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Exploring the Impacts of *Angelica purpurascens* Extracts on Anticholinergic, Antidiabetic, Antibacterial Potential, and Antioxidant Capacity

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Highlights:

- Findings suggest potential for incorporation into nutraceuticals or pharmaceuticals to address health concerns
- Studies indicate promising antidiabetic effects, offering potential for managing blood sugar levels as an alternative or adjunctive treatment for diabetes mellitus
- Extracts show strong antioxidant properties, combating oxidative stress and potentially preventing oxidative stress-related diseases

ABSTRACT:

In Kars-Sarıkamış-Soğanlı, *Angelica purpurascens* (*A. purpurascens*) emerges as a promising natural antioxidant source. Extracts from its leaves, branches, and flowers underwent thorough bioanalytical assessments. The leaf extract exhibited the highest concentrations of herbal flavonoids (45.22 µg QE/mg extract) and total phenolics (28.96 µg GAE/mg extract). Branch extracts demonstrated significant enzymatic activity against AChE and BChE with IC50 values of 37.26 mg/mL and 9.08 mg/mL respectively. The flower extract displayed notable antibacterial properties. This study sheds light on the therapeutic potential of *A. purpurascens* ethanol extracts, suggesting benefits for Alzheimer's, cell damage-induced diseases, and diabetes mellitus. It pioneers new enzymatic and antioxidant insights and contributes to understanding this abundant Türkiye species. *A. purpurascens* holds promise for pharmaceutical exploration, offering potential solutions for challenging health conditions.

Keywords:

- Alzheimer's disease
- *Angelica purpurascens*
- Antibacterial
- Antioxidant
- α-Glycosidase
- Cholinesterase

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INTRODUCTION

Oxygen plays a crucial role in respiration, serving as the catalyst for the oxidation of organic substances and facilitating the combustion of materials like gas, coal, and wood. The primary source of oxygen is the free oxygen generated through photosynthesis, contributing to the atmosphere's average composition of 21% oxygen. Vital for the existence of living organisms, oxygen proves essential in sustaining life. Within our bodies, over 90% of oxygen is consumed by the electron transport chain, also known as the respiratory chain. This intricate process involves the reduction of molecular oxygen to water, utilizing electrons derived from NADH and $FADH_2$, byproducts of metabolic fuels such as glucose, fatty acids, and amino acid carbon skeletons. This reduction not only ensures the conversion of the potent oxidizing capability of oxygen molecules but also transforms it into the high-energy phosphate bond of ATP (Gülçin, 2020).

Despite its indispensability, oxygen's role in human life comes with a caveat. Reactive oxygen species (ROS), predominantly generated by free radicals, pose a potential threat by causing extensive damage during normal metabolic processes, as outlined by Gülçin et al. in 2011 (Gülçin et al., 2011). These ROS, exhibiting higher chemical reactivity than the standard oxygen molecule, underscore the delicate balance required to harness the benefits of oxygen while managing its potentially harmful consequences, as highlighted by Gülçin in 2020 (Gülçin, 2020; F. Topal, 2019b).

Free radicals are known as molecules with one or more unpaired electrons, short lifetime, unstable, low molecular weight and very active. In most cases, free radical generation is part of the pathomechanism. Prolonged exposure to certain environmental pollutants such as cadmium and lead can cause oxidative stress, which is undesirable in biological systems (M. Topal, Ozturk Sarıkaya, & Topal, 2021). Free radicals are molecules or ions that have unpaired electrons and are highly active and unstable to chemical reactions with other molecules (Gülçin, 2012).

In aerobic organisms, various defense mechanisms called "antioxidants" have developed in order to prevent the harmful effects of ROS together with the formation of free oxygen radicals (Halliwell, 1996). According to their structures, antioxidants are in the form of enzymes such as catalase, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and glutathione reductase (GSSG-Rd), vitamins and thiols. They are substances that prevent damage by free radicals to target biomolecules such as lipids, proteins, nucleic acids (Gülcin, 2006). The mechanisms of antioxidant action were first discovered when it was understood that a substance with an antioxidant effect had a high probability of spontaneous oxidation (Göçer, Akıncıoğlu, Öztaşkın, Göksu, & Gülçin, 2013).

AChE (E.C.3.1.1.7) is a crucial enzyme within the cholinesterase mily, contributing to nerve conduction by breaking down acetylcholine. Acetylcholinesterase, responsible for choline ester hydrolysis, is notably abundant in the brain, nerves, and red blood cells. Reduced AChE activity can lead to nervous system disorders and even death. Acetylcholine (ACh) serves as a significant substrate for acetylcholinesterase (Koçyiğit, 2018; M. Topal, 2019; Türkeş et al., 2021). A sudden decline in acetylcholine levels proves fatal, while a gradual decrease is associated with Alzheimer's disease (AD) (F. Topal, 2019c). The human brain, housing around 100 billion neurons, faces neurodegeneration in AD due to cell damage or death (F. Topal, 2019a; M. Topal, 2020).

BChE is prevalent in various tissues like the lung, brain, heart, liver, muscle, kidney, and body fluids such as serum, sweat, and cerebrospinal fluid (Demir & Türkan, 2022). Originating from the liver, BChE is a crucial enzyme capable of hydrolyzing or activating numerous compounds. Its synthesis in the liver makes BChE a marker of liver function in clinical studies. Both AChE and BChE play pivotal

roles in AD, a neurodegenerative ailment marked by cognitive impairment, memory decline, and personality changes (Bingöl et al., 2021; F. Topal, Gulcin, Dastan, & Guney, 2017; M. Topal, 2019).

