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Original Article

Investigation of acetylcholinesterase, butyrylcholinesterase, α-glucosidase, α-amylase, and tyrosinase inhibition and antioxidant activity of methanol and water extracts from aerial parts of *Phlomis lycia* D. DON

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Abstract

Background: Natural antioxidants in the plant kingdom play an essential role in reducing the risk of various chronic diseases and maintaining a healthy life. In addition, acetylcholinesterase, butyrylcholinesterase, α-glucosidase, α-amylase, and tyrosinase are involved in the occurrence and pathology of some as Alzheimer's and diabetes, have become an important strategic target in the effective treatment of these diseases. *Phlomis lycia* D. DON is traditionally used as an appetizer, stimulant, carminative, tonic, and against stomach pain and dyspeptic complaints. There is limited knowledge on this species except for preliminary studies such as botanical and phytochemicals and biological properties. The aerial parts of this plant contained phenolic compounds, such as lignan, phenylethanoid, and iridoid derivatives which can be responsible for the potential biological effects. Therefore, the present study was aimed to investigate the antioxidant and enzyme inhibition activity of the methanol and water extracts obtained from aerial parts of P. *lycia* in the flora of Turkey.

Material and Methods: Antioxidant potentials were determined spectrophotometrically by DPPH and ABTS radical scavenging, iron chelating, and β-Carotene/linoleic acid emulsion method. We evaluated the inhibitory activities of methanol and water extracts of P. *lycia* against various enzymes (acetylcholinesterase, butyrylcholinesterase, α-glucosidase, α-amylase, and tyrosinase) by using 96-well plate methods.

Results: According to the results, the methanol extract was more active than water extract in terms of antioxidant activity. On the other hand, although the methanol and water extracts were observed to demonstrate similar enzyme inhibitory activity, the water extract exhibited more inhibition activity on α-glucosidase.

Conclusions: Our results suggested that *Phlomis lycia* could potentially be used for the isolation of potent antioxidants from the methanol extract. To the best of our knowledge, this study represents the first time that *Phlomis lycia* was reported in the literature with glucosidase inhibition activity.

Keywords: *Phlomis lycia*, Antioxidant activity, Enzyme inhibition, α-glucosidase, α-amylase.

Background

Numerous plants have been used in the cure of diverse ailments for around three thousand years. Research on traditional medicinal plants used against diabetes mellitus has a significant impact on the emergence of therapeutic herbal products that can now be used against diabetes mellitus. This research includes *in-vitro*, *in-vivo*, and clinical trials.[1] Alpha-glucosidase inhibitors (AGI): retard the absorption of carbohydrates by inhibiting α-glucosidase in the small intestine.[2] Acarbose, miglitol, and voglibose belong to the group of AGI drugs, being acarbose the only one used in Turkey. However, these drugs' tolerance is limited due to their gastrointestinal side effects, including abdominal pain, bloating, and diarrhea.[3] Therefore, it is necessary to develop new drugs from AGI that have fewer side effects and are more easily tolerated by the patients.

Alzheimer's disease (AD) is a progressive chronic neurological disease described by decreased cognitive functions, daily activities, behavioral changes, and psychiatric symptoms.[4] Because of the aging of society and lack of effective treatment methods, the incidence of AD has been increasing in recent years.[5] AD is caused by the exacerbation of cholinergic receptors, especially basal forebrain acetyl cholinergic neurons.[6] Acetylcholine is a neurotransmitter released from the synapse of the

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neuron. Acetylcholine is synthesized in the specific cells, called cholinergic neurons, using enzyme choline acetyltransferase, acetyl coenzyme A and the substrate as colinic acid. The enzyme acetylcholinesterase has become an attractive target for discovering rational drug designs and inhibitors based on this mechanism. Although acetylcholinesterase inhibitors are widely used in AD treatment, they can cause serious side effects, such as anorexia, diarrhea, fatigue, nausea, muscle cramps, gastrointestinal, cardiovascular, respiratory system, genital tract, and sleep disorders.[7] Therefore, there is a need for discovering new drugs which are more effective and have higher bioavailability.

Tyrosinase is a polyphenolic oxidase having the binuclear copper active site and plays a significant role in the formation of melanin pigments in mammals.[8] Exhibiting the high activity of tyrosinase leads to skin hyperpigmentation. Therefore, chemical agents with tyrosinase inhibitory effects have been used to suppress melanogenesis and are clinically used in dermatological disorders associated with melanin hyperpigmentation.[9] Kojic acid has been shown to cause cytotoxic and mutagenic effects in the chronic period, although it is one of the popular chemicals used as a cosmetic skin-lightening agent.[10] When these side effects have been considering, the discovery of new natural anti-tyrosinase products with low potential side effects has become more important than the use of chemical agents.

