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Evaluation of inhibitory potency of endemic Onion bulbs: Analysis of phenolic compounds and enzyme inhibition with the computational study

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Keywords
Allium kastambulense; enzyme inhibition; phenolic compound; molecular docking

Abstract

Numerous *Allium* species have been utilized in complementary medicine based on their biological activities. In the present work, the bulb extract of *A. kastambulense* was studied for its phenolic content and enzyme inhibition ability assisted by computer-aided molecular docking studies. The evaluation of enzyme inhibition activity of the bulb extract showed that it has 54.70±1.74 134.3±2.12 IC_{50} for six studied enzymes, respectively. In the reverse-phase HPLC analysis of methanol-chloroform bulb extract, catechin, chlorogenic acid, gentisic acid, vanillic acid, and apigenin were founded to be the represented group of phenolic components. Catechin flavonoid was extensively detected as the most abundant ingredient, and TPC and TFC were calculated at 6.86 ± 0.36 mg g^{-1} and 3.22 ± 0.14 mg g^{-1}. Finally, the catechin ligand has low binding energy values against the studied enzymes with -5.778 - -8.872 kcal/mol.

Endemik Soğanın inhibe edici etkisinin değerlendirilmesi: Hesaplamalı çalışma ile fenolik bileşik ve enzim inhibisyonu analizi

Anahtar kelimeler
Allium kastambulense; fenolik bileşik; enzim inhibisyonu; moleküler kenetlenme

Öz

Çok sayıda *Allium* türü, biyolojik aktivitelerine dayalı olarak tamamlayıcı tıpta kullanılmaktadır. Bu çalışmada, *A. kastambulense* soğan ekstresinin fenolik içeriği ve enzim inhibisyon yeteneği bilgisayar destekli moleküler kenetlenme çalışmaları incelenmiştir. Soğan ekstraktının enzim inhibisyonu aktivitesi, çalışılan altı enzim için 54.70±1.74 - 134.3±2.12 aralığında IC_{50}'ye sahiptir. Metanol-kloroform soğan ekstresinin ters faz HPLC analizinde kateşin, klorojenik asit, gentisik asit, vanilik asit ve apigenin fenolik bileşenleri temsil eden metabolitler olarak bulunmuştur. Bir flavonoid olan kateşin flavonoid en bol bulunan bileşen olarak tespit edildi. TPC ve TFC değerleri sırasıyla 6.86 ± 0.36 mg g^{-1} ve 3.22 ± 0.14 mg g^{-1} olarak hesaplandı. Son olarak, kateşin ligandı, üzerinde çalışılan enzimlere karşı düşük bağlanma enerjisi değerlerine (- 5,778 - -8,872 kcal/mol) sahiptir.

1. Introduction

Allium L. is a member of Amaryllidaceae with more than 900 species. *Allium kastambulense* is an endemic wild onion that grows between Bartın and Kastamonu provinces in the Northwest Anatolian region (Türkiye). Some *Allium* members such as onion and garlic are used in food and cooking. Ethnobotanical studies have also shown that members of Allium have different uses beyond their nutritional value due to their biological activities. To develop novel food ingredients with health-promoting properties, studies on nutraceuticals for researching possible raw materials in the scientific community and food industry are gaining importance (Zeng *et al*. 2017; Armağan 2021; Rocchetti *et al*. 2022). The genus mainly produces organosulfur compounds and various secondary metabolites, including polyphenols, flavonoids, saponins, alkaloids, and peptides. Several preclinical trials have been surveyed on the beneficial effect of cultivated and wild *Allium* plants for biological

activities such as antimicrobial, antioxidant, anticancer, antidiabetic, hypertension, hypercholesterolemia, neuroprotective, and cardioprotective effects (Maccelli *et al*. 2020; Emir and Emir 2021).

