



# Antioxidant activities of *Alyssum virgatum* plant and its main components

Emre Koç<sup>1</sup> · Ayhan Üngördü<sup>2</sup> · Ferda Candan<sup>1</sup>

Received: 5 October 2021 / Accepted: 18 November 2021 / Published online: 27 November 2021  
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

## Abstract

The antioxidant properties of methanol extract of above-ground parts of *Alyssum virgatum*, an endemic plant, were analyzed. Together with their total phenolic, flavonoid, and antioxidant capacities, their effects on reactive oxygen species were determined by experimental methods. The methanol extracts of *A. virgatum* plant appeared to exhibit in-vitro antioxidant activity. In particular, the extract of the plant was found to have a scavenging effect against hydrogen peroxide and hydroxyl radical. Total phenolic content was found to be 161.25 mg gallic acid per gram dry material. Total flavonoid content was found to be 119.89 mg quercetin per gram dry material. Total antioxidant capacity was determined as 94.92 mM  $\alpha$ -tocopherol acetate per gram dry material. Moreover, the amount of the extract that caused 50% inhibition of hydrogen peroxide and hydroxyl radical was assayed as 29.24 mg mL<sup>-1</sup> and 46.04 mg mL<sup>-1</sup>, respectively.

Addition to the experimental studies, DFT, molecular docking, and ADME calculations were performed to determine antioxidant, biological activity, and drug properties of two main phenolic components of *A. virgatum* which are cinnamic acid and ferulic acid. DFT calculations were executed at B3LYP/6–311 + + G(d,p) level in Gaussian 16 software. The HAT, SET-PT, SPLET mechanisms, and the spin density analyses of the main components were investigated in detail. Molecular docking studies of the investigated main components were executed on the antioxidant proteins in Schrodinger 2020–3 program. Additionally, ADME properties of the mentioned main components were determined via QikProp module in the Schrodinger software. All theoretical studies showed that ferulic acid had better antioxidant, biological, and drug activities than cinnamic acid.

**Keywords** *Alyssum virgatum* · Methanolic extract · Antioxidant · DFT · Molecular docking · ADME

## Introduction

Brassicaceae family contains plenty of genders, *Crambe* [1], *Sinapis* [2], *Thlaspi* [3], *Alyssum* [4], and *Brassica* [5]. Regarding its antioxidant, antibacterial, and anticancer properties, family members are rich in terms of various biologically active molecules, such as glucosinolates, flavonoids, phenolic acids, and vitamins [6]. Glucosinolates are secondary metabolites containing sulfur and nitrogen and they

have properties to inhibit tumorigenesis in various types of cancer. Phenolic compounds with antioxidant and antimicrobial properties are also quite common in this family [7].

With the number of its species up to 230, the *Alyssum* L. genus is among the largest known genera in the world and its main distribution area is Eastern Europe and Turkey [8]. This genus is among the large genera of Turkey's flora, and it is indicated by 90 species. Fifty-four of these species are endemic, whereas about a third of these species are endangered and in need of protection [9]. After the latest revisions, this genus was found to have approximately 110 taxa [10].

One of the important defining qualities of secondary metabolites is the fact that they are stored as complex concentrations in high concentrations, and sometimes in organs that do not produce them. Some secondary metabolites are stored as inactive “primary medicines” and they are enzymatically activated in case of risk (burning, infection). Many secondary metabolites interact with proteins, DNA/RNA,

✉ Emre Koç  
emrekoc@cumhuriyet.edu.tr

✉ Ayhan Üngördü  
aungordu@cumhuriyet.edu.tr

<sup>1</sup> Department of Biochemistry, Faculty of Science, Sivas Cumhuriyet University, 58140 Sivas, Turkey

<sup>2</sup> Department of Chemistry, Faculty of Science, Sivas Cumhuriyet University, 58140 Sivas, Turkey

and/or bio membranes. Some of these interactions are very specific in terms of their molecular targets, while others have variable properties [11]. The number of primary metabolites is 2000 in humans, while it is between 5000 and 25,000 in plants. Known plant secondary metabolites are about 100,000 [12]. Based on their biosynthetic origins, plant secondary metabolites may be classed into three major groups: phenolic compounds, terpenoids, and nitrogen-containing molecules. These groups are chemically different from each other and are produced from the metabolic pathways that take place during the process of glycolysis, photosynthesis, and citric acid cycle. Secondary metabolites are synthesized via acetyl-CoA (acetyl coenzyme A), mevalonic acid, shikimic acid, and 1-deoxy-D-xylulose-5-phosphate, which are intermediate products of these processes that are the pathways of primary metabolism [13].

In HPLC analysis for *Alyssum virgatum* plant to be used in the study, cinnamic acid ( $2.05 \pm 1.0$  mg/g extract) and ferulic acid ( $1.85 \pm 0.09$  mg/g extract) are determined as two main phenolic components [14] which are given in Fig. 1.

Since the *Alyssum* L. genus distributed in Turkey is represented by quite a lot of species and the vast majority of them are endemic, the examination of the biological activities of the species of this genus and determination of its active ingredients is of great importance.

Computational chemistry is quite a popular research field in the science world [15–19]. Some properties of molecules have been estimated with the help of computational studies [20–24]. For example; density functional theory (DFT) and molecular docking studies assist to predict the antioxidant and biological activity properties of compounds, respectively [25–31]. In addition to these studies, absorption, distribution, metabolism, and excretion (ADME) results can be also used to examine the drug properties of molecules [31–35].

In this study, scavenging effects on reactive oxygen species and antioxidant activities of methanol extracts of above-ground parts of *A. virgatum*, which is an endemic plant, are analyzed by experimental methods. We experimentally

compared the methanol extract of *A. virgatum* plant with curcumin which are natural antioxidant and hydroxytoluene (BHT) that are synthetic antioxidant. Besides, DFT, molecular docking, and ADME calculations are executed to interpret the antioxidant, biological, and drug activities of cinnamic acid and ferulic acid molecules which are determined as two main phenolic components of *A. virgatum*.

## Methods

### Experimental methods

#### Preparation of the extract

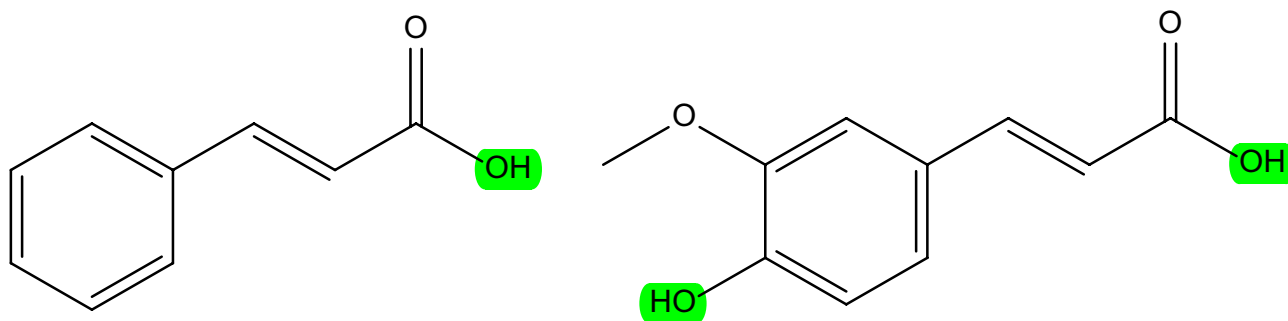
Air-dried *A. virgatum* plant (100 g) was shredded and extracted for approximately 4 h with methanol using a Soxhlet apparatus [36]. Then the extract was drained and evaporated to dryness in a vacuum at 45 °C. The plant extract was lyophilized and stored in the dark at +4 °C.