The genus *Phlomis*, which belongs to the Lamiaceae family, has approximately 100 species and an important natural source among the medicinal plants. The taxa of the genus are distributed throughout Asia, Southern, and Northern Europe.[11] The genus presents 39 taxa and 13 hybrids in Turkey.[12] Several *Phlomis* species have been reported to have been used as stimulants, appetizers, antiallergic, diuretic, diarrhea, antitussive, anti-stomach, pain relief, antidiabetic, and tonic in Turkish traditional medicine.[13] Some *Phlomis* species have particular ethnobotanical uses as herbal teas in their respective countries.[14] Inflorescence and leaves of *Phlomis lycia* D. DON is used against stomach pain and dyspeptic complaint as well as appetizer, stimulant, carminative, and tonic in folk medicine.[14] *P. lycia*, known as 'tüylü çalba', is a shrub to 150 cm and glandular. *P. lycia* is morphologically close to *P. monocephala*, differing primarily from the latter by having leaves cordate or rarely truncate at base and bracteoles as long as calyx.[12,15] Its flowering time is from May to August. *P. lycia* is distributed in South and West Anatolia, Mediterranean, and Aegean regions. Previous phytochemical studies revealed that *Phlomis* species contain flavonoids, iridoids, phenylpropanoids, lignans, and essential oils.[16-20] The abundance of flavonoid and phenolic chemicals in these *Phlomis* species was also demonstrated to have outstanding radical scavenging capability and enzyme inhibition effect.[14,17] According to the literature, there were limited studies on *P. lycia* regarding phylogenetic and isolation of phytochemicals, etc.[11,18] The phenolic compounds (lignan, phenylethanoid, and iridoid derivatives) found in the aerial parts of this plant may be associated with potential bioactivities.[14,17,18] Despite many studies on *Phlomis*, there is no research on antioxidant and enzyme inhibitory activities of *P. lycia*. This study investigates *in vitro* antioxidant potentials and inhibitory effects on diverse enzymes, such as acetylcholinesterase and butyrylcholinesterase against AD; α-glucosidase, and α-amylase against diabetes. In addition, methanol and water extract obtained from aerial parts of *P. lycia* were tested against hyperpigmentation.

Materials and Methods

Chemicals and Equipment

Folin–Ciocalteu's phenol reagent, 2-2′-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman carboxylic acid), sodium carbonate, quercetin, butyryl hydroxytoluene (BHT), ethylenediaminetetraacetic acid (EDTA), galantamine, acarbose, and gallic acid were purchased from Sigma–Aldrich (St. Louis, MO). Hydrochloric acid and methanol were obtained from Merck (Darmstadt, Germany). All chemicals used in the experiments were of analytical grade. To evaporate the combined solvents using Buchi R-300 Rotavapor, Switzerland. Spectrophotometric measurements were performed by a microplate reader (Multiskan Go; Thermo Scientific Inc.).

Plant Materials

The aerial parts of the plant were collected from the natural habitat of the canyon, namely Yarıkpınar, located in the west of Antalya in 12th of April 2018 (The GPS coordinate of locality: 36°30'02.1"N; 30°29'30.2"E). The plant was defined by Prof. Dr. Hayri Duman, who is a botanist at Gazi University (the Department of Botany in Faculty of Science). A voucher specimen was kept in the KNYA Herbarium at Selçuk University (KNYA Herb. No: 26910). The plant materials were dried in the shade place and powdered by a laboratory-type miller.

Preparation of the Extracts

The powdered 10 g of plant material was macerated with methanol according to the method which was proceeded before in our work throughout 24 hours at almost 24°C for two times.[21] Subsequently, each mixture was filtered using filter paper, and the combined solvent was removed by an evaporator under vacuum at 40°C to give the methanol extract. The remaining plant parts were extracted with distilled water twice by the maceration method. Both filtrates were dried, and water extract was obtained. All extracts were deposited at -20°C until performing the

experimental procedures. All the experiments were carried out in three parallel groups and three times.