Herbal extracts have conventionally been used for nutritional values and medicinal purposes. The survey for plant-derived candidates compounds to remedy various diseases such as Alzheimer's, obesity, and hyperpigmentation, which still lack an exact cure, has proceeded for decades (Ocal *et al*. 2022). Some in vitro and in vivo experimental studies show that phytochemical ingredients play a crucial role in preventing these ailments (Gonçalves and Romano 2017; Zengin *et al*. 2018). Some enzymes are among the selected targets to treat various diseases and inhibitory substances have been administered to inhibit these target enzymes such as AChE, collagenase, lipase, and urease. For example, cholinesterase inhibitors such as donepezil used to treat dementia bind to the active side of the target enzymes and reduce the hydrolysis of substrate acetylcholine iodide (Li *et al*. 2016; Takim *et al*. 2021; Makarian *et al*. 2022). Studies on bioactive molecules as traditional, complementary, and functional molecules have continued in medicinal chemistry regarding the easy availability of herbal constituents and leading chemical synthesis. Research on the new active extracts has become requisite in traditional and complementary medicine practices (Baydoun *et al*. 2015; Dutta *et al*. 2021).

The phytochemical ingredients and metabolite quantity of plants vary according to the plant tissues as well as geographical conditions and soil characteristics where plants grow. Surveys on the genus *Allium* collected from diverse ecological environments have been performed on plant secondary metabolites and biological activities. Nonetheless, the bulbs of *A. kastambulense* have been opted to reveal its complementary medicinal importance as no studies on phenolic components and biological activity could be reached on the onion. In the current work; phenolic components, and enzyme inhibition potency of the bulb extracts were investigated with the addition of computational studies.

2. Experimental section

2.1 Plant sample and extraction procedure

The bulbs for *Allium kastambulense* Kollmann were obtained from the plant in Kurucaşile district-Bartın (Türkiye) and the samples were dried at room conditions. The bulbs were granulated with a mechanic grinder and the powder was extracted in methanol-chloroform (4:1) solvent at ambient temperature. The obtained mixture was sonicated for 30 min at 37°C and centrifuged at 4500 x g for 12 min. The upper layer of the sample was filtered with a 0.22 µm pore-sized syringe filter and the organic phase was evaporated lower than 50 °C. The crude bulb extract was kept at -20 °C for following studies.

2.2 Analysis of the total phenolic compound (TPC) and total flavonoid contents (TFC)

The quantification of TPC and TFC in the bulb extracts was achieved by the Folin-Ciocalteu reagent and aluminum chloride ($AlCl_3$) colorimetric method, respectively described by the previous studies (Singleton and Rosi 1965; Pękal and Pyrzynska 2014).

2.3 Phenolic compounds profile of bulb extract

The phenolic compound composition of bulb extract was quantified using the HPLC system coupled with an LC 20AT pump and UV-Vis detector (Shimadzu Scientific Instruments, Japan). The sample solution (20 µL) was loaded onto the equipment set to 1 mL min^{-1} with automatic injection and phenolic compounds in the extracts were identified in comparison with the chromatographic profile, retention times, and UV–vis spectra of standard external metabolites (Elmastaş *et al*. 2017). The quantification of phenolic compounds in the bulbs was computed using the equation for the calibration curve of the obtained compound and the results were specified as mg g^{-1} of dried bulb extract.

2.4 Enzymes inhibition studies

Enzyme inhibitory ability of bulb extract in the work was evaluated using 96-wellplate spectrophotometric method referred in the

following sentences (MultiskanGO Microplate Reader, Thermo Scientific). The cholinesterase (AChE and BChE) inhibitory potency was evaluated using the well-known Ellman's method (Ellman *et al*. 1961). Briefly, both enzymes in Tris-HCI buffer (pH 8.0, 100 mM) were incubated with the extract solutions at 25 °C for 10 min, and substrates (AChI and BChI) were added to wells. The absorbance of the colored mixture was read at 412 nm. The inhibitory assay for collagenase was carried out using synthetic substrate FALGPA (N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala) in tricine buffer (pH 7.5) according to the previously described. The change in absorbance due to hydrolysis of FALGPA was recorded 340 nm (Thring *et al*. 2009). The inhibitory study of pancreatic lipase was performed using substrate p-nitrophenylpalmitate (p-NPP) with referred research (Mohamed El-Korany *et al*. 2020). The wells comprised Tris-HCI (100 mM, pH 8.2), 20 µL of enzyme and bulb extract was incubated for 10 min at 37 °C was incubated and then the absorbance of the reaction by adding p-NPP was monitored at 410 nm. The urease inhibition method was achieved using indophenols technique by measuring the liberated ammonia (Ikram *et al*. 2017). Briefly, urease enzyme from jack bean prepared in KH_2PO_4 buffer (100 mM, pH 8.2) was incubated with the test extracts for 15 min at 37 °C, and then phenol reagent and alkali reagents were added to react with liberated ammonia. The absorbance of colored complex was measured at 630 nm. Tyrosinase inhibition study was done using substrate L-DOPA, mushroom tyrosinase prepared in phosphate buffer (pH 6.8, 100 mM), and bulb extracts. The increased absorbance of the colored compound dopachrome was recorded at 475 nm (Masuda *et al*. 2005).