#### Determination of total phenolic content

The total phenolic content (TPC) of the mentioned methanol extract was found by using a Folin–Ciocalteu reagent (FCR) method, according to Singleton et al. [37]. After that, the sample solution (500  $\mu$ L) and 11.0 mL distilled water was mixed, undiluted FCR (250  $\mu$ L) was added. After 3 min, 750  $\mu$ L of 2% (w/v) sodium carbonate was mixed and then 2 h incubation at room temperature, the absorbance was measured at 760 nm. Gallic acid was preferred as a standard between 0.5 and 100 mg L<sup>-1</sup>, and the results were given as milligram equivalent of the gallic acid per gram dry material.

#### Determination of total flavonoid content

The total flavonoid content was determined as quercetin equivalents per gram of the extract [38]. The sample solution



**Fig. 1** The structures of two main phenolic components of *Alyssum virgatum*. Left: cinnamic acid, right: ferulic acid

in methanol (1.0 mL) was mixed with aluminum chloride %2 (1.0 mL), which was prepared in half the volume of methanol and glacial acetic acid. The mixture was incubated for 10 min at room temperature, and the absorbance was measured at 364 nm. A standard curve of quercetin ranging from 0.5 to 50 mg L<sup>-1</sup> of concentrations was used to find the TFC contents of the extract. The obtained result was expressed as milligram of quercetin equivalents per gram dry material.

### Determination of total antioxidant capacity

The basis of the method is based on the formation of green-colored complex of phosphate/Mo(V) in acidic pH with the reduction of acidic Mo(VI) to Mo(V) [39]. A sample solution (0.2 mL) containing reducing species methanol was combined in an Eppendorf tube with a reagent solution (2.0 mL) which include 4-mM ammonium molybdate, 28-mM sodium phosphate, and 0.6-M sulfuric acid. The tube was capped and incubated for 90 min at 95 °C. Before the absorbance of the aqueous solution of each was measured at 695 nm against a blank, the samples had cooled to room temperature. The total antioxidant capacity of the sample was standardized against  $\alpha$ -tocopherol acetate and expressed as mM  $\alpha$ -tocopherol acetate equivalents per gram dry material.

### Scavenging activity of hydrogen peroxide

The hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging activity of the methanolic extracts was carried out following the procedure of Ruch et al. [40]. The solution of H<sub>2</sub>O<sub>2</sub> (40 mM) was prepared in a phosphate buffer (pH 7.4). The methanolic extract of the plant was added to an H<sub>2</sub>O<sub>2</sub> solution (0.6 mL, 40 mM) and incubated for 10 min at room temperature. The absorbance value of the reaction mixtures was recorded at 230 nm. A buffer solution without H<sub>2</sub>O<sub>2</sub> was used as a blank. The percentage of H<sub>2</sub>O<sub>2</sub> scavenging was calculated as:

$$H_2O_2 \text{ scavenging rate}(\%) = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100 \quad (1)$$

where  $A_{\text{control}}$  is the absorbance of the control, and  $A_{\text{sample}}$  is the absorbance in the methanolic extracts of plants.

### Scavenging activity of hydroxyl radicals

The hydroxyl radicals scavenging activity were measured by Fenton reaction [41]. The reaction mixture contained in 100  $\mu$ L of 3.0-mM deoxyribose, 100  $\mu$ L of 1.0-mM iron(III) chloride, 100  $\mu$ L of 1.0-mM EDTA, 100  $\mu$ L of 1.0-mM ascorbic acid, 100  $\mu$ L of 1.0-mM hydrogen peroxide, and 500  $\mu$ L of 20-mM phosphate buffer, and 2.0 mL of extract at various concentrations. The reaction mixture was incubated for 60 min at 37 °C. Afterwards, 1.0 mL of 1% thiobarbituric acid (TBA) and 1.0 mL of 2.8% trichloroacetic acid (TCA)

were added to the mixture and was boiled for 30 min. The absorbance was measured as a pink malondialdehyde-TBA chromogen at 532 nm. The percentage of hydroxyl radicals scavenging activity was calculated, according to Eq. 1.

### Statistical analysis

The results of the experimental part of this study were reported as the mean  $\pm$  SD of three parallel measurements. The data were statistically analyzed by one-way ANOVA program. The values of  $p < 0.05$  were considered statistically significant.

### Theoretical methods

#### DFT calculations

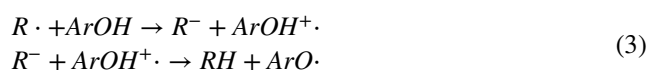
The antioxidant properties of phenolic molecules can be explained via mechanisms which are called hydrogen atom transfer (HAT), single electron transfer followed by proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET) reactions [42–46]. In each of these mechanisms, the radical scavenging abilities of phenolic compounds can be determined. Firstly, HAT mechanism performed in the one-step reaction is given in Eq. 2.



From Eq. 2, it is seen that the phenolic compound (ArOH) by giving a hydrogen atom to free radical ( $R \cdot$ ) via homolytic breaking of the O–H bond quench the radical. The weaker the O–H bond means the easier the radical scavenging. Therefore, the antioxidant activity of ArOH can be estimated with bond dissociation enthalpy (BDE) of the O–H bond.

The second mechanism is SET-PT reactions consisting of two steps as shown in Eq. 2. Its first step is based on the electron transfer from the ArOH molecule. Similarly, the second step of the SET-PT is a proton transfer from the resulting radical cation (ArOH +  $\cdot$ ). For this reason, the ionization potential ( $IP$ ) and proton dissociation enthalpy ( $PDE$ ) values are the important parameters for the antioxidant reactivities.

The antioxidant compounds having the lower  $IP$  and  $PDE$  show the higher radical quenching effect.



The third mechanism, the SPLET mechanism, is governed by proton affinity ( $PA$ ) values, together with the electron transfer enthalpy ( $ETE$ ) values of the phenoxide anion ( $ArO^-$ ). The mentioned parameters can be also used to predict the antioxidant activities of the phenolic molecules.



The aforementioned descriptors obtained from total enthalpy values at 298.15 K were calculated with the help of the following equations:

$$BDE = H_{ArO} + H_{H\cdot} - H_{ArOH} \tag{5}$$

$$IP = H_{ArOH^+} + H_{e^-} - H_{ArOH} \tag{6}$$

$$PDE = H_{ArO} + H_{H^+} - H_{ArOH^+} \tag{6}$$

$$PA = H_{ArO^-} + H_{H^+} - H_{ArOH} \tag{7}$$

$$ETE = H_{ArO} + H_{e^-} - H_{ArO^-} \tag{6}$$

The DFT computations were executed using Gaussian 16 [47] and GaussWiev 6 [48] programs. Becke B3LYP [49, 50] is a popular DFT hybrid function because it has low computational cost and more accuracy, and it is compatible with experimental results [51–56]. Additionally, B3LYP has good performance in geometry optimization and predicts the X–H bond energy [57–59]. Therefore, it was preferred in this study. B3LYP/6–311 + +G(d,p) level was used for the computations of neutral, anionic, cationic, and radical structures of the phenolic compounds. In addition, the spin densities for each atom of the radical molecules were obtained by NBO version 3.1 calculations at the same level of theory. The gas phase enthalpies of hydrogen atom, proton, and electron were used as 0.49765, 0.00236, and 0.00118 hartree, respectively [60, 61].

### Molecular docking

The potential binding mode and the binding interaction of ferulic acid and cinnamic acid ligands with the antioxidant proteins which are urate oxidase (PDB ID: 1R4U) [62], proline-rich tyrosine kinase 2 (PDB ID: 3FZS) [63], glutathione reductase (PDB ID: 1GRS) [64] have been examined using Maestro 12.5, Schrödinger 2020–3 package program [65]. The 3D crystal structure of the mentioned proteins was retrieved from the protein data bank. These proteins obtained from the protein data bank are not ready directly for the use of docking calculations on them. Addition of hydrogens, assigning of the partial charges, and building the side chains along with the filling of missing loops are applied using the Protein Preparation module of Schrödinger Maestro 12.5 version. The water molecules beyond 3 Å of the binding site in the crystal structures are removed. The minimized energy of the protein structure was obtained using the OPLS3e force field at pH: 7.0. The ligands were prepared for

molecular docking with LigPrep using the same force field (pH: 7.0 ± 2.0). The receptor grid was generated by clicking on any atom of the ligand, and the default grid box was created. The grid box was set with 20 × 20 × 20 volumetric spacing for all investigated proteins. The coordinates were taken as x: 31.11, y: 26.98, and z: 38.13 for 1R4U, x: –3.5, y: –3.23, and z: 12.43 for 3FZS, x: 60.71, y: 51.38, and z: 18.86 for 3GRS.