In Vitro Antioxidant Activity

Determination of Total Phenolic Content

The total phenolic contents of the extracts were established using the Folin-Ciocateu (F-C) method applied by Clarke et al.[22] The standard curve was prepared using 1-1000 µg/mL gallic acid solutions in DMSO. Total phenol content was expressed as gallic acid equivalent (mg GAE/g) per weight of the dry extract.

Total Flavonoid Content Determination

To determine the total flavonoid contents of the extracts, we used the modified aluminum chloride colorimetric method by Yang et al.[23] Total flavonoid amounts were calculated as quercetin equivalent per weight of dry extract (mg QE/g).

DPPH Radical Scavenging Activity

DPPH radical scavenging potentials of the extracts were determined by the method of Clarke et al.[22] Instead of the test sample, DMSO was run as a blank, and the quercetin was used as the standard. The DPPH radical scavenging effects (%) of the samples were calculated using the equation below.

DPPH radical scavenging activity (%) = (Control Absorbance - Sample Absorbance)/Control Absorbance × 100

The control absorbance is all solutions that do not contain the test substance. Sample absorbance is that of plant extract or quercetin solution.

ABTS Radical Scavenging Activity

The method applied by Re et al[24] was used with minor modifications to determine the ABTS scavenging activity of the plant extracts. The $ABTS^{+}$ scavenging activity of the plant extract was compared with the reference substance BHT, and the percentage of radical scavenging activity was calculated according to the following formula:

$$ABTS\ radical\ scavenging\ activity\ (\%) = (A_{control}\text{-}A_{test}) / A_{control} \times 100$$

Iron Chelating Activity

To detection of the iron-chelating activity of the extracts, it needs to interact with the formation of the ferrozin-Fe^{2+} complex.[25] EDTA was used as a positive control. The metal chelating capacity is calculated according to the following formula:

$$I\% = (A_{control}\text{-}A_{test}) / A_{control} \times 100;$$

$A_{control}$, absorbance of a non-sample contained test tube; A_{test} is the absorbance of the mixture containing the test samples.

β-Carotene/Linoleic Acid Emulsion Method

The plant extracts and synthetic antioxidant Trolox to be used as a positive control were prepared in methanol (concentration of 2 mg/mL) by following the method described previously.[26] The absorbance of the test tubes and control solution was read at 470 nm for start (t_0) and 120 minutes (t_{120}). The absorbance rate and therefore the oxidation inhibition coefficients were calculated according to the following formula:

$$I\% = a / b \times 100,$$

a is the absorbance after 120 min of incubation; b is the absorbance of the mixture at 0 min.

In Vitro Enzyme Inhibition Activity

Acetylcholinesterase Inhibition Activity

To investigate acetylcholinesterase inhibitions of the plant extracts, we proceeded according to the Ellman's method with slight modification.[27] Galantamine was used as a positive control, and methanol was used as a negative control.

Butyrylcholinesterase Inhibition Activity

Butyrylcholinesterase inhibitory activities of the extracts were detected by the spectrophotometric technique developed by Ellman et al.[27] Butyrylcholinesterase inhibitory effects of the extracts were determined by spectrophotometric measurement at 412 nm.

α-Glucosidase Inhibition Analysis

The α-glucosidase inhibitory effects of the extracts were determined by the 96-well plate method of Lordan et al.[28] In this method, *p*-nitrophenyl α-D-glucopyranoside (PNPG) was used as the substrate. The buffer solution was used as a negative control, and acarbose was used as a positive control. The activity of α-glucosidase inhibition (%) was calculated according to the equation below:

$$I\% = [1\text{-}\ (A_{test}\text{-}A_{test\text{-}control}) / (A_{control}\text{-}A_{blank})]$$

A_{test}: Absorbance of test solutions containing enzyme and substrate

$A_{test\text{-}control}$: Absorbance of test solutions containing substrate but no enzyme

$A_{Control}$: Absorbance of enzyme and substrate without test samples

A_{blank}: Absorbance of reaction system containing the only substrate

α-Amylase Inhibition Activity

The α-amylase inhibitory effects of the extracts were evaluated using the Caraway-Somogi iodine/potassium iodide method reported by Özek with a minor modification.[29] Freshly prepared starch solution (0.05%)

was used as a substrate, and acarbose was used as a positive control. The blank groups include all reagents except for the enzyme solution; the control group contains reagents and substrates instead of sample and enzyme solution. The absorbance of the mixture was read at 630 nm. The percentage of α-amylase inhibition was calculated according to the formula given in the α-glucosidase inhibition assay.

