOD) up to twelve concentration levels for linearity. Each concentration level was studied in triplicate, and linear ranges were determined for each compound. The ranges selected as described in Table 2 of the calibration curves were found to be linear (R^2>0.99), and correlation coefficients (r≥0.99) were acceptable. LOD and LOQ were calculated using the equations:

$$LOD = Mean + 3 \times SDandLOQ = Mean + 10 \times SD$$

Precision studies were evaluated using the RSD% (Relative Standard Deviation). Intra-day and inter-day repeatability was 2.16–13.10% and 2.25–13.85%, respectively, and the calculated recovery value was found between 0.857 and 1.054%. Recovery was used to calculate by the following equation;

$$\text{Recovery}(\%) = ((\text{detectedconcentration} - \text{originalconcentration})/\text{spikedconcentration}) \times 100$$

2.4. Bioactivities

2.4.1. Antioxidant tests

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was studied with some modifications (Sarikurkcu et al., 2018). The extract (10 μL) was mixed with 190 μL DPPH solution (0.004%). The absorbance was read at 517 nm after 30 min incubation at room temperature.

ABTS cation radical scavenging activity was tested with some modifications (Sarikurkcu et al., 2018). $ABTS^{+}$ radical cation was prepared by reacting 7 mmol/L of 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt with 2.45 mmol/L potassium persulfate for 16 h at room temperature, and ABTS solution was diluted with methanol to reach an absorbance of 0.700 ± 0.02 at 734 nm. The extract (10 μL) and 190 μL ABTS solution were added to the wells for 6 min at room temperature. The absorbance was measured at 734 nm.

Ferric reducing antioxidant power (FRAP) was analyzed by making some modifications (Sarikurkcu et al., 2018). The FRAP reagent was made freshly by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM $FeCl_3 \bullet 6H_2O$ at a rate of 10:1:1 (v/v/v). Then, 312.5 μL reagent and 12.5 μL of the extract were added to the wells for 10 min at room temperature. The absorbance was measured at 593 nm.

Cupric ion reducing activity (CUPRAC) was performed with some modifications (Sarikurkcu et al., 2018). The CUPRAC reagent was prepared freshly by mixing 10 mM $CuCl_2$, 7.5 mM neocuproine and 1 M NH_4Ac (pH 7) at a ratio of 10:1:1 (v/v/v). The extract (25 μL) and 175 μL reagent were added to the wells for 30 min at room temperature. The absorbance was measured at 450 nm.

The total antioxidant activity of the samples was carried out by the phosphomolybdenum method with some alterations (Sarikurkcu et al., 2018). The reagent solution to be used in the method comprises 0.6 M H_2SO_4, 28 mM $Na_2HPO_4 \cdot 12H_2O$, and 4 mM ammonium molybdate at a ratio of 1:1:1 (v/v/v). 12.5 μL of the extract and 125 μL of reagent solution were added, and the samples were mixed and incubated for 90 minutes at 95°C. The absorbance was measured at 695 nm.

2.4.2. Enzyme inhibition tests

The tyrosinase inhibitory activity was determined by the dopachrome method using L-DOPA as substrate (Sarikurkcu et al., 2018). 25 μL extract was mixed with 40 μL tyrosinase solution and 100 μL sodium phosphate buffer (pH 6.8), and the mix was incubated at 25° C for 15 min. After adding 40 μL of L-DOPA, the mixture was incubated at 25°C for 10 min. The absorbance was measured at 492 nm. Also, we examined the inhibitory effects of scutellarin on tyrosinase activity. It was dissolved in DMSO at 1 mg/mL, then diluted ten times with pure water. Enzyme activity was assayed at five different scutellarin concentrations.

The α-amylase inhibitory activity was determined by the Caraway-Somogyi iodine/potassium iodide (IKI) method (Sarikurkcu et al., 2018). The extract (25 μL) was mixed with 50 μL α-amylase solution made in a buffer (sodium phosphate, pH 6.9). The mixture was incubated at 37°C for 10 min. The reaction was started by adding 50 μL starch solution to the wells and incubated at 37°C for 10 min. The reaction was then stopped with 25 μL 1 M HCl, and a 100 μL potassium iodide solution was added. The absorbance was measured at 630 nm.

The α-glucosidase inhibitory activity was determined using para-nitrophenyl-α-D-glucopyranoside (pNPG) as substrate (Uysal et al., 2021). The extract (50 μL) was mixed with 50 μL glutathione, 50 μL α-glucosidase solution prepared in a buffer (sodium phosphate, pH 6.8), and 50 μL pNPG. The mix was incubated at 37°C for 15 min, and the reaction was stopped by adding 50 μL sodium carbonate. The absorbance was measured at 400 nm.

Antioxidant and enzyme inhibition tests of the extract were expressed in mg standard equivalent/g extract and compared to Trolox (TE), kojic acid (KE), and acarbose (AKE) used as positive controls. For enzyme inhibition, radical scavenging, and metal chelation tests, the sample concentration that reduced the initial concentration by 50% was defined as the IC_{50}.

2.4.3. Antimicrobial tests

Gram-positive *S. aureus* (ATCC 29213) and *B. cereus* (ATCC 11778), Gram-negative *E. coli* (ATCC 25922) were used to determine the antibacterial effects of the methanol extract against pathogenic bacteria (Javan bakht Dalir et al., 2020; Kocyigit et al., 2018, 2017; Köksal et al., 2017). *C. albicans* (ATCC 10213) was used to determine the antifungal effect of the extract.

The test bacteria used in the study were grown overnight in Mueller-Hinton agar at 37°C. The colonies obtained from the new colony cultures were suspended in sterile saline water and compared with a 0.5 McFarland turbidity tube to prepare a 10^6 and 10^8 CFU/mL dilution for yeast and bacteria. The disc diffusion method was used to determine the antimicrobial activity of *C. sivasica* methanol extract. 25 μL of plant extract was absorbed into 6 mm diameter blank sterile discs and placed in the plates. Bacteria were incubated at 37°C and yeast at 30°C for 18-24 hours. Ampicillin, Gentamicin, and Nystatin standard antibiotic discs were used as positive controls. DMSO was used as a negative control. The inhibition zones were measured by vernier caliper.

A serial dilution technique achieved the minimum inhibition concentration (MIC) in a 96-well microplate (Javan bakht Dalir et al., 2020). The methanol extract of *C. sivasica* was diluted to a final concentration of 1000–1.95 $\mu g/mL$ in a 96-well microplate to obtain ten times two-fold dilutions. 20 μL of bacterial and yeast cultures were added to 100 μL of the methanol extract and 180 μL of a medium, separately and a total volume was completed to 300 μL. Medium containing 20 μL of cell culture without extract was used as a negative control. Initial absorbance values were read from the microplate reader at 620 nm. Microplates were incubated for 24 h at 37°C. At the end of the incubation period, the absorbance values were reread and recorded. After incubation, MIC values were defined as the lowest concentration of extract that completely inhibited the microorganism's proliferation.

2.5. Docking studies

Molecular docking studies were performed to determine phytochemicals found in the extract, which play an essential role in enzyme interactions. The 3D structures of compounds were retrieved from the PubChem database (www.pubchem.ncbi.nlm.nih.gov) as SDF formats. The crystal structures of the enzymes for the docking studies were downloaded from the Protein Data Bank (http://www.rcsb.org) with identification codes: α-glucosidase (PDB code: 3A4A), α-amylase (PDB code: 4W93) and tyrosinase (PDB code: 2Y9X) (Ismaya et al., 2011; Williams et al., 2015; Yamamoto et al., 2010). These structures were imported at Molegro Virtual Docker software (Maiti and Bidinger, 1981). The structural errors of proteins were checked; incorrect residues have been rearranged and optimized both within themselves and with neighboring residues with which they interact. Cavities of the reference ligand bind in the crystal structure were selected as docking regions. For the validation of docking protocols, we performed by redocking with the co-crystallized ligand. The molecular interactions between enzymes and phytochemicals with higher binding affinity to the proteins were analyzed using Discovery Studio Visualizer 2020 software.

2.6. Statistical analysis

All assays were performed in triplicate. The results are expressed as the mean and standard deviation values (mean ± SD). Tukey's honestly significant difference post hoc test was used to determine whether the results were statistically different from each other. The Pearson correlation coefficients were calculated to evaluate the relationship between TPC, TFC, and the biological activities examined. Biplot-Principal component analysis (PCA) - was applied to the data to form groups with similar biological activity characteristics. Statistical procedures were carried out with R software (v.4.0.3).

3. Results and discussion

3.1. TPC, TFC, and LC–ESI-MS/MS

Centaurea species are widely used as a traditional medicine to treat many diseases (Uysal et al., 2021). This situation requires the screening of *Centaurea* species for compounds that could be drugs in the industry. Therefore, in this study, first, the phytochemical profiling of *C. sivasica* with TPC, TFC, and LC-ESI-MS/MS was performed. The TPC and the TFC are listed in Table 1.