### ADME study

A set of ADME properties of the ferulic acid and the cinnamic acid were calculated using a QikProp tool in the Schrödinger program [66]. QikProp module generates relevant descriptions and uses them to carry out ADME estimations and uses the method of Jorgensen to obtain pharmacokinetic properties and descriptors.

## Results and discussion

### The TPC, TFC, and TAC investigations

The antioxidant properties of plants can be referred to the phenolic compounds due to their redox potentials that allow them to act as metal chelating, quenching of singlet oxygen, hydrogen donors, and reducing agents. These species which are called as secondary plant metabolites contribute to the antioxidant ability of plants [67–70]. Furthermore, phenols play a role in reducing the harmful effects of reactive oxygen species (ROS) radicals. Phenol scavenging activity is dependent on the position of the hydroxyl substituents on the aromatic ring of phenolic molecule [71, 72].

Folin-Ciocalteu technique was used to determine the TPC. TPC was calculated with a regression equation based on a standard curve using gallic acid at different concentration ( $R^2 = 0.998$ ) and expressed as milligram of gallic acid equivalents per gram dry material, i.e., mg GAE g<sup>-1</sup> DW. The phenolic concentration of methanolic extract of *A. virgatum* was determined as 161.25 mg GAE/g DW in Table 1.

**Table 1** The experimental TPC, TFC, and TAC values of methanolic extract of *A. virgatum* plant

Plant extract	TPC (mg gallic acid g <sup>-1</sup> dry material)	TFC (mg quercetin g <sup>-1</sup> dry material)	TAC (mM α-tocopherol acetate g <sup>-1</sup> dry material)
<i>Alyssum virgatum</i>	161.25 ± 0.73 <sup>a</sup>	119.89 ± 1.02 <sup>b</sup>	94.92 ± 1.27 <sup>c</sup>

The values are mean of triplicates ± SD

Mean values followed by different superscripts

<sup>a>b>c</sup> indicate an important statistical difference ( $p < 0.05$ )

The results of TFC obtained in the quantitative analysis are given in Table 1. TFC was determined comparing with a calibration curve of quercetin at 0–50 mg L<sup>-1</sup> ( $R^2=0.996$ ) and then expressed in mg quercetin equivalents per gram dry material, i.e., mg QUE g<sup>-1</sup> DW. From Table 1, the flavonoid content of *A. virgatum* is found as 119.89 mg QUE/g DW. The achieved results indicate that the phenolic equivalent of gallic acid content was higher than the flavonoid equivalent of quercetin content in *A. virgatum* ( $p < 0.05$ ).

The total antioxidant capacity of the methanol extract of *A. virgatum* plant was determined by the phosphomolybdenum with using  $\alpha$ -tocopherol as a standard. The  $\alpha$ -tocopherol is an antioxidant species with chain-breaking properties which inhibits the spread of free radical reactions. Additionally, it is a significant vitamin which is soluble in fat [73]. The basic of the method depends on the formation of green-colored complex of phosphate/Mo(V) in acidic pH with the reduction of acidic Mo(VI) to Mo(V). The TAC was determined comparing with a calibration curve of  $\alpha$ -tocopherol acetate at 100–500 mg L<sup>-1</sup> ( $R^2=0.995$ ). The obtained results were indicated as mM  $\alpha$ -tocopherol acetate per gram dry material. The TAC value methanol extract of *A. virgatum* is presented in Table 1. From Table 1, it can be stated that the mentioned methanolic extract has a TAC of 94.92 mM  $\alpha$ -tocopherol acetate per gram dry material. It should be indicated that there are statistical differences between the TPC, the TFC, and the TAC values ( $p < 0.05$ ).

### The scavenging activity of hydrogen peroxide and hydroxyl radical

The scavenging activity of the *A. virgatum* plant extract was analyzed by using hydroxyl radical ( $\cdot\text{OH}$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). The  $\text{H}_2\text{O}_2$  is not a free radical. However, it can form a radical by many oxidase enzymes such as superoxide dismutase. The scavenging activities of  $\text{H}_2\text{O}_2$  of the plant extract and positive controls are shown in Table 2. Additionally, it is stated that the plant extract and positive controls have an observable maximum inhibition of between 85 and

**Table 2** The ROS scavenging activities of the mentioned plant extract and positive controls

Sample	Hydrogen peroxide $\text{H}_2\text{O}_2$ IC <sub>50</sub> (mg mL <sup>-1</sup> )	Hydroxyl radicals ( $\cdot\text{OH}$ ) IC <sub>50</sub> ( $\mu\text{g}$ mL <sup>-1</sup> )
<i>Alyssum virgatum</i>	29.24 ± 2.49 <sup>b</sup>	46.04 ± 3.95 <sup>b</sup>
BHT	48.78 ± 2.72 <sup>c</sup>	52.78 ± 3.18 <sup>b</sup>
Curcumin	16.35 ± 1.37 <sup>a</sup>	19.21 ± 1.84 <sup>a</sup>

The values are mean of triplicates ± SD

Mean values followed by different superscripts

<sup>a>b>c</sup> show a significant statistical difference ( $p < 0.05$ )

95%. To make comparisons to *A. virgatum*, the natural antioxidants such as curcumin and synthetic antioxidant butylated hydroxytoluene (BHT) were used. The IC<sub>50</sub> value of the methanol extract was analyzed as 29.24 mg mL<sup>-1</sup> for  $\text{H}_2\text{O}_2$  scavenging activity. The IC<sub>50</sub> value (16.35 mg mL<sup>-1</sup>) of curcumin was substantially lower than that (48.78 mg mL<sup>-1</sup>) of BHT. These results show that curcumin has the strongest hydrogen peroxide scavenging activity among the investigated species. Ranking of  $\text{H}_2\text{O}_2$  scavenging activity is *Curcumin* > *A. virgatum* > BHT ( $p < 0.05$ ). It should be stated that *A. virgatum* is stronger  $\text{H}_2\text{O}_2$  scavenger than BHT which is synthetic antioxidant.

Hydroxyl radical is an oxygen-containing chemical species, and it is the most reactive free radicals in biological cells, which causes lipid oxidation and huge biological damage [74]. All of the samples show inhibition of between 75 and 85%. Table 2 shows the hydroxyl scavenging activity of positive controls and the plant extract. The lower IC<sub>50</sub> means better protective effect of the extracts against hydroxyl radical. The IC<sub>50</sub> values of hydroxyl scavenging effect of curcumin and BHT are obtained as 19.21 and 52.78  $\mu\text{g}$  mL<sup>-1</sup>, respectively ( $p < 0.05$ ). However, there are no statistical differences between the IC<sub>50</sub> values of the *A. virgatum* and the BHT ( $p \geq 0.05$ ). These results also indicate that curcumin has a stronger hydroxyl radical scavenger than the plant extract and BHT.