Tyrosinase Inhibition Activity

The tyrosinase enzyme inhibitory activity of the extracts was performed according to the method described by Jeong et al.[30] The absorbance was then measured at 492 nm with a microplate reader. Kojic acid was used as a positive control. Inhibition value (%) was calculated by the following formula:

I% = [1- (Aa-Ab) / (Ac-Ad)] × 100,
Aa: Absorbance of test samples containing enzyme,
Ab: Absorbance of test samples without enzyme,
Ac: Absorbance of enzyme solution without test sample,
Ad: Absorbance of both test samples and enzyme-free mixture.

Statistical Analysis

All determinations of antioxidant and enzyme inhibition activity were performed in triplicate. The results are represented as mean ± standard deviation. Where appropriate, data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test or Tukey's multiple comparison test. The software employed for statistical analysis was GraphPad Prism, version 8.

Results and Discussion

In Vitro Antioxidant Activity

Total Phenolic and Flavonoid Content

Total phenol contents were calculated and given as mg gallic acid equivalent per gram of dry extract using calibration curve of gallic acid (Y = 0.003X + 0.0578, r^2 = 0.998). Total flavonoid quantities were detected from the calibration curve of quercetin with Y = 0.0059X + 0.0739 (r^2 = 0.9971), and presented as mg quercetin equivalent per gram of dry extract. As seen from the results, the amount of flavonoids (118.91 ± 2.10 mg QE/g) was higher in the water extract than the methanol extract, while the phenolic content was higher in the methanol extract (114.58 ± 5.52 mg GAE/g) than the water extract in Figure 1. This means that the flavonoid-rich compounds with higher polarity are present in the aqueous extract, and the more non-polar phenolic-rich components are passed into the methanol extract. As far as our literature survey, there are not many studies on the antioxidant activity of *Phlomis* species. Zhang and Wang reported the total phenolic and flavonoid contents of methanol and acetone extracts of *P. umbrosa*, and *P. megalantha*. It was found that the methanol and acetone extracts of *P. megalantha* contain 55.20, and 43.42 mg GAE/g, respectively, as well as 35.91, and 54.33 g (-)-epicatechin equivalents (EE)/g, respectively. It was also exhibited that these *Phlomis* species had remarkable radical scavenging activity attributed to their rich flavonoid and phenolic compounds.[17] In another work, the methanol, ethyl acetate, butanol, and water extracts of the aerial parts of *P. thapsoides* collected from Uzbekistan, were evaluated on their biological activities. The ethyl acetate fraction was shown the highest total phenolic content with 362.06 mg GAE/g, although other fractions were found to range between 37.67 and 69.55 mg GAE/g.[31] *Phlomis* species included phytochemical investigations, including *P. lycia*, phenylethanoid, iridoid, monoterpenoid, diterpenoid, and lignane glycosides, well as flavonoid derivatives, and phenolic compounds were isolated and identified.[17,32,33] Saracoğlu et al investigated the phenolic constituents of *P. lycia* from Antalya. Seven phenolic compounds, such as lignan, phenylethanoid, and iridoid derivatives, were isolated from aerial parts of the plant.[18]

Determination of DPPH Radical Scavenging Activity

DPPH radical scavenging activities of methanol and water extracts of *P. lycia* were shown in the concentration range of 50-1000 µg/mL in Figure 2. The methanol extract had higher DPPH radical scavenging activity

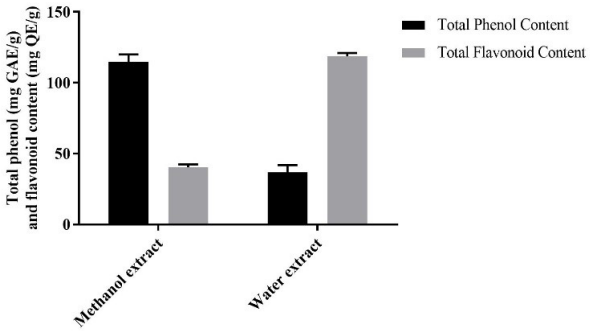

Figure 1. Total Phenol and Flavonoid Content of Methanol and Water Extracts of *P. lycia*