TPC was 21.42 mg GAE/g extract, and TFC was 19.73 mg RE/g extract of the methanol extract. The results suggest that TPC and TFC in the *Centaurea* species can vary, but it can be said that the TFC ratio is high in general (Zengin et al., 2019a, 2018b). The high levels of flavonoids defined in the methanol extract according to the LC-ESI-MS/MS results support the high amount of TFC. According to the biplot-PCA (Fig. 6), TPC, TFC, and the studied tests were on the same side in high methanol extract concentrations (1000-500 μg/mL). It indicates that high TPC and TFC have a high value for these tests.

Phenolics and flavonoids were quantitatively determined in the extract of *C. sivasica*, as seen in Table 2 and Fig. 1B. Fifty-seven compounds as phenolics and flavonoids were quantitatively analyzed in the plant extract using the LC-ESI-MS/MS system and also chromatograms of the standard phenolics, and the extract are given in Fig. 1A. A high amount of scutellarin (27843.91 μg/g), quercimeritrin (3629.85 μg/g), chlorogenic acid (2519.68 μg/g), and baicalin (920.49 μg/g) were found in the composition of the extract, respectively. In addition, that, kaempferol-3-glucoside (365.25 μg/g), shikimic acid (347.39 μg/g), quercetin-3-galactoside (346.16 μg/g), fisetin (246.11 μg/g), quercetin-3-β-D-glucoside (120.39 μg/g), cynarin (77.81 μg/g), rosmarinic acid (65.45 μg/g), tamarixetin (43.77 μg/g), and coumarin (37.31 μg/g) was found to the low amount in the composition of the extract as regards results of LC–ESI–MS/MS analysis (given in Table 2). Finally, since quercetin-3-β-D-glucoside and isoquercitrin are compounds that are similarly obtained from different companies, their levels were found to be (120.39 μg/g) and (121.49 μg/g), respectively.

Scutellarin, quercimeritrin, and chlorogenic acid were found as significant components in the methanol extract of the *C. sivasica* species. Chlorogenic acid (11.1 mg/g) levels were also determined in ethyl acetate extract of *C. triumfetti* (Acet, 2021). Phenolic compounds, which are chlorogenic acid from *C. depressa* (Demir et al., 2009), chlorogenic acid, kaempferol-3-glucoside, and quercetin-3-β-D-glucoside from *C. isaurica* (Flamini et al., 2004) like our findings have been reported in previous studies on *Centaurea*. However, extensive phenolic compounds from methanol extract of the *C. sivasica* species have not been reported.

Table. 1
TPC and TFC of *C. sivasica*.

	Methanol extract
Total phenolics (mg GAE/g extract)	21.42 ± 0.10
Total flavonoids (mg RE/g extract)	19.73 ± 0.17

The results are given as the mean ± SD.

3.2. Antioxidant effects

Phenolic and flavonoid compounds can scavenge free radicals as both primary and secondary antioxidants. That is why they are considered an indispensable ingredient in many foods with medicinal benefits (Amarowicz and Pegg, 2019). With this approach, the antioxidant properties of the methanol extract of *C. sivasica* were determined as both mg TE/g extract and IC_{50}. Trolox's IC_{50} values as a positive control in all antioxidant tests were also given (Table 3). The relationships between TPC, TFC, and antioxidant tests with the Pearson correlation test (Fig. 2) and biplot-PCA (Fig. 6) were evaluated.

The DPPH and ABTS scavenging activities for the methanol extract were 2.72 and 69.58 mg TE/g extract, respectively. DPPH and ABTS radical scavenging activity were lower than that detected in other studies related to various *Centaurea* species (Erel et al., 2014; Kenny et al., 2014; Köse et al., 2016). IC_{50} values were 23.4 and 387.1 μg/mL, respectively. Good correlations were established between TPC, TFC and DPPH (p = 0.01, r = 0.80–0.82). A very good correlation was found between TPC, TFC and ABTS (p = 0.001, r = 0.96–0.97). The stronger relationship between the ABTS test and TPC and TFC compared to the DPPH test can be because the ABTS test is superior to the DPPH test when applied mainly to plant extracts taken with absolute methanol (Shalaby and Shanab, 2013).

The FRAP, CUPRAC were used to determine the reducing power activity of the methanol extract. The FRAP and CUPRAC activities were 44.78 and 141.18 mg TE/g extract, respectively. FRAP and CUPRAC activity was similar to some *Centaurea* species (Zengin et al., 2018b). IC_{50} values were 419.7 and 345.1 μg/mL, respectively. A very good correlation has been found between TFC, TPC and metal-reducing power (p = 0.001, r = 0.92–0.99). The high chelation activity of the high phenolic and flavonoid contents can explain this strong relationship in the methanol extract (Heim et al., 2002).

According to the phosphomolybdenum method, the methanol extract's antioxidant power was ascertained as 109.25 mg TE/g extract. It can be said that this is lower than the other *Centaurea* studied (Zengin et al., 2019a, 2018b). The IC_{50} value was 474.7 μg/mL. The method measures the reduction degree of Mo (VI) to Mo (V) with antioxidant compounds such as phenolic and non-phenolic compounds, carotenoids, etc., under acidic pH. Considering that the phosphomolybdenum method is also a potent test for determining total antioxidant capacity (Zengin et al., 2019b), a perfect correlation was

Table. 2
Validation parameters of compounds by LC–MS/MS method and analysis of phenolic compounds in *C. sivasica*.