2.6 Computational molecular docking study

The molecular docking simulation was implemented on studied enzymes to evaluate the in-silico inhibition ability of catechin, which is the most abundant in the bulb extract, for learning protein-ligand interactions by using Autodock vina v.1.2.0 (Troot and Olson 2010; Eberhardt *et al*. 2021). Three-dimensional structure of target proteins AChE, BChE, collagenase, lipase, tyrosinase, and urease were uploaded the PDB protein data bank (https://www.rcsb.org/). The geometry optimization of catechin were realized using Chimera software (Pettersen *et al*. 2004). Undesired and water molecules were removed, and polar hydrogen bonds were added to molecular docking process. The grid box of ligand complexes was defined with the previous literature. The binding energy, visualization, and interaction for catechin were evaluated via Discovery Studio (DS) 3.5 (San Diego: Accelrys Software Inc. 2012).

3. Results and Discussion

3.1 Phenolic components and evaluation of biochemical inhibitory potency of the bulb extract

The quantification of phenolic components was assayed in the bulb extract of *A. kastambulense* with reverse-phase HPLC analysis, and these compounds are given in Table 1. Catechin, chlorogenic acid, gentisic acid, vanillic acid, and apigenin were profiled as the main phytochemical ingredients. Catechin was extensively defined to be the most abundant antioxidant secondary metabolite in the bulb extracts, and its quantity was calculated as 25.21±0.98 µg g^{-l}. The TPC and TFC of the bulbs were computed as 6.86 ± 0.36 mg g^{-1} and 3.22 ± 0.14 mg g^{-1} equilibrated with gallic acid and quercetin, respectively (Table 1). In the study examining metabolomics profiles in different organs of *A. ampeloprasum* L. in İzmir (Türkiye) using LC-MS/MS technique, they have detected phenolic compounds like benzoic acid, catechin, and vanillic acid and have suggested a link between bioactive ingredients and the locality where plants are collected (Emir *et al*. 2022). Phytochemical ingredient analyses are carried out in various plant taxa to correlate with the biological potency of plants. Đordevski *et al*. (2022) identified with the UHPLC system that flower extract *A. scorodoprasum* has 18 phenolic constituents including apigenin, depending on the extraction solvents such as ethanol, ethanol:water, butanol and botanol:water (Đorđevski *et al*. 2023). In similar works, researchers investigated phenolic profiles by LC-ESI-MS/MS in bulb extract of *A. nigrum* L. and *A. subhirsutum* L.

and they have determined about thirty phenolic compounds such as benzoic acids, p-coumaric acid, vanillic acid, quercetin, and catechin. They have expressed that the TPC of both plants was 45.6 ± 1.9 and 15.8 ± 0.9 mg g^{-1}, and TFC for them was 8.2 ± 0.7 and 5.7 ± 0.8 mg g^{-1}, respectively (Emir *et al*. 2020).

Table 1. The main components, TPC and TFC in the bulb extract of *A. kastambulense*

Compounds	*Retention time (min)*	Amount (µg/g)
Cathecin	10.558	25.21±0.22
Chlorogenic acid	11.627	6.34±0.11
Gentisic acid	11.771	8.42±0.13
Vanillic acid	13.583	9.50±0.15
Apigenin	47.475	2.71±0.01
TPC (mg/g DW)		6.86 ± 0.36
TPC (mg/g DW)		3.22 ± 0.14