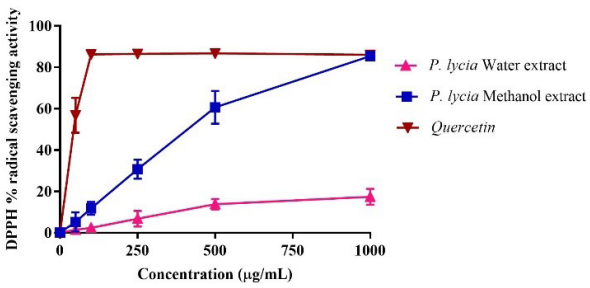

Figure 2. DPPH Radical Scavenging Activity of Methanol and Water Extracts of *P. lycia*

than the water extract. Otherwise, the methanol extract exhibited almost equivalent activity of quercetin at the highest concentration. As comparing the literature, there were limited works on antioxidant potentials of different *Phlomis* species evaluated in terms of DPPH assay. The methanol extract of *P. samia*, and the isolates such as samioside and acteoside were found as active with more than 70% radical scavenging activity. The IC_{50} values for them were detected to be 66.0 µg/mL (23.8 µM) for the methanol extract, as well as 18.0 (23.8 µM), and 30.5 µg/mL (48.9 µM) for samioside and acteoside, respectively. In addition, when the results compared the standards caffeic acid (66.7 µM) and gallic acid (17.6 µM), it was revealed that the most promising sample was found as samioside.[33] In other phenolic principles of *P. caucasica* study screening free radical scavenging activity, two phenylethanoid glycosides, it was reported that the highest active antioxidants were forsythoside B, and acteoside (RC_{50} = 4.97, and 4.27 µg/ml, respectively).[32] The methanol and acetone extracts of *P. megalantha* and *P. umbrosa* were tested with DPPH assay. It was found that the highest radical scavenging activity was observed in the acetone extract of *P. megalantha* (IC_{50} = 13.8 µg/mL) that was similar to positive reference α-tocopherol (IC_{50} = 12.1 µg/mL). Otherwise, the strongest activity was observed in positive references ascorbic acid, and BHT with IC_{50} values 4.9, and 7.2 µg/mL. Among the five pure phenolic compounds, protocatechuic acid had the most significant radical scavenging ability, followed by rosmarinic acid, chlorogenic acid, and rutin, except for benzoic acid.[17]

Determination of ABTS Radical Scavenging Activity

ABTS radical scavenging activity method is one of the commonly used methods in determining antiradical activity. The ABTS radical is more polar than the DPPH free radical and will be useful in determining the radical scavenging activity of extracts obtained using more polar solvents such as water. Figure 3 showed the ABTS radical scavenging activity of the *P. lycia* methanol and water extracts with the reference substance BHT. When our findings were compared with each other, both extracts and BHT increased in radical scavenging activity proportional to concentration. However, the methanol extract was more effective than the water extract at low concentrations, and ABTS radical scavenging activity nearly reached the same level at 1000 µg/mL. In the literature, the ethyl acetate, methanol, water, and butanol extracts of the aerial parts of *P. thapsoides* were detected as active against ABTS radical with IC_{50} values 9.48, 30.97, 49.67, and 52.32 µg/mL, respectively. Among them, the highest antioxidant effect was observed in the ethyl acetate fraction (IC_{50}=9.48 µg/mL), which was as effective as the Trolox reference with IC_{50} value 1.63 µg/mL.[31]

Iron Chelating Activity

Iron chelators function as antioxidants by scavenging reactive oxygen species (ROS) and reduce the amount of available iron, thereby decreasing the quantity of OH generated in Fenton reactions.[34] Many researchers have reported that the antioxidant and inhibition of lipoxygenase by chelation/reduction of iron in their active site of flavonoids can be attributed to its iron chelation activity.[35] Figure 4 showed the iron-chelating activity of the methanol and water extracts of *P. lycia* and reference compound used as EDTA. *P. thapsoides* was tested on ferric reducing antioxidant power assay for its various extracts in the literature. The ethyl acetate, methanol, butanol, and water extracts had found as 9.33, 4.29, 3.19, and 3.15 mM $FeSO_4$ equivalent/mg sample comparing with quercetin with 24.04 mM $FeSO_4$ equivalent/mg sample.[31] In the literature, the ferric reducing/antioxidant power of the extracts of *P. megalantha* and *P. umbrosa* were reported. The acetone and methanol extracts of *P. megalantha* (reducing power 2.3, and 2.1, respectively) were found more active than *P. umbrosa*. Phenolic compounds, protocatechuic acid, rutin, chlorogenic acid, and rosmarinic acid were detected as remarkably reducing power.[17]