Codes	Name	Compounds/ plant extract (μg/g)	Rt	Precursor Ions (m/z) ->Product Ions (m/z)	Ion Polarity	LOD (μg/L)	LOQ (μg/L)	Linearity Range (μg/L)	R^2	Recovery (%)	Intra-Assay Precision (RSD %)	Inter-Assay Precision (RSD %)
1	Ascorbic acid	ND	1,47	175.1 ->114.9	Negative	48,1	141,2	250-4000	0,99	0.857	13,10	13,85
2	Shikimic acid	347,397	1,18	173.0 -> 93.1	Negative	68,25	210,24	500-8000	0,991	0.924	4,75	5,05
3	Gallic acid	ND	1,74	169.0 -> 125.0	Negative	4,8	15,25	31,25-1000	0,999	0,982	3,2	2,8
4	Protocatechuic acid	ND	2,77	152.9 -> 108.9	Negative	4,62	14,77	31,25-1000	0,997	0,958	8,88	7,95
5	Gentisic acid	ND	3,15	153.0 -> 109.0	Negative	9,45	32,5	125-2000	0,996	0,996	7,2	7,5
6	Catechin	ND	3,51	288.9 -> 245.1	Negative	28,74	69,24	250-8000	0,999	0,962	5,93	5,58
7	4-hydroxybenzoic acid	ND	4,54	137.0 -> 93.1	Negative	19,25	54,12	250-8000	0,999	0,981	2,27	2,25
8	Chlorogenic acid	2519,683	5,30	353.0 -> 191.0	Negative	23,4	74,1	125-4000	0,998	0,976	8,5	8,86
9	4-hydroxybenzaldehyde	ND	5,77	121.0 -> 92.0	Negative	8,78	26,7	62,5-2000	0,998	1,054	7,14	8,02
10	Vanillic acid	ND	5,86	167.0 -> 151.8	Negative	22,54	52,1	125-4000	0,999	0,993	3,33	3,8
11	Caffeic acid	ND	6,05	178.9 -> 135.1	Negative	2,63	10,8	31,25-1000	0,999	0,99	3,4	4,1
12	Epicatechin	ND	6,83	290.9 -> 138.8	Positive	8,45	19,69	62,5-4000	0,998	1,02	6,1	5,8
13	Syringic acid	ND	6,34	197.1 -> 181.8	Negative	26,98	83,2	250-8000	0,994	0,943	9,83	10,23
14	p-Coumaric acid	ND	8,50	163.0 -> 119.0	Negative	2,25	7,8	15.625-1000	0,999	1,009	5,78	5,21
15	Salicylic acid	ND	8,89	137.0 -> 93.1	Negative	15,94	47,84	125-4000	0,999	0,988	2,16	3,5
16	Taxifolin	ND	9,11	304.8 -> 258.9	Positive	39,3	139,2	500-8000	0,998	1,028	6,8	6,78
17	Polydatine	ND	9,74	390.9 -> 228.9	Positive	0,97	2,83	31,25-1000	0,996	0,997	7,66	7,52
18	Trans-ferulic acid	ND	9,58	193.1 -> 133.9	Negative	12,45	35,32	62,5-4000	0,997	0,956	8	8,2
19	Sinapic acid	ND	9,90	223.1 -> 208.0	Negative	25,6	90,09	250-4000	0,999	1,018	5,52	5,4
20	Quercimeritrin	3629,853	10,38	464.8 -> 302.9	Positive	3,13	10,21	31,25-2000	0,998	0,984	7,34	7,89
21	Coumarin	37,314	10,64	147.1 -> 91.3	Positive	5,63	15,62	62,5-2000	0,999	1,016	4,73	4,05
22	Scutellarin	27843,914	11,20	462.8 -> 286.8	Positive	2,3	6,2	12,5-800	0,997	0,992	9,66	8,81
23	O-coumaric acid	ND	11,30	163.0 -> 119.1	Negative	6,06	13,36	62,5-1000	0,998	1,001	4,74	4,6
24	Cynarin	77,812	11,37	516.8 -> 162.9	Positive	9,39	28,3	62,5-2000	0,994	0,977	5,66	5,89
25	Protocatechuic ethyl ester	ND	11,34	181.0 -> 107.9	Negative	0,56	2,1	15,625-250	0,996	0,958	7,45	6,98
26	Quercetin-3-galactoside	346,16	11,89	464.8 -> 302.8	Positive	0,38	2,06	6,25-800	0,998	0,946	6,37	6,52
27	Quercetin-3-β-D-glucoside	120,385	11,91	464.8 -> 302.9	Positive	1,04	3,12	12,5-800	0,999	1,008	3,57	3,02
28	Isoquercitrin	121,488	11,91	464.9 -> 302.8	Positive	0,95	3,23	12,5-800	0,999	1,007	3,21	3,3
29	Rutin	ND	11,98	608.9 -> 299.4	Negative	21,3	62,5	250-8000	0,999	0,994	8,96	8,15
30	Resveratrol	ND	11,98	227.0 -> 142.9	Negative	12,18	38,44	125-2000	0,998	1,017	6,46	5,23
31	Naringin	ND	12,03	580.7 -> 272.8	Positive	14,68	43,8	62,5-8000	0,998	0,989	2,62	3,66
32	Rosmarinic acid	65,448	12,17	358.9 -> 160.7	Positive	22,72	69,78	125-4000	0,998	1,013	5,66	5,28
33	Quercetin-3-D-xyloside	ND	12,51	432.7 -> 299.5	Negative	45,85	125,8	500-8000	0,999	0,938	7,25	7,1
34	Hesperidine	ND	12,53	611.0 -> 302.9	Positive	10,6	38,3	62,5-2000	0,999	0,995	4,8	4,98
35	Baicalin	920,486	12,53	446.8 -> 270.9	Positive	0,39	2,21	6,25-800	0,999	0,979	2,81	3,01
36	Neohesperidin	ND	12,86	610.7 -> 302.9	Positive	18,93	69,5	250-4000	0,998	0,962	6,96	6,88
37	Kaempferol-3-glucoside	365,253	13,35	448.8 -> 286.9	Positive	0,61	2,31	6,25-200	0,999	0,985	5,43	5,52
38	Fisetin	246,107	13,53	286.8 -> 137.1	Positive	20,8	68,5	125-4000	0,996	0,942	9,75	8,9
39	Oleuropein	ND	13,84	539.1 -> 275.1	Negative	9,68	36,2	125-4000	0,996	1,036	6,68	6,2
40	Trans-cinnamic acid	ND	14,35	147.1 -> 103.1	Negative	60,35	190,1	500-8000	0,997	1,015	4,14	4,5
41	Ellagic acid	ND	15,25	301.0 -> 145.0	Negative	72,5	226,5	500-8000	0,992	1,021	6,2	6,8
42	Quercetin	ND	15,03	300.7 -> 150.9	Negative	4,54	12,6	15,625-1000	0,999	1,006	2,88	2,65
43	Naringenin	ND	15,53	270.9 -> 119.1	Negative	2,8	7,81	31,25-4000	0,999	1,02	3	2,8
44	Silibinin	ND	15,93	482.8 -> 163.1	Positive	2,96	9,74	62,5-2000	0,999	0,996	4,06	4,25
45	Hesperetin	ND	16,25	300.9 -> 164.0	Negative	15,7	33,25	62,5-4000	0,995	0,938	11,53	12,02
46	Morin	ND	15,97	302.8 -> 153.0	Positive	3,19	12,6	62,5-2000	0,998	0,985	3,13	3,75
47	Kaempferol	ND	16,62	284.9 -> 116.9	Negative	37,26	128,1	500-8000	0,998	0,998	5,02	5,63
48	Tamarixetin	43,765	16,20	315.0 -> 299.9	Negative	4,73	15,68	31,25-8000	0,999	1,001	3,13	3,09
49	Baicalein	16,197	18,14	271.0 -> 123.0	Positive	1,95	6,3	50-800	0,999	1,012	5,76	5,22
50	7-hydroxyflavone	ND	18,97	238.9 -> 137.1	Positive	2,19	5,87	6,25-200	0,994	0,985	7,28	7,21
51	6-hydroxyflavone	ND	19,60	239.0 -> 103.1	Positive	1,97	6,15	12,5-200	0,996	0,952	10,5	9,65
52	Biochanin A	17,659	20,59	284.9 -> 151.9	Positive	2,45	7,81	62,5-2000	0,999	1,021	4,43	5
53	Chrysin	28,374	20,85	254.9 -> 153.0	Positive	4,84	15,63	31,25-1000	0,999	1,034	5,9	6,21
54	Flavone	0,8	21,69	223.0 -> 77.2	Positive	1,52	6,02	6,25-200	0,999	1,005	2,6	2,82
55	5-hydroxyflavone	ND	23,69	238.9 -> 103.1	Positive	7,81	23,76	62,5-2000	0,999	1,024	4,15	4,3
56	6,2,4-trimetoxyflavone	0,951	24,57	312.9 -> 148.0	Positive	1,55	4,88	12,5-400	0,999	1,026	4,13	4,18
57	Diosgenin	0,833	34,51	415.0 -> 271.0	Positive	3,13	8,19	25-800	0,999	1,013	3,38	3,25

R_t, retention time, LOD, and LOQ: limit of detection and limit of quantification, respectively.

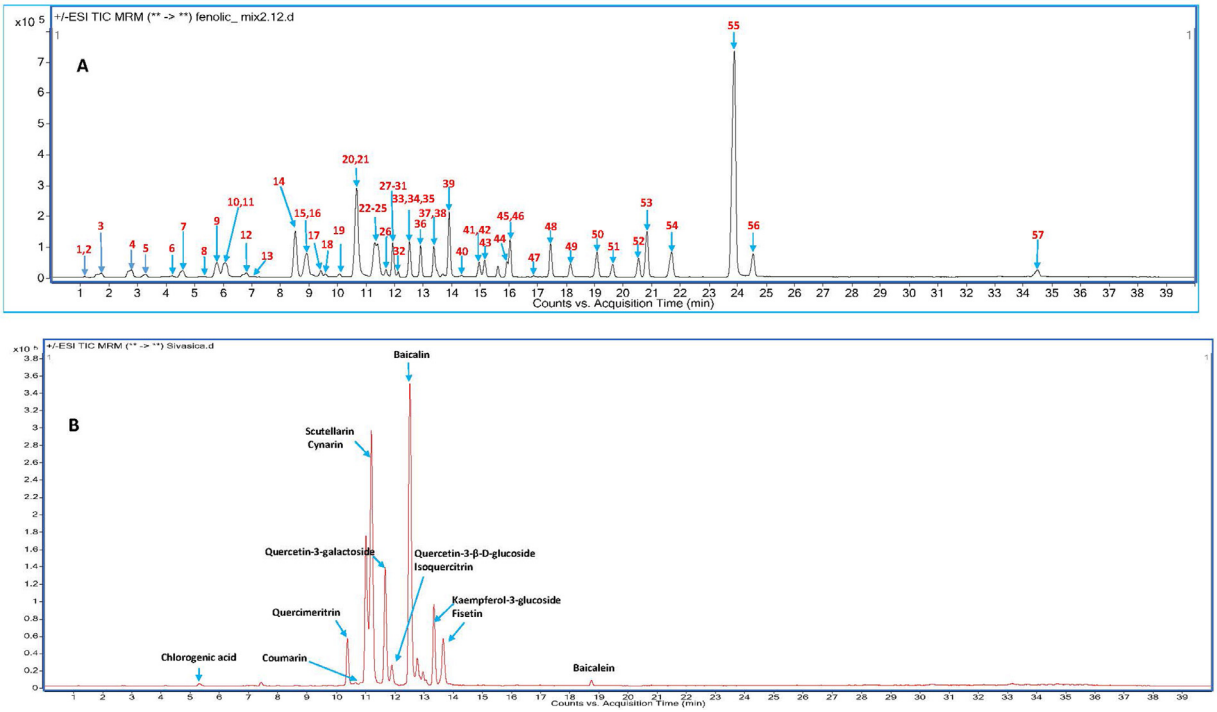

Fig. 1. LC–ESI–MS/MS MRM chromatograms of (A) the standard compounds (1. Ascorbic acid, 2. Shikimic acid, 3. Gallic acid, 4. Protocatechuic acid, 5. Gentisic acid, 6. Catechin, 7. 4-hydroxybenzoic acid, 8. Chlorogenic acid, 9. 4-hydroxybenzaldehyde, 10. Vanillic acid, 11. Caffeic acid, 12. Epicatechin, 13. Syringic acid 14. p-Coumaric acid, 15. Salicylic acid, 16. Taxifolin, 17. Polydatine, 18. Trans-ferulic acid, 19. Sinapic acid, 20. Quercimeritrin, 21. Coumarin, 22. Scutellarin, 23. O-coumaric acid, 24. Cynarin, 25. Protocatechuic ethyl ester, 26. Quercetin-3-galactoside, 27. Quercetin-3-β-D-glucoside, 28. Isoquercitrin, 29. Rutin, 30. Resveratrol, 31. Naringin, 32. Rosmarinic acid, 33. Quercetin-3-D-xyloside, 34. Baicalin, 35. Hesperidine, 36. Neohesperidin, 37. Kaempferol-3-glucoside, 38. Fisetin, 39. Oleuropein, 40. Trans-cinnamic acid, 41. Ellagic acid, 42. Quercetin, 43. Naringenin, 44. Silibinin, 45. Hesperetin, 46. Morin, 47. Kaempferol, 48. Tamarixetin, 49. Baicalein, 50. 7-hydroxyflavone, 51. 6-hydroxyflavone, 52. Biochanin A, 53. Chrysin, 54. Flavone, 55. 5-hydroxyflavone, 56. 6,2,4-trimetoxyflavone and 57. Diosgenin). (B) compounds in the methanol extract of *C. sivasica*.