### HAT mechanism

BDE values of the O–H bond is an important descriptor to interpret the HAT reaction. The lower BDE values mean the higher antioxidant activity. While cinnamic acid has one O–H bond which is in the carboxyl group, ferulic acid has two O–H which are in the hydroxyl and carboxyl groups. The BDE values for the mentioned compounds are calculated in the gas phase, and they are presented in Table 3. Referring to Table 3, it is seen that the BDE value of the cinnamic acid is calculated as 99.35 kcal mol<sup>-1</sup>. The mentioned BDE values for hydroxyl and carboxyl groups of ferulic acid molecule are obtained as 88.22 and 99.19 kcal mol<sup>-1</sup>, respectively. It is noticed from Table 3 that the studied BDE values of the ferulic acid are smaller than those of cinnamic acid. The lowest BDE of the O–H bond belongs to the OH in the hydroxyl group of ferulic acid. The low BDE value of

**Table 3** The calculated BDE values (kcal mol<sup>-1</sup>) of the cinnamic acid and ferulic acid at 25 °C in the gas at B3LYP/6-311 + +G(d,p) level

	BDE
Cinnamic acid	
OH (carboxyl) ferulic acid	99.35
OH (hydroxyl)	82.22
OH (carboxyl)	99.19

**Table 4** The computed IP and PDE values (kcal mol<sup>-1</sup>) of the studied molecules in vacuo at B3LYP/6–311 + +G(d,p) level

	IP	PDE
Cinnamic acid	197.80	216.05
OH (carboxyl) Ferulic acid	178.84	
OH (hydroxy)		217.89
OH (carboxyl)		234.85

the O–H bond in the hydroxyl group of ferulic acid can be explained by aromatic conjugation because it stabilizes the radical. For that reason, the mentioned O–H bond is broken easier. According to the BDE values of the examined molecules, it is clear that ferulic acid has better antioxidant property than cinnamic acid. In other words, it can be stated that ferulic acid exhibits the best antioxidant activity in the main phenolic components of *A. virgatum*.

### SET–PT mechanism

The first step of SET-PT reactions is related to the IP value of the compound. The lower IP shows the easier the electron donating ability. The IP values of studied molecules are presented in Table 4. The order of the IPs is ferulic acid < cinnamic acid at the B3LYP method. Considering IP values, it can be seen that that electron donating ability of ferulic acid is better than cinnamic acid molecules. Therefore, it can be said that scavenging activity of ferulic acid is better than those of cinnamic acid. These results are compatible with BDE values in Table 3.

The second step in the SET-PT mechanism is concerned with PDE values of molecules. The lower PDE means the easier reaction. PDEs of molecules are given in Table 4. From Table 4, it is seen that the lowest PDE value in ferulic acid is close to that of cinnamic acid. When IP and PDE values of the studied phenolic compounds are considered together, it can be stated that ferulic acid has better antioxidant scavenging reactivity than cinnamic acid. The results of this mechanism are compatible with those of HAT reactions.

### SPLET mechanism

The first step of SPLET mechanism is PA reaction. In this reaction, it is apparent that the compound with lower PA has higher proton affinity. The lower proton affinity purports the higher antioxidant activity. The PA values of the investigated molecules are shown in Table 5. It is apparent from Table 5 that ferulic acid, which has low PA exhibit better antioxidant properties than cinnamic acid. ETE reaction is the second step of SPLET. The molecule with lower ETE gives easier reaction. From Table 5, it is noticed that ferulic acid has the

**Table 5** The obtained PA and ETE values (kcal mol<sup>-1</sup>) of the mentioned compounds in the vacuum at the same level of theory

	PA	ETE
Cinnamic acid	334.68	79.17
OH (carboxyl) Ferulic acid		
OH (hydroxy)	327.77	68.96
OH (carboxyl)	336.46	77.23

lowest ETE value. According to ETE reaction, the ferulic acid shows better radical scavenging ability than cinnamic acid. When PA and ETE values of the studied molecules are considered together, it can be noticed that ferulic acid has better antioxidant scavenging reactivity than cinnamic acid. The results of this mechanism are quite compatible with those of HAT and SPLET mechanisms.

### Spin densities

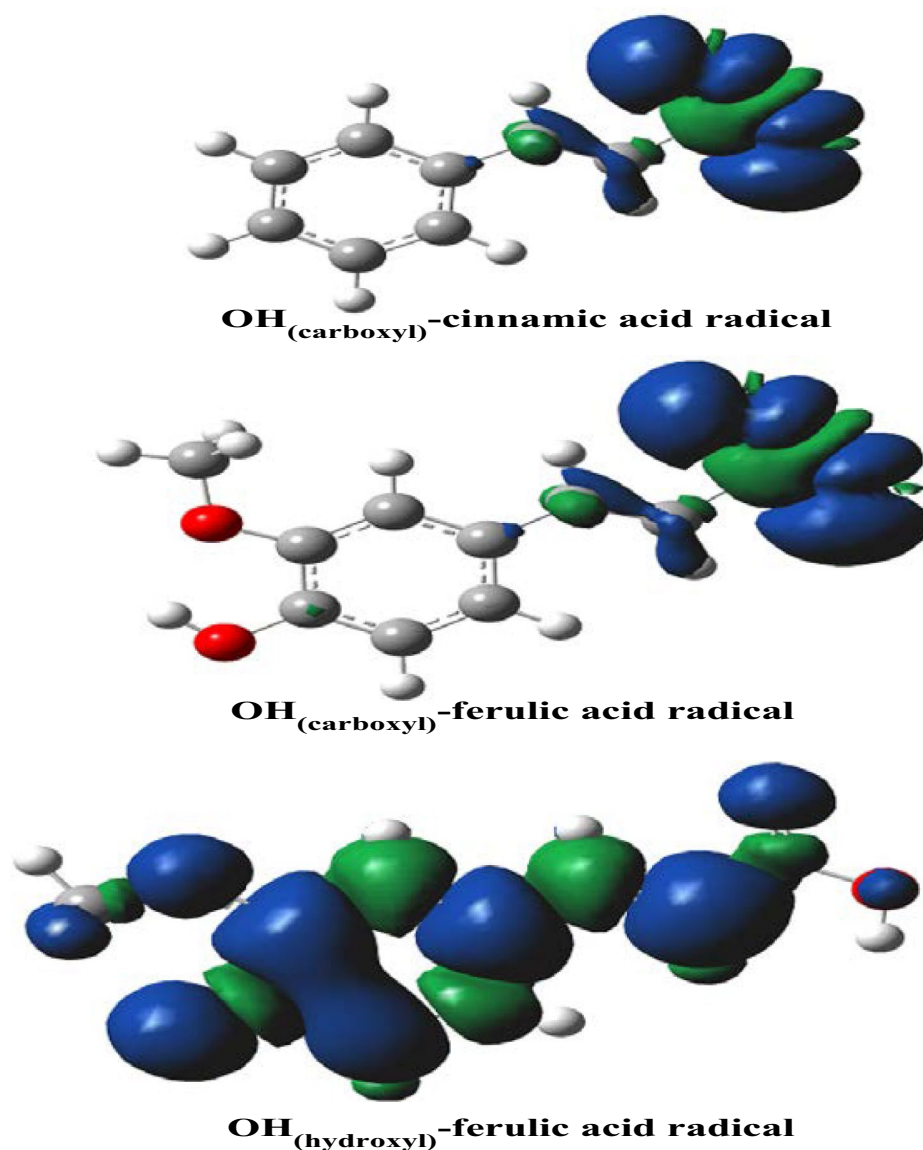
Spin density is the electron density applied to free radicals. It is well-known that spin density is an important descriptor to characterize the stabilities of free radicals. The delocalization of higher unpaired electrons means the lower the energy of the radical. In other words, the molecule having higher delocalization occurs easily. To show spin densities of the molecules, free radicals are obtained by hydrogen abstraction of the OH. Then the natural bond analysis calculation of the formed free radical is performed. Using NBO analysis, the spin density distributions are achieved.

The spin density distributions of the cinnamic acid and the ferulic acid radical are obtained in the abovementioned way and shown in Fig. 2. Figure 2 shows that spin density distributions created by removing the H atom from the carboxyl group of cinnamic acid and ferulic acid are similar to each other. In these radicals, spin densities are delocalized around the carboxyl group because two electronegative oxygen atoms in the carboxyl group quite attract electrons. On the other hand, spin density distribution formed by removing the H atom from the hydroxyl group of the ferulic acid is delocalized on the whole molecule because an oxygen atom connected to the phenolic ring attracts fewer electrons than two oxygens. So, the spin electron density spreads over the entire molecule. This distribution can be clearly seen in Fig. 1. Therefore, it can be stated that best stable molecule is hydroxyl-ferulic acid radical. Consequently, it can be said that ferulic acid has a higher radical quenching effect than cinnamic acid.