β-Carotene/Linoleic Acid Emulsion Effect

The β-carotene/linoleic acid method is a method of inhibiting lipid peroxidation, which is important in determining the type of antioxidant that terminates radical degradation by introducing $H^{\cdot}$ radical into the medium. In addition, singlet in lipids gives an idea to determine the sputtering of oxygen. Figure 5 showed the

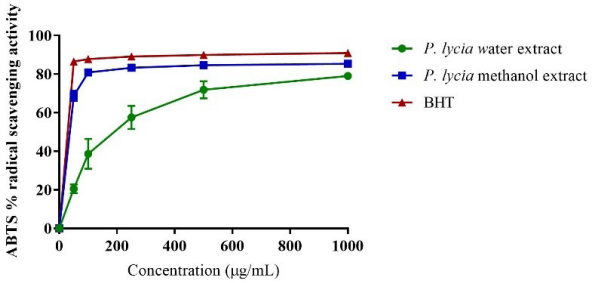

Figure 3. ABTS Radical Scavenging Activity of Methanol and Water Extracts of *P. lycia*.

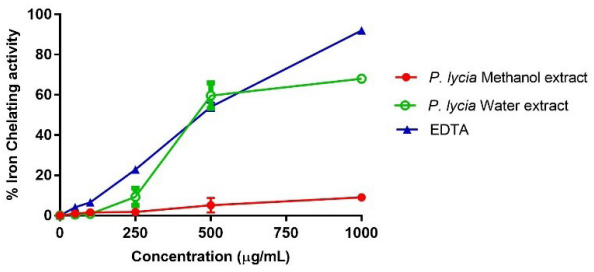

Figure 4. Iron Chelating Activity of Methanol and Water Extracts of *P. lycia* and Reference Compound EDTA.

β-carotene/linoleic acid emulsion effect of the methanol and water extracts of *P. lycia*. When the results were evaluated, the water and methanol extracts showed similar effects, and both extracts were found to be less effective than the reference substance Trolox. In the literature, the inhibition of linoleic acid oxidation of the extracts of *P. megalantha* and *P. umbrosa* were presented. The acetone and methanol extracts of these plants were inhibited with from 82.4 to 95.5% compared with positive references BHT, and α-tocopherol with 93.3 and 88.1%. Phenolic pure compounds, protocatechuic acid, chlorogenic acid, benzoic acid, rutin, and rosmarinic acid, also exhibited from 37.0 to 56.2% inhibitions.[17]

According to our findings, our work was corroborated with the previous works on some of *Phlomis* species for their antioxidant potentials. We also revealed that *P. lycia* may promise radical scavenging potentials attributed to their rich phenolic and flavonoid constituents.

Enzyme Inhibitory Activity

Anticholinesterase Inhibition

The acetylcholinesterase and butyrylcholinesterase inhibition activity results of the extracts together with the drug galantamine used in the treatment of AD were presented in Figure 6. Against acetylcholinesterase, the methanol extract and water extracts showed significantly lower activity than the reference substance galantamine, while the water extract was found to be more effective than the methanol extract. In both extracts, the inhibition effects of these enzymes showed an increase in proportion to the increase in concentration depending on the concentration. In the acetylcholinesterase inhibition activity, both extracts showed almost the same activity (Table 1). In a recent study conducted by Şenol et al, investigated on neurobiological effects of *P. grandiflora* in terms of tyrosinase and anticholinesterase activity, among ethanol, *n*-hexane, chloroform, ethyl acetate, and n-butanol extracts, only chloroform extract was found as active against butyrylcholinesterase with 59.28% inhibition at 200 µg/ml concentration[16]. However, in our study, the water extract *P. lycia* was found as more active than methanol extract in terms of butyrylcholinesterase inhibition activity.

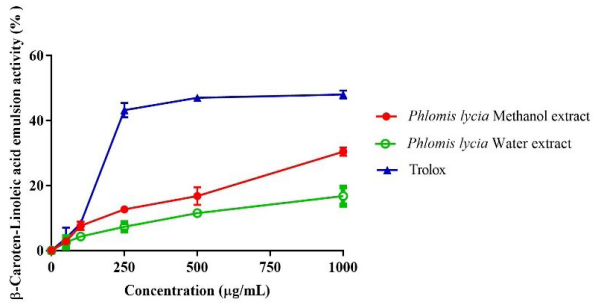

Figure 5. β-Carotene/Linoleic Acid Emulsion Effect of Methanol and Water Extracts of *P. lycia* and Reference Compound Trolox.