Table. 3
The antioxidant capacity of *C. sivasica*.

	Methanol extract		Trolox
Method	(mg TE/g extract)	IC_{50} (μg/mL)	IC_{50} (μg/mL)
DPPH	2.72 ± 0.01	123.4 ± 1.11[a]	2.93[b]
ABTS FRAP	69.58 ± 0.04 44.78 ± 0.14	387.1 ± 1.09[a] 419.7 ± 1.02[a]	34.37[b] 20.96[b]
CUPRAC	141.18 ± 0.60	345.1 ± 1.01[a]	68.25[b]
Phosphomolybdenum	109.25 ± 0.18	474.7 ± 1.05[a]	145.4[b]

The results are expressed as the mean ± SD.
The values indicated by the different superscripts within the same line are different according to Tukey's honestly significant difference post hoc test at a 5% significance level.
IC_{50} (μg/mL), 50% inhibition concentration for all samples.

found between TPC and TPC and the phosphomolybdenum method ($p = 0.001$, $r = 0.98–0.99$).

As seen in Table 3, the IC_{50} values of Trolox in antioxidant tests were lower than methanol extract. Phosphomolybdenum test of the methanol extract was the closest test to Trolox in terms of IC_{50} value compared to other antioxidant tests. According to Tukey's honestly significant difference post hoc test results, antioxidant tests of methanol extract and Trolox were determined to be statistically different at a 5% significance level.

In this study, it is impossible to determine the components that contribute to the methanol extract's antioxidant activities. However, high correlation coefficients were found between TPC, TFC, and antioxidant activities. TPC, TFC, and antioxidant tests at high concentrations of the methanol extract were located on the same side in biplot-PCA. Also, the high antioxidant properties of the compounds (scutellarin, quercimeritrin, chlorogenic acid, and baicalin) found in high amounts in methanol extract were detected in previous studies, supporting these strong relationships determined statistically (Ahmed et al., 2016; Liang and Kitts, 2015; Liu et al., 2018; Shieh et al., 2000).

3.3. Enzyme inhibition effects

Tyrosinase, α-amylase, and α-glucosidase inhibitory activity tests were performed to determine the skin whitening and anti-diabetic properties of *C. sivasica* methanol extract. Results were found as both mg AKE/g extract, mg KE/g extract, and IC_{50} values. Also, IC_{50} values were given for positive controls (Table 4). The relationship between TPC, TFC, and enzyme inhibition tests was figured out by the Pearson correlation test (Fig. 3) and the biplot-PCA (Fig. 6).

Tyrosinase inhibitory activity was 36.81 mg KE/g extract of the methanol extract. This value was higher than other *Centaurea* species studied previously (Zengin et al., 2019a, 2018b). The IC_{50} value of the methanol extract was 81.62 μg/mL, and the IC_{50} value of kojic acid used as a positive control was 1.05 μg/mL. Thus, the methanol extract did not show as high inhibitory activity as kojic acid.

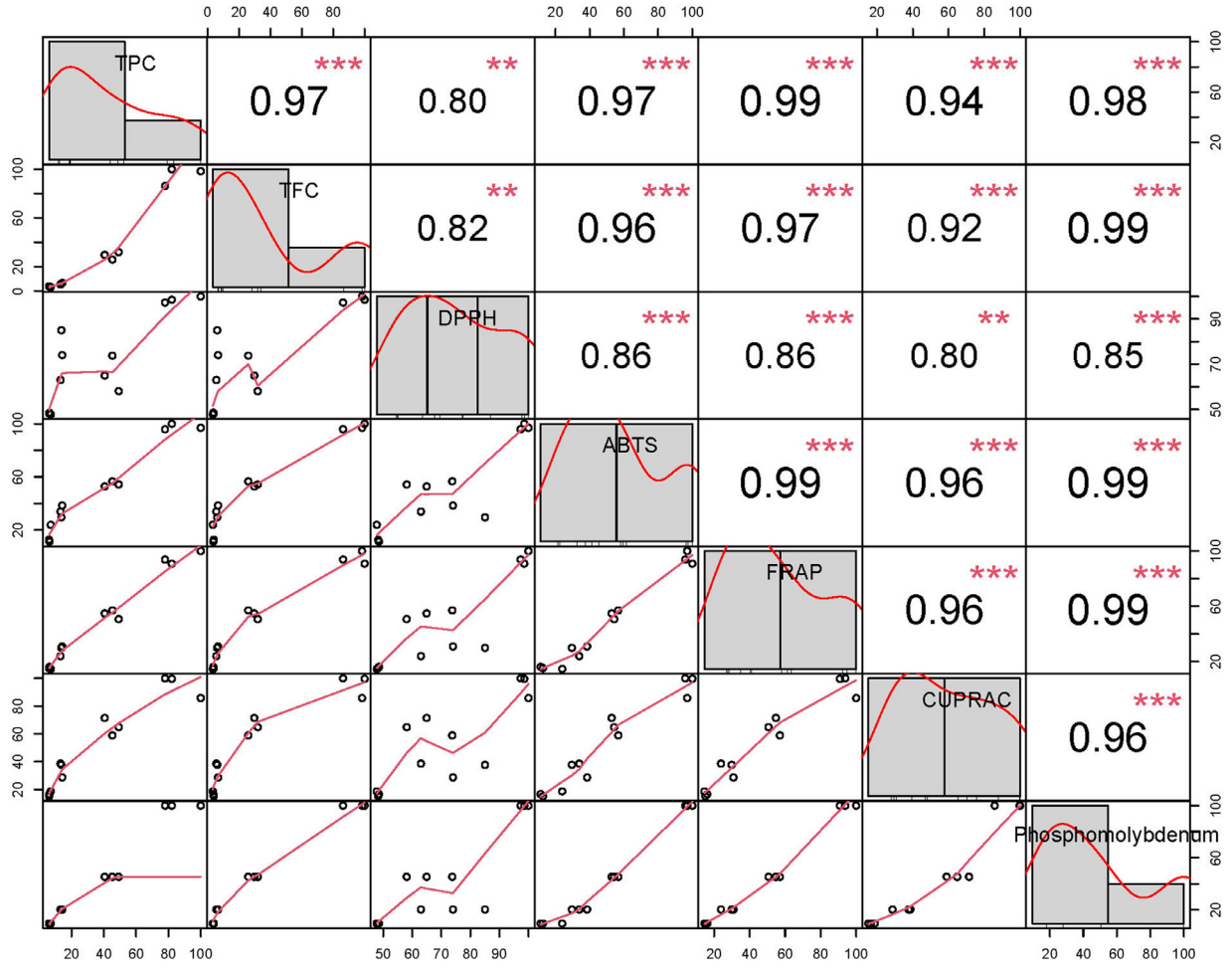

Fig. 2. The Pearson correlation coefficients ($p = 0.001^{***}$, $p = 0.01^{**}$) between TPC, TFC and, antioxidant tests.

Table. 4
Enzyme inhibitory activity of *C. sivasica* methanol extract and positive controls.

	Methanol extract			Kojic Acid	Acarbose
Enzyme	(mg KE/g extract)	(mg AKE/g extract)	IC_{50} (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)
Tyrosinase	36.81 ± 1.21	–	81.62 ± 1.10[a]	1.05 ± 0.04[b]	–
α-amylase	–	376.39 ± 1.82	279.40 ± 1.06[a]	-	249.50 ± 1.14[a]
α-glycosidase	–	27.77 ± 1.34	252.60 ± 1.24[a]	-	17.50 ± 1.40[b]

The results are shown as the mean ± SD.
The values indicated by the different superscripts within the same line are different according to Tukey's honestly significant difference post hoc test at a 5% significance level.
IC_{50} (μg/mL), 50% inhibition concentration for all samples.