### Molecular docking study

The molecular docking studies are based on ligand-antioxidant protein interactions. In this article, the aforementioned proteins are preferred to evaluate the biological activities of the ferulic acid and the cinnamic acid. The docking

**Fig. 2** The spin densities of the investigated molecules at B3LYP/6-311+ +G(d,p)



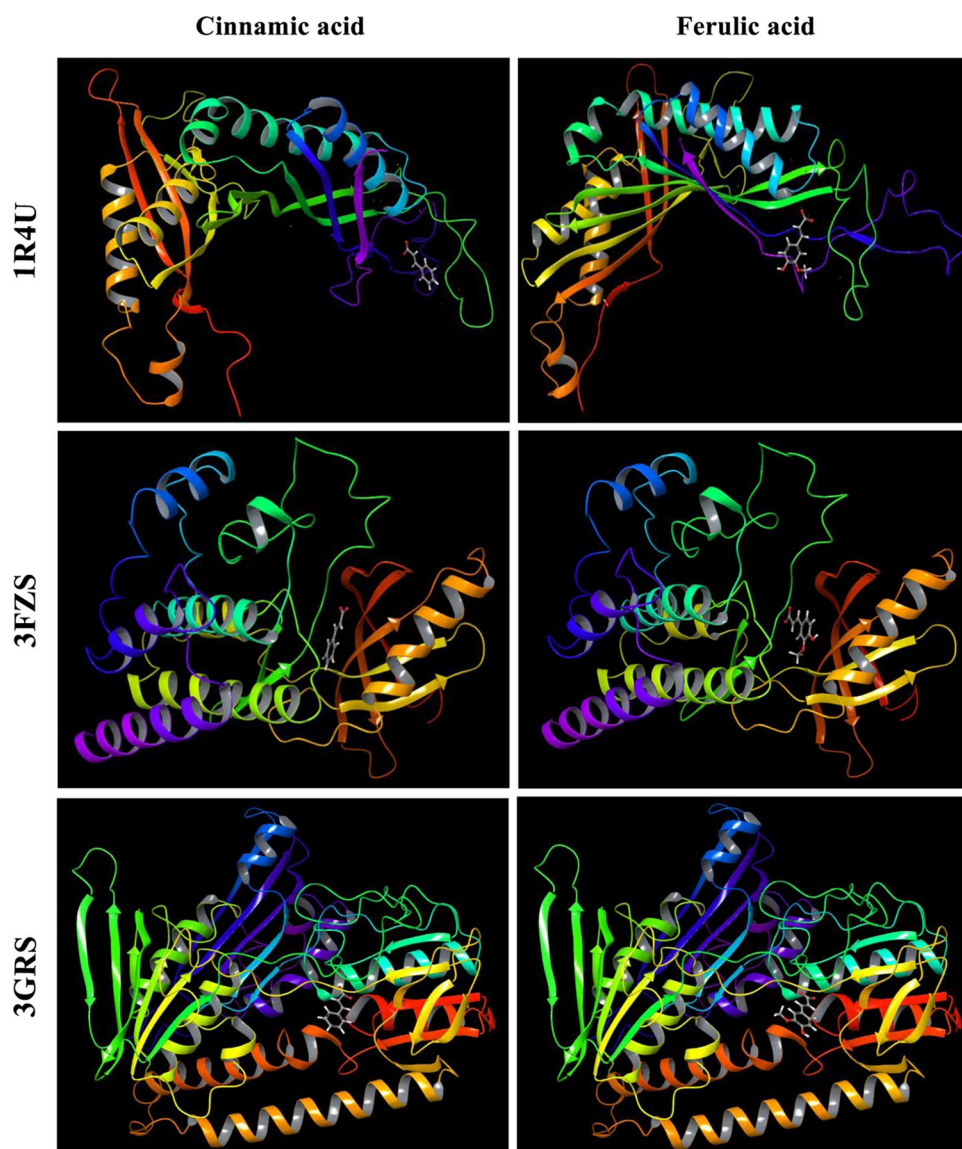
calculations of the ferulic acid and cinnamic acid ligands to the active site of the mentioned proteins are executed by Schrodinger program, Maestro 12.5 version. The pictorial demonstration (3D) and the ligand interaction diagram (2D) of the docked molecules are obtained as shown in Figs. 3 and 4, respectively.

As shown in Fig. 4, cinnamic acid in 1R4U forms hydrogen bonding with amino acid ARG 176 and the water molecule; ferulic acid in the same protein forms hydrogen bonding with ARG 166 and VAL 227. In 3FZS protein, while cinnamic acid forms salt bridge bonding with LYS 457, ferulic acid forms hydrogen bonding with GLU 464 and the water compound. Finally, cinnamic acid in 3GRS forms hydrogen bonding with amino acid GLY 31, ASP 331, and two water molecules; ferulic acid in the same

enzyme forms hydrogen bonding with GLU 50, GLY 31, and three water compounds.

Besides aforementioned interactions, the significant parameters are achieved from interactions of the target protein with the mentioned ligands. Among these, the most important parameter is Docking score. The resulting docking results of the mentioned ligands are tabulated in Table 6. It is seen from Table 6 that the docking scores of cinnamic acid and ferulic acid are in the range of  $-3.403$  to  $-5.921$ . According to the Docking scores, it is clear that biological activity of ferulic acid in investigated antioxidant proteins is higher than those of cinnamic acid. These data are compatible with the aforementioned DFT results. Also, it is indicated that ferulic acid shows the best activity in 3GRS enzyme.

**Fig. 3** 3D demonstration of the docking of the mentioned antioxidant proteins with the ligands



Additionally, it is apparent that Glide energies of cinnamic acid are smaller than that of ferulic acid. According to these results, it can be said that the biological activity of ferulic acid is higher than that of cinnamic acid.

### ADME analysis

The ADME properties of the examined ligands are determined *in silico* by using QikProp module of Schrodinger suite 2020–3. Some of the obtained ADME properties are given in Table 7. Referring to Table 7, the calculated dipole moment of cinnamic acid and ferulic acid are 6.700 and 6.384, respectively. While the predicted number of hydrogen

bonds that would be donated by the solute to water compounds in an aqueous solution of the cinnamic acid and the ferulic acid is 1 and 2, the obtained number of hydrogen bonds that would be accepted by the solute to water compounds in an aqueous solution of the mentioned ligands is 2 and 3.5. Finally, it is noticed from Table 7 that % human oral absorption of the aforementioned ligands are in the range of 67–80%. According to the values, it can be said that almost all the ADME properties of the molecules are within the recommended values. Additionally, it is apparent from Table 7 that the drug activity of ferulic acid is higher than that of cinnamic acid. Consequently, it can be stated ferulic acid has better drug activity than that of cinnamic acid.