In the current work, the enzyme inhibitory potency of the bulb extract was examined against six medicinal enzymes to uncover its complementary herbal medicinal importance. In the screening work, the bulb extract exhibited inhibitory effects, and the obtained IC_{50} values were 59.93±1.77 78.00±1.89, 54.76±1.73, 54.70±1.74, 134.3±2.12, and 91.05±1.95 µg mL^{-1} for collagenase, lipase, tyrosinase, urease, AChE, and BChE, respectively (Table 2, Figure 1). The evaluation of enzyme inhibition potential of various plants in pharmaceutical research and traditional medicine has been investigated in previous studies. The literature survey indicated that the extract of *Allium ampeloprasum* L. has inhibitory effects against AChE, BChE, and tyrosinase with IC_{50} values in the range of 34.55 ± 1.8 - 42.975 ± 3.2, 30.075 ± 1.7 - 41 ± 2.5, 490.66 ± 6.2 - 207.85 ± 7.2 µg/mL, respectively (Emir *et al*. 2022). The previous study conducted in two Kazakh species of *Allium* declared that the extracts of *A. galanthum* and *A. turkestanicum* have tyrosinase inhibitory activity at 100 µg/mL, and bulb ether extract of *A. galanthum* has the more efficient inhibitory ability (Kadyrbayeva *et al*. 2021). In various previous studies, it has been stated that plants such as *A. mongolicum*, *A. scabriflorum*, *A. cappadocicum*, *A. paniculatum*, *A. ascalonicum*, and *A. ursinum* belonging to the genus *Allium* have inhibitory effects at different concentrations on the activities of some enzymes such as cholinesterase, urease, lipase, and tyrosinase (Kim 2007; Shabana *et al*. 2010; Nikkhahi *et al*. 2018; Wang *et al*. 2019; Phetmanee *et al*. 2020; Marrelli *et al*. 2022; Rocchetti *et al*. 2022). In comparison with the mentioned studies, the present results possessed the bulb extract of *A. kastambulense* has a reasonable inhibitory effect against the studied enzymes consistent with previous studies.

Table 2. IC_{50} values of bulb extract of *A. kastambulense*

Enzymes	Bulb extract (µg mL^{-1})	
	IC_{50}	r^2
AChE	134.3±2.12	0.985
BChE	91.05±1.95	0.986
Collagenase	59.93±1.77	0.983
Lipase	78.00±1.89	0.987
Tyrosinase	54.76±1.73	0.988
Urease	54.70±1.74	0.984

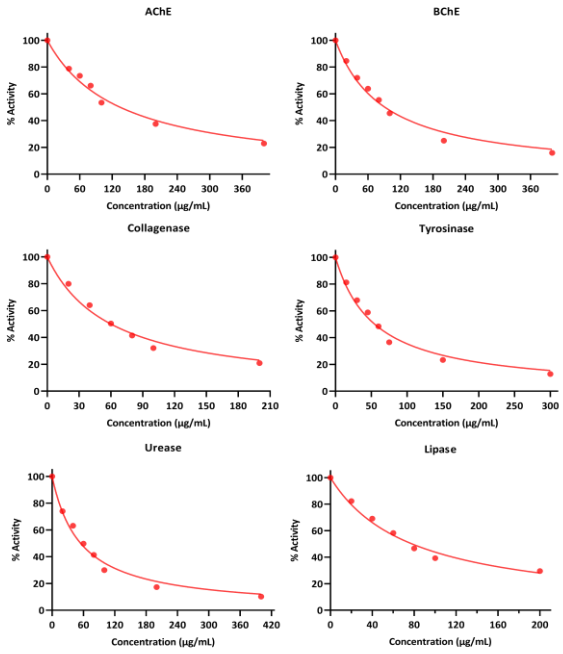

Figure 1. Inhibitory concentration of onion extracts

3.2 Molecular docking of catechin as a main chemical compound in the bulb extract

In the present work, molecular docking simulations were done to investigate the binding energy between catechin and six enzymes using Autodock Vina v1.1.2 (The Scripps Research Institute, USA). Three-dimensional structures were retrieved by using entry IDs for AChE, BChE, try, lipase,

collagenase, and urease, which the IDs are 4EY6, 1P0I, 2Y9X, 6OB0, 7Z5U, and 3LA4, respectively (https://www.rcsb.org/, accessed on 24 February 2023). The structure of the target ligand, catechin (CID: 9064) was downloaded from PubChem. The molecular docking utilized to examine the interaction between the target ligand and proteins was used to explore the mechanisms of action of the catechin after the in vitro enzyme activity tests. For every simulation, the ligand is docked separately with the studied enzymes, and then the obtained lowest scores corresponding to the best binding affinity are given in Table 3.