According to the Tukey test results, it was determined that the inhibitory activities of methanol extract and kojic acid were statistically different from each other. According to the correlation test result, an excellent correlation was found between the TPC, TFC, and tyrosinase inhibitory effect of the methanol extract (p=0.001, r=0.92-0.98). Simultaneously, according to the biplot-PCA results, the fact that TPC, TFC, and tyrosinase inhibitor tests were in the same region in high doses of methanol extract indicates that TPC and TFC have a statistically high value for tyrosinase inhibition.

In the methanol extract, α-amylase inhibitory activity was 376.39 mg AKE/g extract, and α-glucosidase inhibitory activity was 27.77 mg AKE/g extract. According to some literature results, α-glucosidase inhibitory activity was lower, and α-amylase inhibitory activity was similar (Zengin et al., 2018b, 2018a). It was noteworthy that in terms of α-amylase inhibition, the IC_{50} value (279.40 μg/mL) of the methanol extract was close to the IC_{50} value (249.50 μg/mL) of acarbose used as a positive control, and there was no significant difference between them according to the Tukey test (Table 3). When evaluated in terms of α-glucosidase inhibition, a difference was found between the IC_{50} (252.60 μg/mL) value of methanol extract and the IC_{50} value (17.50 μg/mL) of acarbose. According to the correlation test results, it can be said that TPC and TFC have a good α-amylase and α-glucosidase inhibitory effect. It also appears that TFC and TPC in biplot-PCA have a high value in inhibiting α-amylase and α-glucosidase enzymes. According to these statistical results, it can be said that there are compounds useful in inhibiting these two enzymes in the methanol extract.

It should also be considered that interactions of different phytochemical compounds in the extract may have affected the inhibition of the enzymes studied.

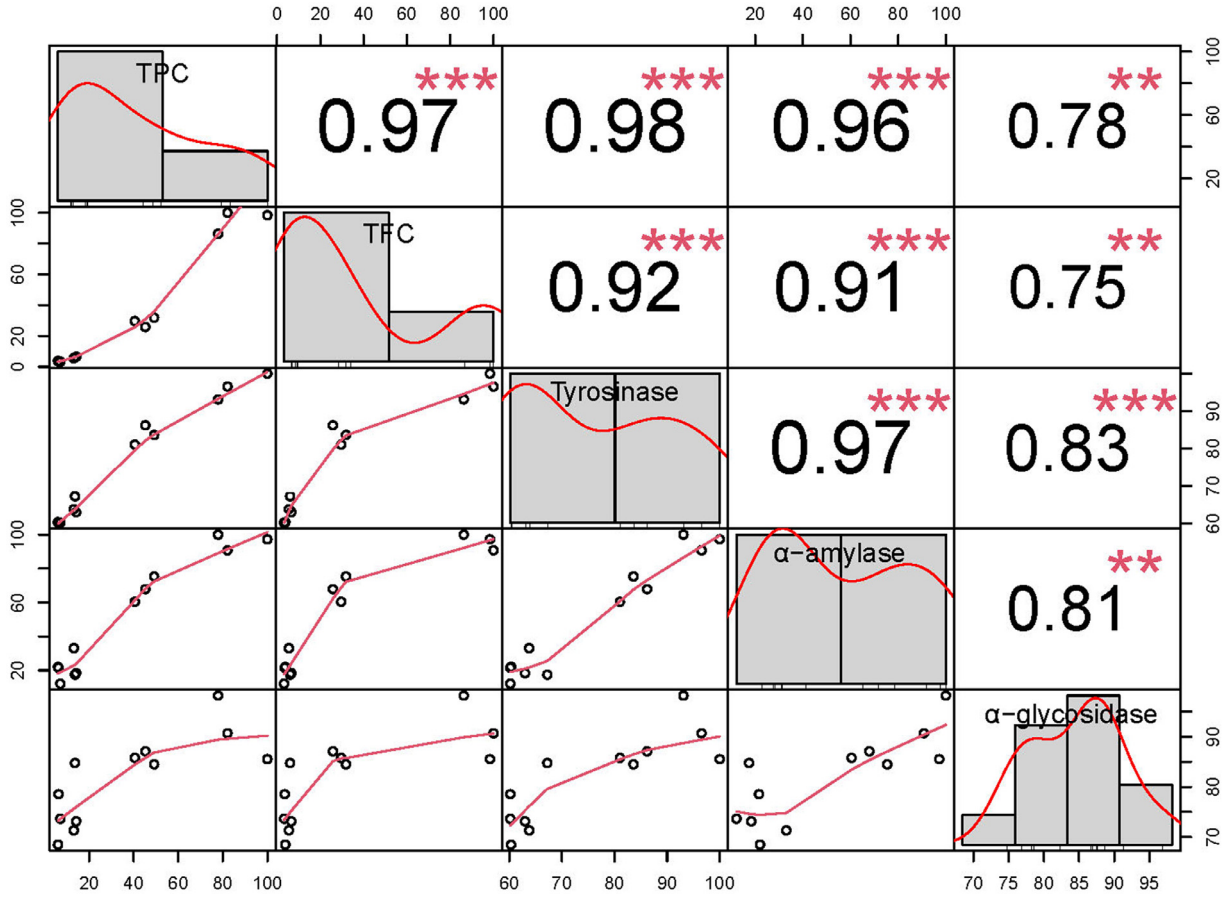

Fig. 3. The Pearson correlation coefficients ($p = 0.001^{***}$, $p = 0.01^{**}$) between TPC, TFC and, tyrosinase, α-amylase, and α-glucosidase enzyme inhibitions.

Scutellarin was found mostly in the extract, and since tyrosinase was the enzyme most affected by the extract, we determined the inhibition potential of scutellarin on the enzyme. The results showed a high inhibition effect of the scutellarin on this enzyme with IC_{50} values 43.32 μM or 20.38 μg/ml (Fig. 4).

3.4. Antimicrobial activities

It was determined that the methanol extract of *C. sivasica* showed varying inhibition zones and MIC values on test microorganisms and was listed in Table 5. Also, the relationship between TPC, TFC, and microorganism growth inhibition tests was evaluated by the Pearson correlation test (Fig. 5) and the biplot-PCA (Fig. 6).

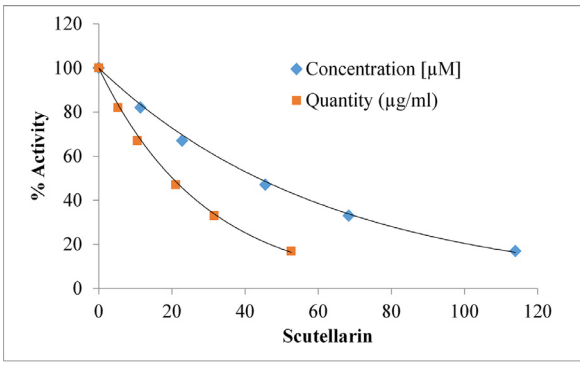

Fig. 4. The inhibition effect of scutellarin on tyrosinase activity.

Inhibition zone diameters between 15.0 mm and 16.3 mm were obtained in testing Gram-positive (*B. cereus, S. aureus*) bacteria, Gram-negative (*E. coli*) bacterium, *C. albicans*. Any inhibition zones were not detected in DMSO, which was used as the negative control. The most sensitive bacterium was identified as *B. cereus*. Compared with antibiotics used as positive controls, it was only found *B. cereus* to be more effective than Ampicillin (7 mm). The MIC values of methanol extract against Gram-positive bacteria (*B. cereus, S. aureus*), Gram-negative bacterium (*E. coli*), and *C. albicans* was found as 500 μg/mL, 500 μg/mL, 1000 μg/mL, 500 μg/mL, respectively (Table 4).

In previous studies, extracts from other *Centaurea* species appear to be particularly effective against Gram-positive bacteria (Güven et al., 2009; Özcan et al., 2019). Our Pearson correlation analysis and biplot-PCA results also support this by showing a better correlation (p=0.001, r=0.90-0.95) and a high value between TPC, TFC, and inhibition of Gram-positive bacteria (*B. cereus, S. aureus*) according to the Gram-negative bacterium (*E. coli*).

It can be said that the methanol extract of *C. sivasica* has an evident inhibitory effect on *C. albicans*, as is the case with other generally working *Centaurea* species (Özcan et al., 2019; Uysal et al., 2021). In Pearson correlation analysis and biplot-PCA, high TPC and TFC concentrations were strongly associated with *C. albicans* inhibition. Considering this study and studies with other *Centaurea* species, it can be said that *Centaurea* species have strong antifungal activities. It is necessary to find new and unique substances from *Centaurea* species in subsequent studies and investigate their antifungal activity.

Since microorganisms have a higher chance of developing resistance to the most known and commonly used antimicrobials, there is a need for new molecules to eliminate such resistance. Many phenolic compounds may also be useful in combating human pathogens,

Table. 5
The antimicrobial activity of *C. sivasica* methanol extract.