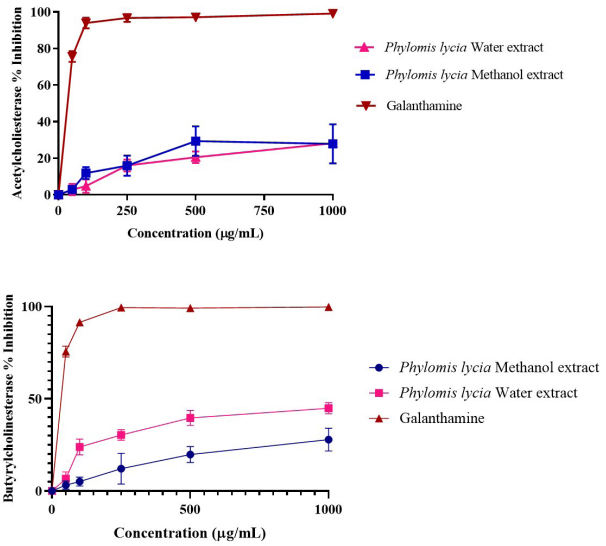

Figure 6. Acetylcholinesterase and Butyrylcholinesterase Inhibition Activities of Methanol and Water Extracts of *P. lycia*.

α-Amylase and α-Glucosidase Inhibition Activity

In this study, the potential hypoglycemic activity of methanol and water extracts obtained from the aerial parts of *P. lycia* was determined by two important enzymes in terms of antidiabetic activity: α-amylase and α-glucosidase inhibition activity, which play a crucial role in the control of blood sugar. Acarbose was used as the reference substance in both enzyme inhibition activity tests. The inhibition effects of the extracts against these two enzymes were compared with acarbose. As shown in Figure 7, the standard substance, acarbose, and both extracts demonstrated an increase in the effect of enzyme inhibition depend on the increase of concentration. In the α-glucosidase inhibition activity test, methanol and water extracts showed a much lower inhibition effect than acarbose (Table 1). As for α-glucosidase inhibition activity test, water extract exhibited a higher inhibition effect than methanol extract. In the literature survey on the genus *Phlomis*, Safamansouri et al investigated several Iranian traditional herbs for their α-amylase inhibitory activities. Among them, *P. bruguieri* and *P. persica* were found as more active than other *Phlomis* species in the literature.[36] In another study, Yang et al identified 20 compounds as α-glucosidase inhibitors from ethyl acetate fraction of *P. tuberosa* using sepbox chromatography and Thin-layer chromatography bioautography.[37] In another study, Jabeen et al isolated 12 compounds as α-glucosidase inhibitors from *P. stewartii*, and showed α-glucosidase inhibitory activity with IC_{50} values ranging from 14.5 to 355.4 µM.[38] In addition, Sarkhail et al have reported that the antidiabetic effects of methanol extract of *P. anisodonta* on streptozotocin-induced diabetic rats were evaluated. Oral administration of *Phlomis* extracts showed blood glucose reducing effect and increased hepatic antioxidant enzymes at doses of 200-400 mg/kg.[39]

Table 1. Enzyme Inhibition Activities of the Extracts of *P. lycia* and Reference Compounds

Extracts and Standards	Concentration (µg/mL)	Inhibition%				
		α-Amylase	α-Glucosidase	Acetylcholinesterase	Butyrylcholinesterase	Tyrosinase
P. lycia methanol extract	100	6.60 ± 1.77	11.50 ± 3.89	11.89 ± 3.26	5.15 ± 2.40	27.45 ± 1.02*
	250	11.25 ± 2.73	12.51 ± 3.58	15.94 ± 5.49	12.11 ± 8.32	29.83 ± 0.46**
	500	19.79 ± 3.55	23.38 ± 1.66	27.88 ± 10.71***	19.78 ± 4.37**	30.06 ± 2.58**
	1000	26.35 ± 2.69**	58.53 ± 5.14***	29.37 ± 8.07***	27.86 ± 6.19***	33.87 ± 0.17***
P. lycia water extract	100	3.28 ± 2.24	11.58 ± 1.42	4.82 ± 3.73	23.89 ± 4.28	27.81 ± 0.13*
	250	6.43 ± 2.71	13.84 ± 0.24	16.07 ± 3.29	30.39 ± 2.85***	32.87 ± 0.84***
	500	12.67 ± 9.34	18.73 ± 2.00	20.52 ± 3.16***	39.64 ± 4.09***	36.09 ± 0.26***
	1000	23.52 ± 7.28*	28.78 ± 4.06***	28.25 ± 1.84***	44.91± 3.03***	38.25 ± 0.37***
Acarbose	100	18.05 ± 8.31	26.09 ± 2.16***			
	250	42.22 ± 13.59***	32.89 ± 3.96***			
	500	84.38 ± 5.59***	46.93 ± 2.80***			
	1000	93.34 ± 0.40***	51.04 ± 4.68***			
Galantamine	100			93.95 ± 2.95***	91.42 ± 1.28***	
	250			96.72 ± 2.15***	99.48 ± 0.19***	
	500			97.11 ± 0.78***	99.19 ± 0.96***	
	1000			98.12 ± 0.83***	99.75 ± 0.33***	
Kojic acid	100					44.25 ± 6.54***
	250					63.48 ± 1.27***
	500					74.50 ± 1.30***
	1000					82.22 ± 1.34***