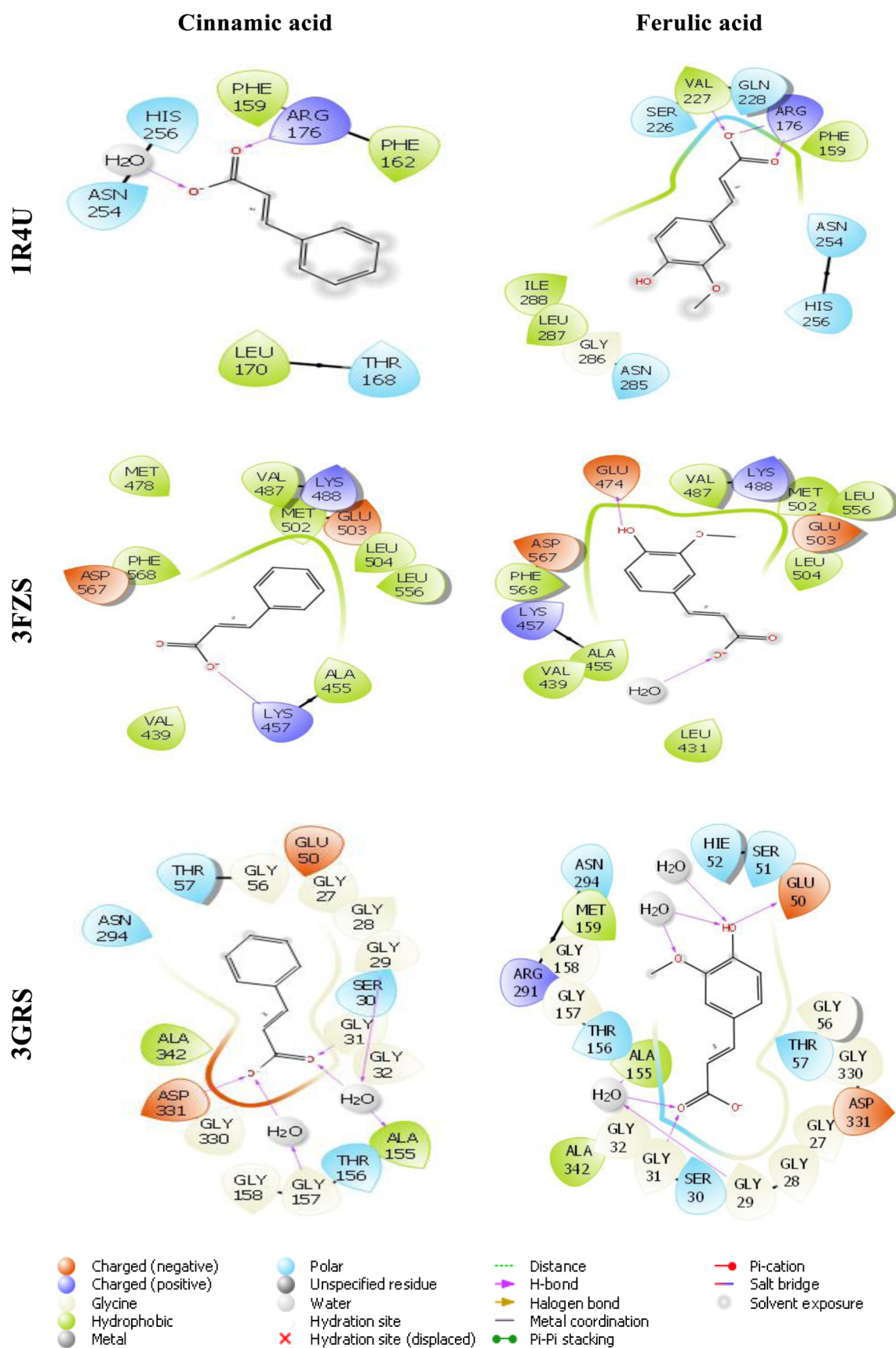


Fig. 4 2D presentation of the interaction between the receptor region of the target protein with the investigated molecules

**Table 6** The docking results obtained from interaction of the ferulic acid and cinnamic acid with the mentioned proteins

Protein	Ligand	Docking score	Glide hbond	Glide emodel	Glide ligand efficiency	Glide energy
1R4U	Cinnamic acid	−3.403	−0.360	−23.118	−0.309	−15.916
	Ferulic acid	−5.244	−0.537	−32.627	−0.375	−20.450
3FZS	Cinnamic acid	−4.904	−0.015	−24.889	−0.446	−18.341
	Ferulic acid	−5.806	−0.160	−35.788	−0.415	−26.344
3GRS	Cinnamic acid	−4.795	−0.534	−45.589	−0.436	−31.345
	Ferulic acid	−5.921	−0.506	−56.333	−0.423	−36.562

**Table 7** The ADME parameters of the mentioned ligands

Compound	Dipole	Mol MW	Donor HB	Accept HB	QPlogPo/w	No. of meta	QPlogKhsa	Rule of Five	% Human oral absorption
Cinnamic acid	6.700	148.161	1	2	1.897	0	−0.518	0	79.497
Ferulic acid	6.384	194.187	2	3.5	1.371	2	−0.612	0	67.241
Recommended	1–12.5	130–725	0–6	2–20	−2–6.5	1–8	−1.5–1.5	Max 4	>80% is high <25% is poor

## Conclusions

In the present study, it is determined that the methanolic plant extract has had rich content in terms of phenolic and flavonoid molecules. The phenolic compounds were higher than the flavonoid content in both experimental results and HPLC analyses.

The *A. virgatum* showed the scavenging activity of ROS. Curcumin was more effective than both the plant extract and positive control BHT at scavenging activity ROS. But the plant extract exhibited more scavenging activity than synthetic antioxidant BHT for hydrogen peroxide. However, there were no statistical differences between the values of the *A. virgatum* and the BHT at scavenging effect of hydroxyl radical.

The results of the present study indicate that methanol extracts of *A. virgatum* plant can be useful as an antioxidant source. For that reason, using this valuable strain in pharmaceutical products and food, their cultivation and conservation are of great importance. Further work is required involving isolation and purification of chemical compounds, so that the plants can be used as a natural antioxidant agent.

Cinnamic acid and ferulic acid, which are phenolic compounds are the main components of the *A. virgatum*. The antioxidant properties of the mentioned phenolic molecules can be explained by means of the HAT, SET-PT, SPLET mechanisms, and the spin density distributions which are investigated by DFT results. Based on the DFT study, it has been seen that ferulic acid is a better antioxidant than cinnamic acid.

The molecular docking studies of the antioxidant proteins with the main components are carried out. The docking results suggest that the biological activity of ferulic acid is better than cinnamic acid.

The in silico ADME predicts of the investigated molecules are calculated. From computed ADME properties, it is seen that the ferulic acid has better drug activity than the cinnamic acid.

**Acknowledgements** We convey our special thanks to Dr. Bayram ATASAGUN (Selçuk University, Department of Vocational School of Health Services) identifying the plant species of this research.

**Author contribution** Emre Koç: Conceptualization, methodology, software, formal analysis, investigation, writing — original draft, writing — review and editing; Ayhan Üngördü: Conceptualization, methodology, software, formal analysis, investigation, writing — original draft, writing — review and editing, visualization; Ferda Candan: investigation, writing — review and editing, supervision.

**Funding** This work is supported by the Scientific Research Project Fund of Sivas Cumhuriyet University under the project number RGD-020. The numerical calculations reported in this paper were fully/partially performed at TUBITAK ULAKBIM, High Performance and Grid Computing Center (TRUBA resources).