	The methanol extract Inhibition zones (mm)	Positive control Inhibition zones (mm)			Negative control Inhibition zones (mm)	The methanol extract MIC (μg/mL)
		Ampicillin	Gentamycin	Nystatin	DMSO	
B. cereus	16.3 ± 0.05	7	23		–	500 ± 28.58
S. aureus	16.0 ± 0.05	32	20		–	500 ± 15.40
E. coli	15.0 ± 0.06	16	23		–	1000 ± 11.53
C. albicans	15.6 ± 0.05	–	–	30	–	500 ± 31.45

The results are expressed as the mean ± SD.

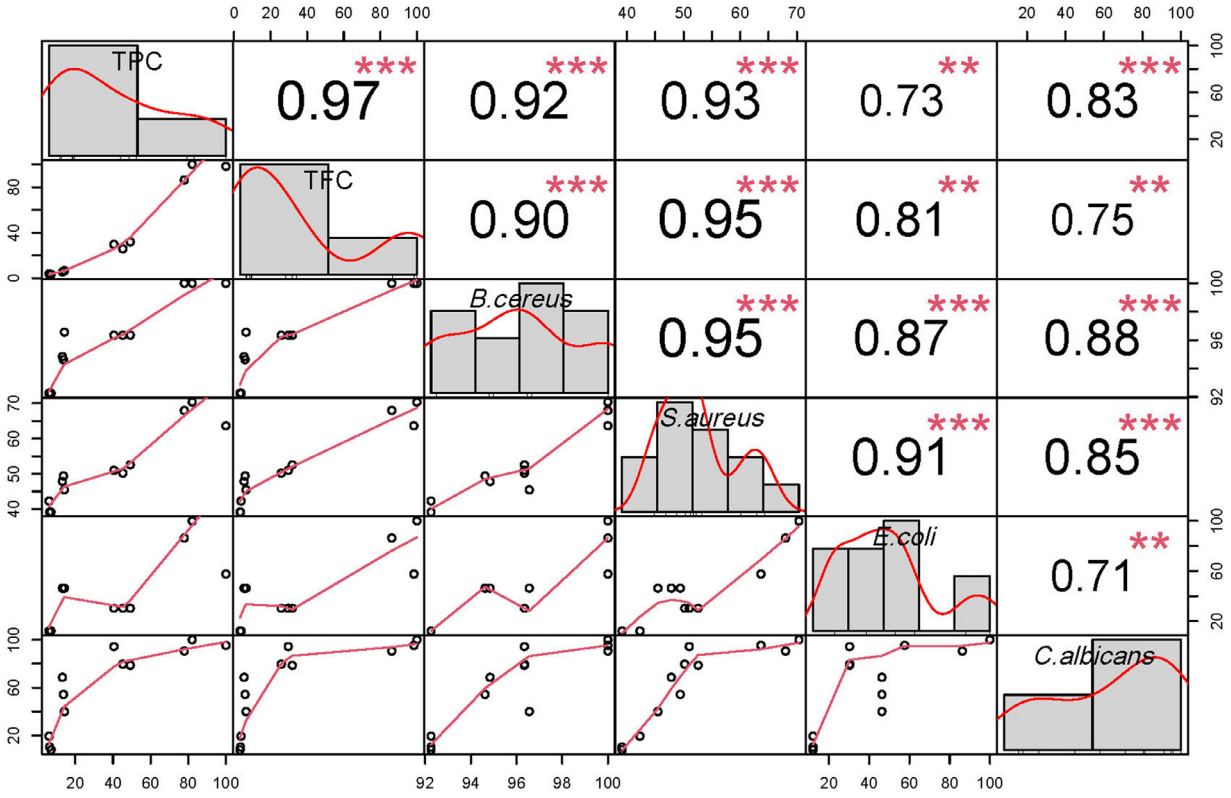

Fig. 5. The Pearson correlation coefficients between TPC, TFC and *B. cereus, S. aureus, E. coli, C. albicans* growth inhibitions ($p = 0.001^{***}$, $p = 0.01^{**}$).

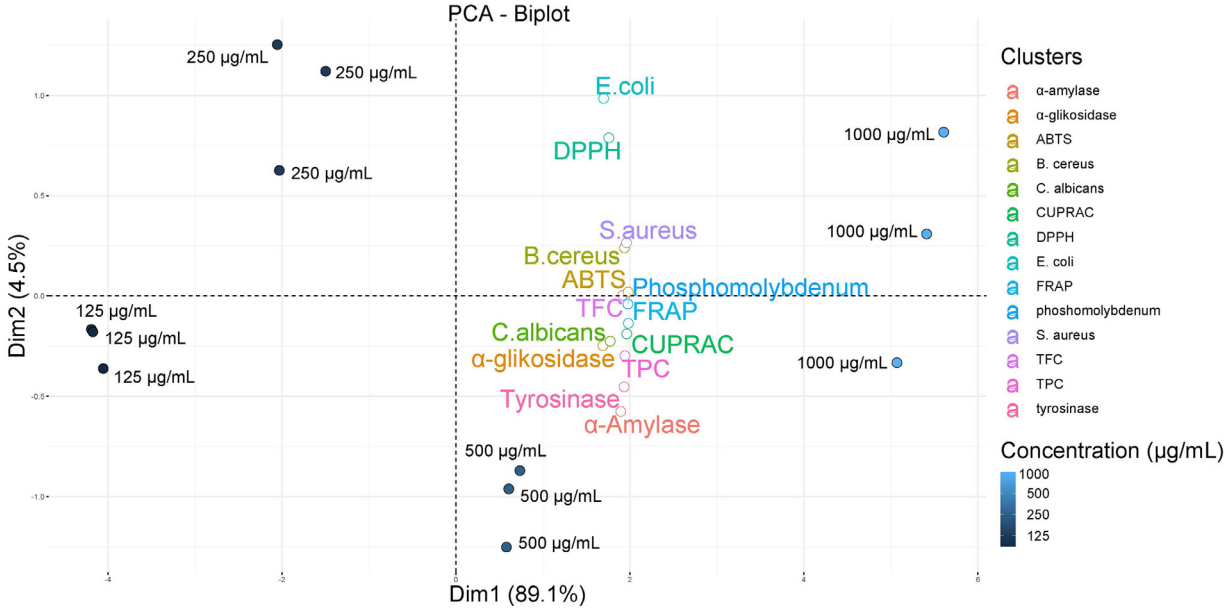

Fig. 6. An overview of the data, via the application of principal component analysis (PCA) to TPC, TFC, antioxidant properties (DPPH, ABTS, FRAP, CUPRAC, phosphomolybdenum method), enzyme inhibitor activities (tyrosinase, α-amylase, α-glucosidase), antimicrobial effects (*B. cereus, S. aureus, E. coli, C. albicans*) results found for different concentrations of methanol extract (1000-125 μg/mL), taking three from each concentration. A different color was created for each concentration of methanol extract.

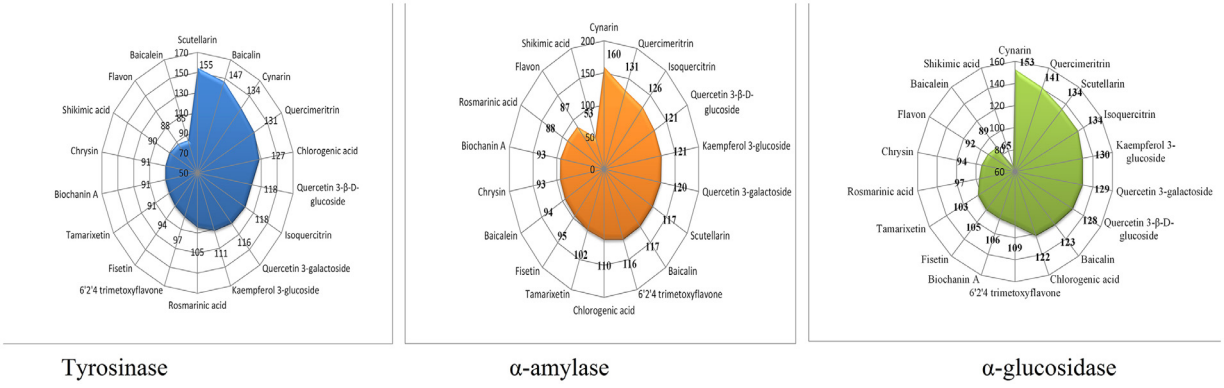

Fig. 7. The docking results of phytochemicals founded methanol extract of *C. sivasica* against three enzymes.