The predicted binding motif indicates that the ligand binds within the catalytic cavity via conventional hydrogen bonds, carbon-hydrogen bonds, pi-pi stacked, and pi-alkyl. These interactions with amino acids in the targets play a key role in the inhibitory effect of the ligand. Alignment and position of substituents on compounds may be considered responsible for ligand binding with studied enzymes. In the binding model, the ligand was positioned with the active site of studied enzymes, and ligand-protein interactions showed various interactions such as H-bond, Pi-Pi, amide-π, alkyl, π-alkyl, and π-anion involved with amino acids in the protein structures (Figures 2-7).

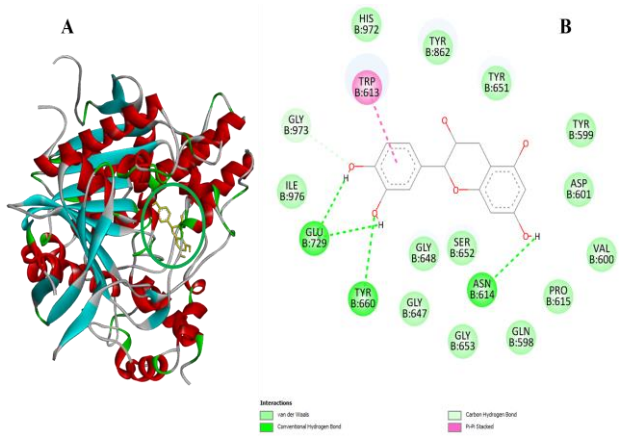

Figure 2. The interaction view of catechin with AChE

Table 3. Docking score and interaction description of ligand catechin with studied enzymes

Enzymes	Score (Kcal/mol)	Interaction description
AChE	-8.836	**H-bond:** Try660, Glu729, Asn614, Glu973; **π-π:** Trp613
BChE	-8.221	**H-bond:** Try125, Glu194, Gly112, Ala325, Pro282, Trp79 **π-π:** Trp79, Phe326; **π-Alkyl:** Phe326,
Try	-7.038	**H-bond:** His262, His84; **π-π:** His262, Phe263; **Amide-π:** Ser281, Val282; **π-Alkyl:** Val282, Ala285, Val247
Lipase	-8.819	**H-bond:** Ser130, Trp833; **π-π:** Tyr92; **Alkyl:** Ile192; **π-Alkyl:** Trp53, Tyr92, Ile192
Collagenase	-8.832	**H-bond:** Glu95, Tyr93; **π-Anion:** Glu152; **π-π:** Trp136; Tyr196; **π-Alkyl:**His124
Urease	-8.324	**H-bond:** Gln81, Glu735, Asp723, Val80; **π-π:** Phe705; **Alkyl:** Val737; **π-Alkyl:** Lys709, Val737