**Data availability** N/A.

**Code availability** GaussView 6.0, Gaussian 16, Schrodinger 2020–3.

## Declarations

**Conflict of interest** The authors declare no competing interests.

## References

- Sharma HK, Kumar A, Singh V, Meena H, Meena B, Sharma P, Rai P (2022) Genetic resources of Brassicas. *Cash Crops*. Springer 285–337
- Al-Shehbaz IA (2021) Nomenclatural Adjustments in *Eutrema*, *Ceratocnemum*, *Rhamphospermum*, and *Sinapis* (Brassicaceae, Cruciferae). *Harv Pap Bot* 26:1–4
- Dueli GF, DeSouza O, Ribeiro SP (2021) Metal bioaccumulation alleviates the negative effects of herbivory on plant growth. *Sci Rep* 11:1–11
- Malik G, Hooda S, Majeed S, Pandey VC (2022) Understanding assisted phytoremediation: potential tools to enhance plant performance. *Assisted Phytoremediation*. Elsevier 1–24
- Ahlatw Y, Li S, Timilsena PR, Pliakoni ED, Brecht JK, Liu T (2022) Identification of senescence-associated genes in broccoli (Brassica oleracea) following harvest. *Postharvest Biol Technol* 183:111729
- Vaughn SF, Berhow MA (2005) Glucosinolate hydrolysis products from various plant sources: pH effects, isolation, and purification. *Ind Crops Prod* 21:193–202
- Kim YS, Milner J (2005) Targets for indole-3-carbinol in cancer prevention. *J Nutr Biochem* 16:65–73
- Al-Shehbaz I, Beilstein MA, Kellogg E (2006) Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. *Plant Syst Evol* 259:89–120
- Orcan N, Binzet R (2009) *Alyssum misirdalianum* (Brassicaceae), a new species from Southern Turkey. *Novon: A J Botanical Nomenclature* 19:494–496
- Güner A, Aslan S (2012) *Türkiye bitkileri listesi:(damarlı bitkiler)*. Nezahat Gökyiğit Botanik Bahçesi Yayınları
- Taiz L, Zeiger E (2010) Responses and adaptations to abiotic stress. Sinauer Associates, Inc, Plant Physiology, Fifth Edition Sunderland, MA, pp 755–778
- Hegeman AD (2010) Plant metabolomics—meeting the analytical challenges of comprehensive metabolite analysis. *Brief Funct Genomics* 9:139–148
- Dewick PM (2002) Medicinal natural products: a biosynthetic approach. John Wiley & Sons
- Ozay C, Mammadov R (2016) Assessment of some biological activities of *alyssum l.* Known as madwort *Acta poloniae pharmaceutica* 73:1213–1220
- Rahuman MH, Muthu S, Raajaraman BR, Raja M (2020) Quantum computational, spectroscopic and molecular docking studies on 2-acetylthiophene and its bromination derivative. *J Mol Struct* 1212:128129
- Loganathan L, Natarajan K, Muthusamy K (2019) Computational study on cross-talking cancer signalling mechanism of ring finger protein 146, AXIN and Tankyrase protein complex. *J Biomol Struct Dyn* 38(17):5173–5185
- Dhevaraj J, Gopalakrishnan M, Pazhamalai S (2019) Synthesis, characterization, molecular docking, ADME and biological evaluation of 3-(4-(tetrazol-1-yl)phenyl)-5-phenyl-1H-pyrazoles. *J Mol Struct* 1193:450–467
- Singh SP, Singh NI, Nongalleima K, Doley P, Singh CB, Sahoo D (2018) Molecular docking, MD simulation, DFT and ADME-toxicity study on analogs of zerumbone against IKK- $\alpha$  enzyme as anti-cancer agents. *Netw Model Anal Hlth* 7(1):1–8
- Elancheran R, Saravanan K, Divakar S, Kumari S, Maruthanila VL, Kabilan S, Ramanathan M, Devi R, Kotoky J (2017) Design, synthesis and biological evaluation of novel 1, 3-thiazolidine-2, 4-diones as anti-prostate cancer agents. *Anti-Cancer Agent Me* 17:1756–1768
- Abdel-Kader NS, Abdel-Latif SA, El-Ansary AL, Sayed AG (2019) Combined experimental, DFT theoretical calculations and biological activity of sulfaclozine azo dye with 1-hydroxy-2-naphthoic acid and its complexes with some metal ions. *New J Chem* 43:17466–17485
- Krishnan KG, Ashothai P, Padmavathy K, Lim WM, Mai CW, Thanikachalam PV, Ramalingan C (2019) Hydrazone-integrated carbazoles: synthesis, computational, anticancer and molecular docking studies. *New J Chem* 43:12069–12077
- Khan SA, Rizwan K, Shahid S, Noamaan MA, Rasheed T, Amjad H (2020) Synthesis, DFT, computational exploration of chemical reactivity, molecular docking studies of novel formazan metal complexes and their biological applications. *Appl Organomet Chem* 34(3):e5444
- Kuruville TK, Muthu S, Prasana JC, George J, Saji RS, Geoffrey B, David, RHA (2019) Molecular docking, spectroscopic studies on 4-[2-(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one and QSAR study of a group of dopamine agonists by density functional method. *Spectrochim Acta A* 222:117185
- Abrigach F, Karzazi Y, Benabbes R, El Youbi M, Khoutoul M, Taibi N, Karzazi N, Benchat N, Bouakka M, Saalaoui E (2017) Synthesis, biological screening, POM, and 3D-QSAR analyses of some novel pyrazolic compounds. *Med Chem Res* 26:1784–1795
- Ungordu A, Tezer N (2017) The solvent (water) and metal effects on HOMO-LUMO gaps of guanine base pair: a computational study. *J Mol Graph Model* 74:265–272
- Tezer N (2009) Density functional theory and ab-initio computational study of molecular structure, tautomerism, and geometrical isomerism of ethynyl-bridged dipyrindiones: in the gas phase and dielectric media. *J Mol Struct-Theochem* 895:100–106
- Lone SH, Bhat MA, Lone RA, Jameel S, Lone JA, Bhat KA (2018) Hemisynthesis, computational and molecular docking studies of novel nitrogen containing steroidal aromatase inhibitors: testolactam and testololactam. *New J Chem* 42:4579–4589
- Polo E, Ibarra-Arellano N, Prent-Penalosa L, Morales-Bayuelo A, Henao J, Galdamez A, Gutierrez M (2019) Ultrasound-assisted synthesis of novel chalcone, heterochalcone and bis-chalcone derivatives and the evaluation of their antioxidant properties and as acetylcholinesterase inhibitors. *Bioorg Chem* 90:103034
- Abrigach F, Rokni Y, Takfaoui A, Khoutoul M, Doucet H, Asehraou A, Touzani R (2018) In vitro screening, homology modeling and molecular docking studies of some pyrazole and imidazole derivatives. *Biomed Pharmacother* 103:653–661
- Kaddouri Y, Abrigach F, Ouahhoud S, Benabbes R, El Kodadi M, Alsalmeh A, Al-Zaqri N, Warad I, Touzani, R (2021) Synthesis, characterization, reaction mechanism prediction and biological study of mono, bis and tetrakis pyrazole derivatives against *Fusarium oxysporum* f. sp. *Albedinis* with conceptual DFT and ligand-protein docking studies. *Bioorg Chem* 110:104696
- Kaddouri Y, Abrigach F, Yousfi EB, Hammouti B, El Kodadi M, Alsalmeh A, Al-Zaqri N, Warad I, Touzani R (2021) New heterocyclic compounds: synthesis, antioxidant activity and computational insights of nano-antioxidant as ascorbate peroxidase inhibitor by various cyclodextrins as drug delivery systems. *Curr Drug Deliv* 18:334–349
- Wang L, You ZH, Li LP, Yan X, Zhang W, Song KJ, Song CD (2020) Identification of potential drug-targets by combining evolutionary information extracted from frequency profiles and molecular topological structures. *Chem Biol Drug Des* 96(2):758–767
- Zahran EM, Abdelmohsen UR, Shalash MM, Salem MA, Khalil HE, Desoukey SY, Fouad MA, Krischke M, Mueller M, Kamel MS (2020) Local anaesthetic potential, metabolic profiling, molecular docking and in silico ADME studies of *Ocimum forskolei*, family Lamiaceae. *Nat Prod Res* 1–7
- Zhang J, Shan YY, Pan XY, Wang C, Xu WF, He LC (2011) Molecular docking, 3D-QSAR studies, and in silico ADME