Note: * means statistically significance as $P < 0.05$; ** means statistically significance as $P < 0.01$; *** means statistically significance as $P < 0.001$.

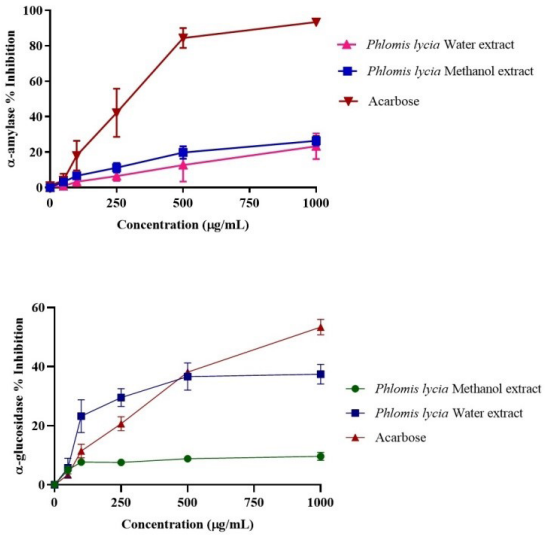

Figure 7. α-Amylase and α-Glucosidase Inhibition Activity Graphs of Methanol and Water Extract of *P. lycia* and Reference Compound Acarbose.

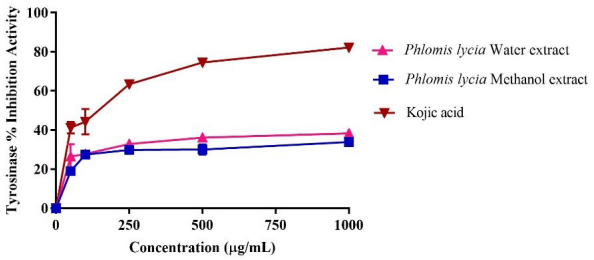

Figure 8. Tyrosinase Inhibition Activity Graphs of Methanol and Water Extracts of *P. lycia* and Reference Compound Kojic Acid.

Tyrosinase Inhibition Activity

In the tyrosinase inhibition activity test, L-DOPA was used as a substrate, and kojic acid was used as a reference to determine the mono- and diphenolase activity of tyrosine obtained from the mushrooms. As shown in Figure 8, the tyrosinase inhibition activities of the methanol and water extracts of *P. lycia* were lower active than the standard substance kojic acid. However, when these two extracts were compared, no difference was observed in their inhibition effects against tyrosinase (Table 1). In a recent study, Salimi et al reported that the methanol extract of *P. kurdica* demonstrated mushroom tyrosinase inhibitory activity in a dose-dependent manner and inhibited the tyrosinase enzyme about 28% at the concentration of 0.25 mg/mL.[40] These results showed consistency with the results of our study.

Conclusions

Our results revealed that methanol and water extracts of *P. lycia* showed strong antioxidant activities and

moderate enzyme inhibitory effects. These findings had also been compared to standard antioxidants and positive references for acetylcholinesterase, butyrylcholinesterase, α-glucosidase, α-amylase, and tyrosinase inhibitors. In addition, the extracts showed remarkable radical scavenging activities attributable to high total polyphenol and flavonoid contents. The methanol extract was observed to be more active than water extract in terms of antioxidant activity as well as *α*-glucosidase and *α*-amylase enzyme inhibition effects. Our results showed that the plant might be helpful as a biological source for developing a natural agent to prevent Alzheimer's, skin disorders related to melanin hyperpigmentation and diabetes mellites associated with oxidative stress and enzymatic reaction.

Competing Interests

None.

Acknowledgments

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