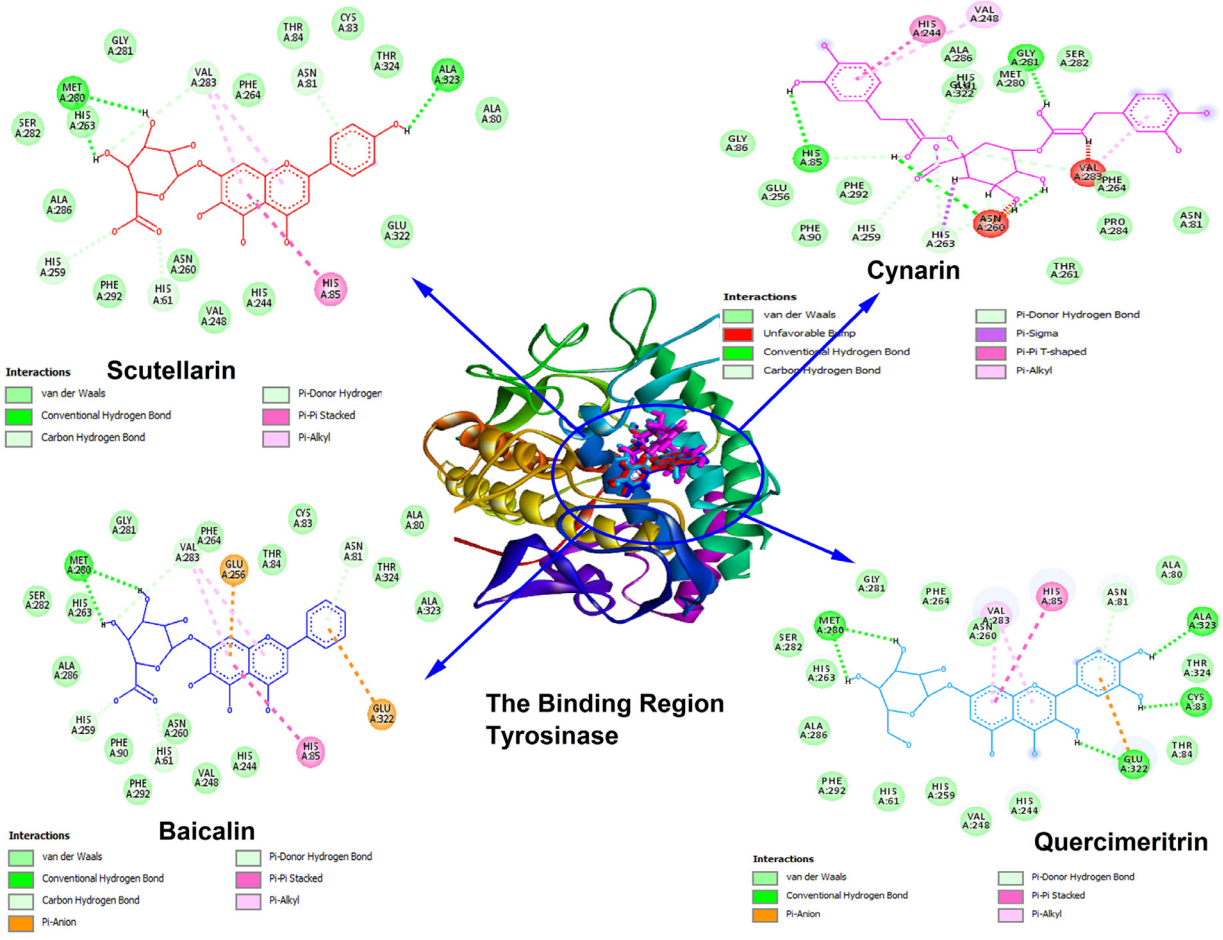

Fig. 8. Representation of docked ligand-protein complex and interaction of scutellarin, cynarin, baicalin, and quercimeritin with amino acid residues of tyrosinase.

given that they have potent antibacterial activity in protecting plants against many pathogens. Besides, given that bacteria or other pathogens do not readily develop resistance to plant-derived compounds, they may be important in enhancing antimicrobial therapy (Biharee et al., 2020). From this point of view, it has been determined in previous studies that many compounds in the methanol extract of *C. sivasica* have antibacterial and antifungal effects with different mechanisms (Biharee et al., 2020; Kang et al., 2010; Sung and Lee, 2010).

3.5. *In silico*

We conducted molecular docking studies to investigate the inhibition potential of phytochemicals found in the extract against target enzymes and explain the inhibition differences. The binding MolDock Scores obtained by Molegro Virtual Docker the phytochemicals with the target enzymes are shown in Fig. 7. The results revealed that cynarin, scutellarin, quercimeritrin have the most binding affinity against three enzymes. When in vitro enzyme inhibition and docking results

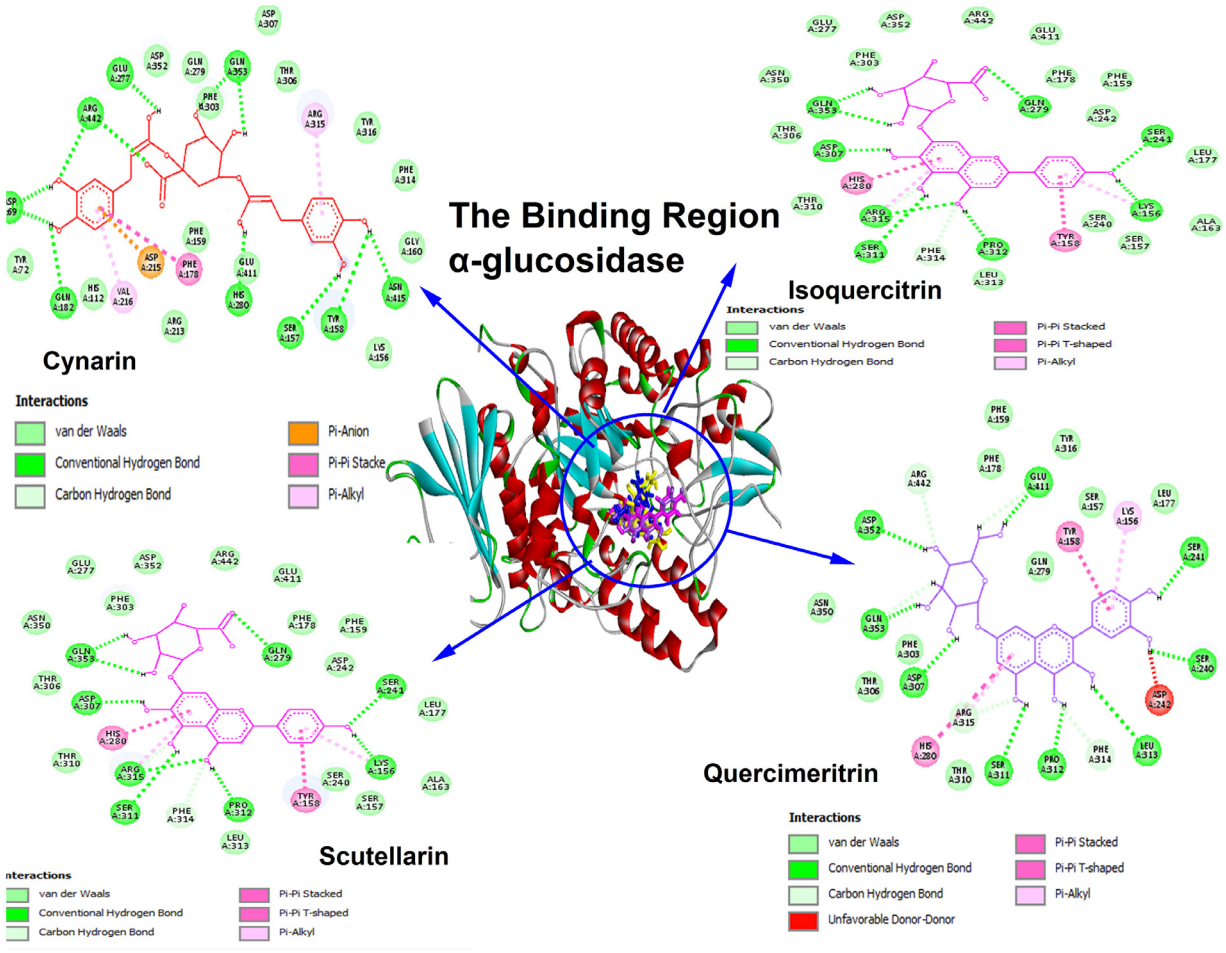

Fig. 9. Representation of docked ligand-protein complex and interaction of cynarin, scutellarin, isoquercitrin, and quercimeritin with amino acid residues of α-glucosidase.

are evaluated together, compounds such as scutellarin, quercimeritrin, chlorogenic acid, and baicalin are found in excess in the extract and have the highest potential to inhibit the tyrosinase enzyme. Similarly, these compounds have more potential to inhibit α-glycosidase than α-amylase. These results showed similar trends with an in vitro study reported in the literature (Li et al., 2018). It may be deducted from this data that in silico studies can explain the different effects of the extract on enzymes.