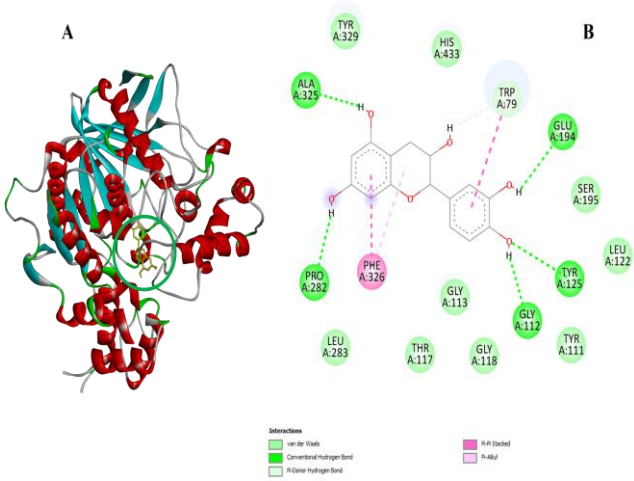

Figure 3. The interaction view of catechin with BChE

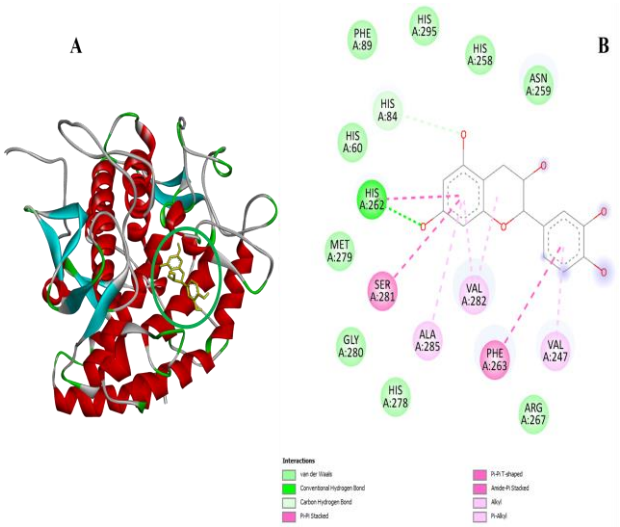

Figure 4. The interaction view of catechin with Try

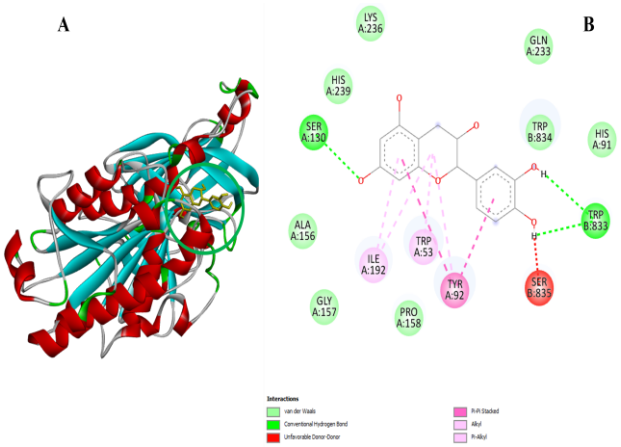

Figure 5. The interaction view of catechin with Lipase

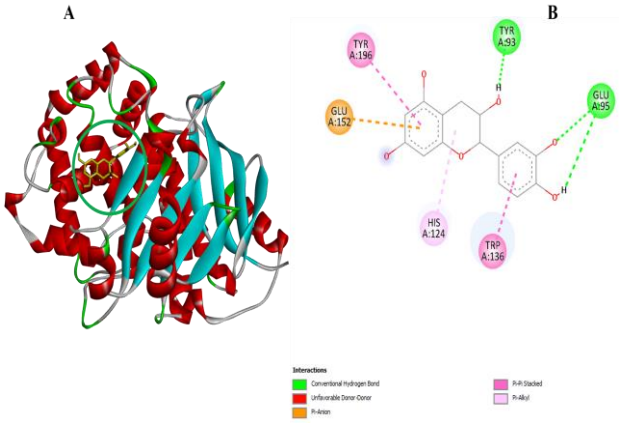

Figure 6. The interaction view of catechin with Collagenase

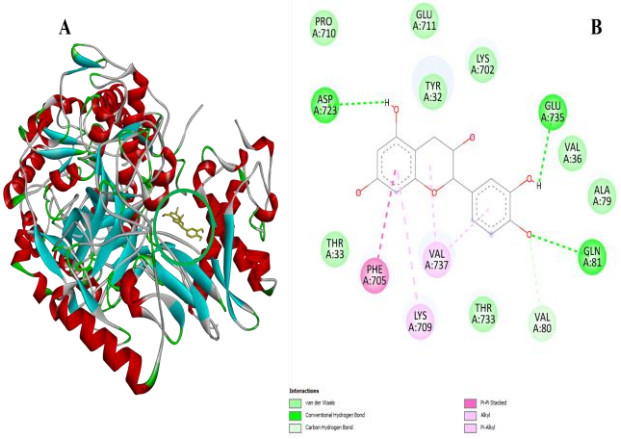

Figure 7. The interaction view of catechin with Urease

4. Conclusion

In the current work, phenolic contents and biological activity of bulb extract were investigated, and molecular docking study of catechin flavonoid - a type of secondary metabolite against the studied enzymes was performed. Briefly, the results of endemic bulb extract (*A. kastambulense*) owning enzyme inhibition capacity were explained for the first time in addition to computer-aided molecular docking details. According to the obtained molecular docking scores, the catechin may be related to the inhibitory potency of the bulb extract. Overall, since these findings are the results of in vitro laboratory studies, the complementary medical importance of bulb extract can be further explored in the future with additional in vitro and in vivo pharmacological analyzes.

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