- prediction of p-aminosalicylic acid derivatives as neuraminidase inhibitors. *Chem Biol Drug Des* 78:709–717
35. Kaddouri Y, Bouchal B, Abrigach F, El Kodadi M, Bellaoui M, Touzani R (2021) Synthesis, molecular docking, MEP and SAR analysis, ADME-Tox predictions, and antimicrobial evaluation of novel mono-and tetra-alkylated pyrazole and triazole ligands. *J Chem* 2021
  36. Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Sökmen A, Akpulat HA (2003) Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. (Asteraceae). *J Ethnopharmacol* 87:215–220
  37. Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol* 299:152–178
  38. Lamaison J, Carnat A, Petitjean-Freytet C (1990) Tannin content and inhibiting activity of elastase in Rosaceae. *Ann Pharm Fr* 48:335–340
  39. Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem* 269(2):337–341
  40. Ruch RJ, Cheng SJ, Klaunig JE (1989) Prevention of cyto-toxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis* 10:1003–1008
  41. Yu WL, Zhao YP, Shu B (2004) The radical scavenging activities of radix puerariae isoflavonoids: a chemiluminescence study. *Food Chem* 86:525–529
  42. Marković Z, Milenković D, Đorović J, Marković JMD, Stepanić V, Lučić B, Amić D (2012) PM6 and DFT study of free radical scavenging activity of morin. *Food Chem* 134:1754–1760
  43. Benayahoum A, Bouakkaz S, Bordjiba T, Abdaoui M (2019) Antioxidant activity and pKa calculations of 4-mercaptostilbene and some derivatives: a theoretical approach. *J Mol Liq* 275:221–231
  44. Chen J, Yang J, Ma L, Li J, Shahzad N, Kim CK (2020) Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. *Sci Rep* 10:1–9
  45. Shaikh SAM, Singh BG, Barik A, Balaji NV, Subbaraju GV, Naik DB, Priyadarsini KI (2019) Unravelling the effect of  $\beta$ -diketo group modification on the antioxidant mechanism of curcumin derivatives: a combined experimental and DFT approach. *J Mol Struct* 1193:166–176
  46. Koç E, Üngördü A, Candan F (2019) Antioxidant properties of methanolic extract of ‘*Veronica multifida*’ and DFT and HF analyses of its the major flavonoid component. *J Mol Struct* 1197:436–442
  47. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Petersson GA, Nakatsuji H, Li X, Caricato M, Marenich AV, Bloino J, Janesko, BG, Gomperts R, Mennucci B, Hratchian HP, Ortiz JV, Izmaylov AF, Sonnenberg JL, Williams-Young D, Ding F, Lipparini F, Egidi F, Goings J, Peng B, Petrone A, Henderson T, Ranasinghe D, Zakrzewski VG, Gao J, Rega N, Zheng G, Liang W, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Throssell K, Montgomery Jr JA, Peralta JE, Ogliaro F, Bearpark MJ, Heyd JJ, Brothers EN, Kudin KN, Staroverov VN, Keith, TA, Kobayashi, R, Normand, J, Raghavachari, K, Rendell AP, Burant JC, Iyengar SS, Tomasi J, Cossi M, Millam JM, Klene M, Adamo C, Cammi R, Ochterski JW, Martin RL, Morokuma K, Farkas O, Foresman JB, Fox DJ (2016) Gaussian 16 Rev. C.01. Wallingford, CT
  48. Dennington R, Keith T, Millam J (2016) GaussView 6.0. 16, Semicem. Inc, Shawnee Mission KS
  49. Becke AD (1993) A new mixing of Hartree-Fock and local density-functional theories. *J Chem Phys* 98:1372–1377
  50. Lee C, Yang W, Parr RG (1988) Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys Rev B* 37:785
  51. Tirado-Rives J, Jorgensen WL (2008) Performance of B3LYP density functional methods for a large set of organic molecules. *J Chem Theory Comput* 4:297–306
  52. Ahmad F, Alam MJ, Alam M, Azaz S, Parveen M, Park S, Ahmad S (2018) Synthesis, spectroscopic, computational (DFT/B3LYP), AChE inhibition and antioxidant studies of imidazole derivative. *J Mol Struct* 1151:327–342
  53. Ali A, Asif M, Alam P, Alam MJ, Sherwani MA, Khan RH, Ahmad S (2017) DFT/B3LYP calculations, in vitro cytotoxicity and antioxidant activities of steroidal pyrimidines and their interaction with HSA using molecular docking and multispectroscopic techniques. *Bioorg Chem* 73:83–99
  54. Najafi M, Najafi M, Najafi H (2012) DFT/B3LYP study of the substituent effects on the reaction enthalpies of the antioxidant mechanisms of Indole-3-carbinol derivatives in the gas-phase and water. *Comput Theor Chem* 999:34–42
  55. Ungordu A, Tezer N (2017) Effect on frontier molecular orbitals of substituents in 5-position of uracil base pairs in vacuum and water. *J Theor Comput Chem* 16(07):1750066
  56. Ungordu A, Tezer N (2017) DFT study on metal-mediated uracil base pair complexes. *J Saudi Chem Soc* 21:837–844
  57. Fiorucci S, Golebiowski J, Cabrol-Bass D, Antonczak S (2007) DFT study of quercetin activated forms involved in antiradical, antioxidant, and prooxidant biological processes. *J Agric Food Chem* 55:903–911
  58. Klein E, Lukeš V, Ilčín M (2007) DFT/B3LYP study of tocopherols and chromans antioxidant action energetics. *Chem Phys* 336:51–57
  59. Klein E, Lukeš V (2006) DFT/B3LYP study of O-H bond dissociation enthalpies of para and meta substituted phenols: correlation with the phenolic C–O bond length. *J Mol Struct (Theochem)* 767:43–50
  60. Bartmess JE (1994) Thermodynamics of the electron and the proton. *J Phys Chem* 98:6420–6424
  61. Klein E, Rimarcik J, Lukes V (2009) DFT/B3LYP study of the O-H bond dissociation enthalpies and proton affinities of para- and meta-substituted phenols in water and benzene. *Acta Chim Slovaca* 2:37–51
  62. Retailleau P, Colloc’h N, Vivarès D, Bonneté F, Castro B, El Hajji M, Mornon JP, Monard G, Prangé T, (2004) Complexed and ligand-free high-resolution structures of urate oxidase (Uox) from *Aspergillus flavus*: a reassignment of the active-site binding mode. *Acta Crystallogr D Biol Crystallogr* 60:453–462
  63. Han S, Mistry A, Chang JS, Cunningham D, Griffor M, Bonnette PC, Wang H, Chrnyk BA, Aspnes GE, Walker DP (2009) Structural characterization of proline-rich tyrosine kinase 2 (PYK2) reveals a unique (DFG-out) conformation and enables inhibitor design. *J Biol Chem* 284:13193–13201
  64. Karplus PA, Schulz GE (1987) Refined structure of glutathione reductase at 1.54 Å resolution. *J Mol Biol* 195:701–729
  65. Schrödinger Release 2020–3 (2020) Maestro. Schrödinger, LLC
  66. Schrödinger Release 2020–3 (2020) QikProp. Schrödinger, LLC
  67. Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yasuhara T, Yoshida T, Okuda T (1989) Effects of the interaction of tannins with co-existing substances. VI.: effects of tannins and related polyphenols on superoxide anion radical, and on 1,1-diphenyl-2-picrylhydrazyl radical. *Chem Pharm Bull* 37:2016–2021
  68. Albayrak S, Atasagun B, Aksoy A (2017) Comparison of phenolic components and biological activities of two *Centaurea* sp. obtained by three extraction techniques. *Asian Pac J Trop Med* 10:599–606

69. Zengin G, Atasagun B, Aumeeruddy MZ, Saleem H, Mollica A, Bahadori MB, Mahomoodally MF (2019) Phenolic profiling and in vitro biological properties of two Lamiaceae species (*Salvia modesta* and *Thymus argaeus*): a comprehensive evaluation. *Ind Crops Prod* 128:308–314
70. de Ancos B, González EM, Cano MP (2000) Ellagic acid, vitamin C, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. *J Agric Food Chem* 48:4565–4570
71. Cai Y, Luo Q, Sun M, Corke H (2004) Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci* 74:2157–2184
72. Silva FA, Borges F, Guimarães C, Lima JL, Matos C, Reis S (2000) Phenolic acids and derivatives: studies on the relationship among structure, radical scavenging activity, and physicochemical parameters. *J Agric Food Chem* 48:2122–2126
73. Ramanathan K, Anusuyadevi M, Shila S, Panneerselvam C (2005) Ascorbic acid and  $\alpha$ -tocopherol as potent modulators of apoptosis on arsenic induced toxicity in rats. *Toxicol Lett* 156:297–306
74. Zhang X, Barraza KM, Beauchamp J (2017) Field-induced droplet ionization illuminates stepwise oxidation of cell membrane lipids by hydroxyl radicals at the air-water interface. In: 254th American Chemical Society National Meeting & Exposition, August 20–24, Washington, DC

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.