Molecular docking studies have revealed that scutellarin, baicalin, cynarin, quercimeritrin, and chlorogenic acid may be the compounds in the extract most likely to affect tyrosinase. Their interaction types and attachment positions are shown in Fig. 8. It is not possible cynarin affects enzyme activity due to its less amount. Scutellarin, the topmost docked compound to tyrosinase, was detected to utilize various interactions with the binding and catalytic amino acids of the enzyme. It interacted to via hydrogen bonds with Met 280 and Ala 923; carbon-hydrogen bond with Asn 81, Val 283, His 259, and His 61; van der Waals interactions with Ser 282, Gly 281, Phe 264, Thr 84, Cys 83, Thr 324, Ala 80, Glu 322, His 244, Val 248, Asn 260, Phe 292, Ala 286; $\pi-\pi$ stacked hydrophobic interactions with His 85; and π-alkyl hydrophobic interactions with Val 283 (Fig. 8). In vitro study of scutellarin supported it is in silico results. Therefore, it can be suggested that the reason for the extract's strong effect on tyrosinase enzyme activity may be scutellarin as the most abundant phenolic. Scutellaria is a major bioactive flavonoid in the Chinese medicinal herb *Scutellaria baicalensis* Georgi (Lamiaceae). It also reported that Scutellaria baicalensis extracts showed anti-browning effects (Kudo et al., 2017; Park et al., 2013). Baicalin has a similar chemical structure to scutellarin. It exhibited similar interactions compared with scutellarin. Scutellarin made a second hydrogen bond thanks to the -OH group at the 4 position. The previous study reported a dose-dependent fashion, the potent inhibition effects of baicalin on tyrosinase activity (Jeong et al., 2015).

We have applied molecular docking of all phytochemicals found in the extract with α-glucosidase, and the results are presented in Fig. 7. Interaction details of cynarin, quercimeritrin, scutellarin, and isoquercitrin are demonstrated in Fig. 9. Phytochemicals abundant in the extract, such as scutellarin, chlorogenic acid, baicalin, and quercimeritrin, have MolDock Score close to each other at between 144 and 122. Quercimeritrin sits at the active site of α-glucosidase, and the complex was stabilized by nine conventional and five carbon-hydrogen bonds and several hydrophobic interactions. Nine hydrogen bonds were formed with Asp 352, Glu 411, Ser 241, Ser 240, Leu 313, Pro 312, Ser 311, Asp 307, Gln 353, Asp 352 residues. Other charged and polar residues such as Asn 350, Phe 303, Thr 306, Thr 310, Leu 177, Ser 157, Tyr 316, Phe 159, and Phe 178 were involved in making van der Waal's interactions. The residues making hydrophobic interactions were his 280, Tyr 158, and Lys 156. The primary compound scutellarin is stabilized by eight hydrogen bonds, van der Walls, and mixed π hydrophobic interactions. The inhibitory effect of scutellarin against α-glucosidase was previously reported (Li et al., 2018). Previous in vitro reports and now conducted docking results revealed the inhibitory potential of scutellarin on α-glucosidase. Therefore, it is thought that scutellarin is an essential contributor to α-glucosidase inhibition due to

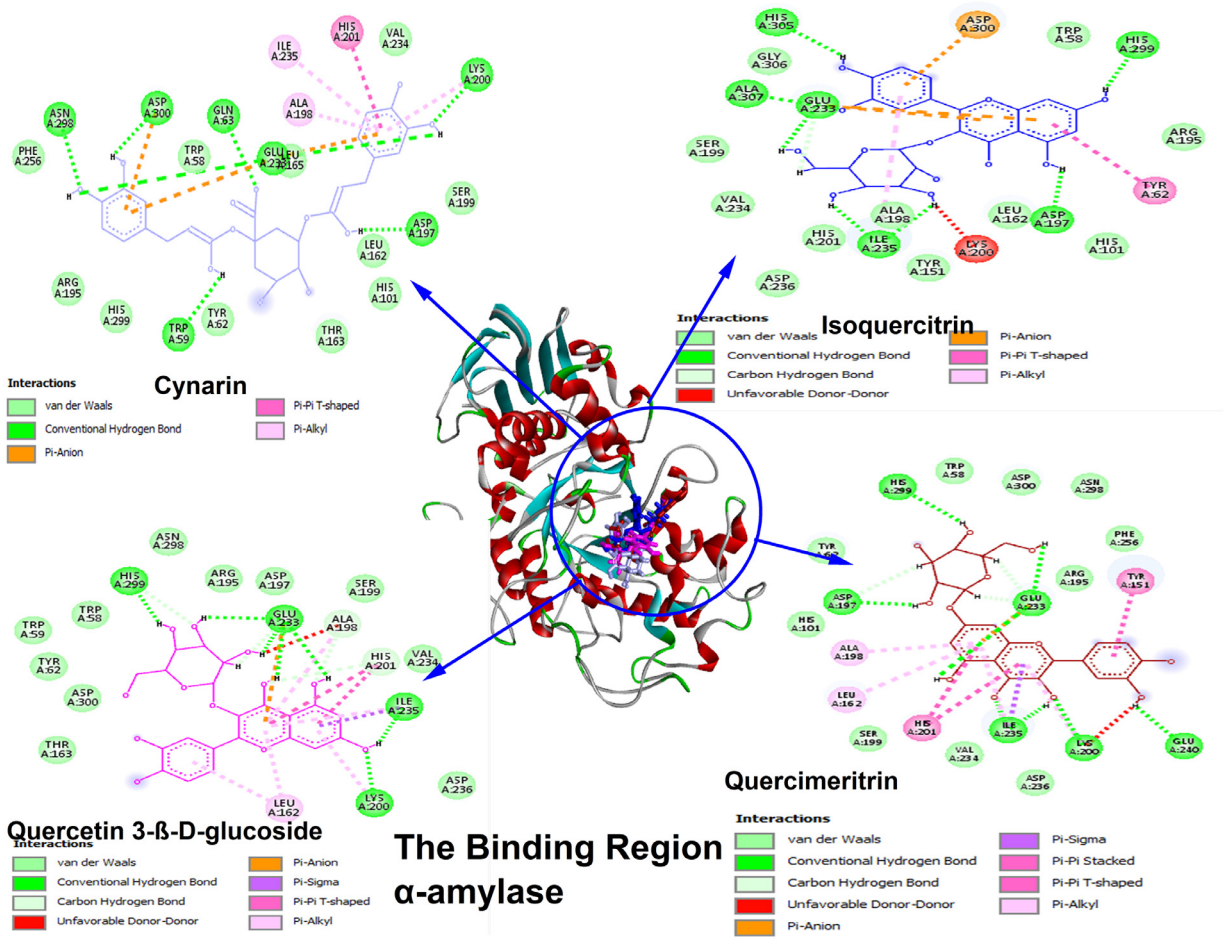

Fig. 10. Representation of docked ligand-protein complex and interaction of cynarin, isoquercitrin, quercetin 3-β-D-glucoside, and quercimeritin with amino acid residues of α-amylase.

both its excess amount and its inhibitory potential. Also, it has been evaluated that other compounds significantly contribute to α-glucosidase inhibitory activities of extract as the synergistic.

Generally, compounds detected in the extract have a low affinity to α-amylase. Compounds such as cynarin, isoquercitrin, and quercetin 3-β-D-glucoside have a high potential to affect the enzyme (Fig. 10). Nevertheless, their contribution to total inhibition will be minimal because of their small amount. Low inhibition potentials of compounds scutellarin, baicalin, baicalein, and chrysin have been reported in in vitro studies (Liu et al., 2018). These studies support the effectiveness of docking software.

The 2D interaction models of some phytochemicals at the molecular level are shown in Fig. 10. These data are shown that the O-glucoside forms of quercetin have more potent inhibition potential. Quercimeritrin is a single molecule have with both high inhibition potential and abundance. Results have shown that His 299, Glu 233, Asp 197, Glu 233, Glu 240, Lys 200, and Ile 235 were critical residues in hydrogen bonding of quercimeritrin with the enzyme. Other than this, the entire quercimeritrin exhibited several hydrophobic interactions.

4. Conclusion

In this study, antioxidant, tyrosinase, α-amylase, α-glucosidase inhibitory effects, and antimicrobial activities of the methanol extract of *C. sivasica* were tested.

Based on molecular docking studies, important phytochemicals contributing significantly to possible tyrosinase, α-amylase, and α-glucosidase inhibitory activities were identified. It has been thought that the phenolic and flavonoid contents contribute significantly to the antioxidant activity of the extract and can be used in the food industry to extend the shelf-life of foods. According to the results obtained from molecular docking and enzyme inhibition studies, scutellarin, a high amount in the extract, and extract have been an effective tyrosinase inhibitor. Due to this feature, scutellarin and the extract can be used as an alternative skin whitening agent in the cosmetics industry. It is evaluated that the extract had an important α-amylase and α-glucosidase inhibitory effect and could potentially be used to treat diabetes. Besides, It has been deduced that the effect of the extract, especially on Gram-positive bacteria (*B. cereus, S. aureus*) and *C. albicans*, would be evaluated in developing a potential antimicrobial drug. It has been concluded that although the extract, in general, has a high activity, a more detailed analysis is required to identify the compounds responsible for the activity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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