Diabetes stands as a significant health concern, rooted in the insufficiency, deficiency, or ineffectiveness of insulin produced by pancreatic beta cells. In this condition, the body fails to convert energy into glucose, resulting in elevated glucose levels. The hormone insulin cannot adequately manage this glucose, leading to irregular releases (F. Topal, 2019c). The α-Glucosidase enzyme (E.C.3.2.1.20) localized on the small intestine's brush surface breaks down complex carbohydrates. Inhibitors of this enzyme indirectly assist in preventing hyperglycemia (M. Topal, 2019).

Angelica species belonging to the Apiaceae family are plants used medicinally in Western and Eastern countries. There are about 110 species of Angelica, which grows very widely in Europe and Asia in the northern hemisphere. Especially in China, Korea and Japan, Angelica species have been used for traditional medicinal purposes for thousands of years, and due to their therapeutic importance, biological and chemical researches on these plants are carried out today (Kim, Lim, & Lee, 2020).

Angelica purpurascens (Avé-Lall.) Gill *(A. Purpurascens)* is a synonym for *Xanthogalum purpurascens*, known as angelica in Türkiye. *A. purpurascens* is widely cultivated in Türkiye, especially in the Northeast Black Sea region. Although there are many studies on the literature review, the composition of essential oils and biological components, the data on the activities of Angelica species, essential oil composition and antioxidant activities of *A. purpurascens* are limited (Türkuçar, Karaçelik, & Karaköse, 2021).

Enzyme inhibition studies are very important in the diagnosis and treatment of many diseases. Since there is a need to identify natural and safe food antioxidant sources, especially those of plant origin, it is thought that our study will contribute to the literature. *A. purpurascens* is a common plant in Türkiye. No activity and antioxidant capacity have been studied in the literature on ethanol extracts of *A. purpurascens* plant. Several studies were conducted in solvents such as methanol and ethyl acetate. However, in these studies, the reduction capacity according to the Fe^{3+}-Fe^{2+} transformation method, Cu^{2+}-Cu^{+} reduction capacity according to the CUPRAC method, DMPD radical removal studies, enzyme inhibition studies were not examined, especially to determine the antioxidant capacity. Inhibition studies were conducted on AChE, BChE and α-Glycosidase enzymes, which are important for our metabolism, and the effects of these enzymes on metabolic diseases were discussed.

MATERIALS AND METHODS

Materials and methods

Preparation of plant extracts

The plant *A. purpurascens* was collected near Soğanlı in the Sarıkamış district of Kars. This plant species has not been determined before in this region. The people of this region define this plant as "wild kekire". It was collected on 03/07/2021 at the 12th km of Sarıkamış. Herbarium number was determined as "F.Topal 2" in Erzincan Binali Yıldırım University Herbarium. The leaves, branches and flowers of the dried plant were mixed with ethyl alcohol and crushed in a blender. Afterwards, the mixture was filtered through cheesecloth and the remaining pulp was continued to be extracted again with ethyl alcohol under the same conditions. It will be filtered again through the cheesecloth. The extracts were then filtered through filter paper and the filtrates were taken into balloons. Ethyl alcohol was then removed by passing through the evaporator.

Fe^{3+}-Fe^{2+} reduction capacity

To initiate the process, a solution was formulated and subsequently transferred to test tubes at various concentrations. Following this, each tube received 0.2 M phosphate buffer and 1% potassium ferricyanide before undergoing an incubation period. Subsequent to these steps, 10% TCA was introduced into the reaction mixture, and the final mixture had 0.1% $FeCl_3$ added. The resulting absorbance was then measured at 700 nm against a blank (Oyaizu, 1986).

Cu^{2+}-Cu^{+} reduction capacity (CUPRAC method)

For the Cu^{2+}-Cu^{+} reduction capacity (CUPRAC method), a modified procedure based on Apak et al.'s method was employed. $CuCl_2$, neocuprine solution, and CH_3COONH_4 buffer solution were successively added to tubes containing various concentrations of the prepared stock solution. After incubation, absorbance values were recorded at 450 nm against the blank (Apak, Güçlü, Özyürek, Esin Karademir, & Erçağ, 2006; Gülçin, 2009).

DPPH free radical removal activity

A 1 mM solution of $DPPH^{\cdot}$ was used as a free radical. A stock solution of 1 mg/mL concentration, which was prepared before, was used as a sample. The stock DPPH solution was then added to each sample tube. After incubation, absorbance was measured at 517 nm versus blank (Blois, 1958).

ABTS radical removal activity

ABTS radical removal activity was determined according to Re et al.'s method. Initially, an ABTS solution was prepared, and ABTS radicals were generated by adding 2.45 nM persulfate solution to it. The sample, with varied concentrations, was exposed to ABTS addition and subsequent incubation, and absorbances at 734 nm against the blank were assessed (Re et al., 1999).

DMPD radical removal activity

DMPD radical removal activity, following Fogliano et al.'s method, involved obtaining the colored radical cation ($DMPD^{\cdot+}$). Plant extracts and standard antioxidants were placed in test tubes at different concentrations, and $DMPD^{\cdot+}$ solution was added. After incubation, absorbance values were measured at 505 nm ((Fogliano, Verde, Randazzo, & Ritieni, 1999).

Determination of total phenolic compound amount

Total phenolic compound amount determination utilized gallic acid as the standard. A standard graph was prepared from gallic acid, and plant extract stock solution was used. After being placed in a metric cup, the volume was adjusted to 23 mL with distilled water. Folin-Ciocalteu reagent and 2% Na_2CO_3 were introduced, and after mixing at room temperature, absorbance readings at 760 nm were recorded against a blank of pure water. The gallic acid equivalent (GAE) corresponding to the sample absorbance values was determined using the equation derived from the standard graph (Kalın, Gülçin, & Gören, 2015).

Determination of total flavonoid amount

Total flavonoid amount determination, as per Kalın et al.'s method, involved adding 1000 µg of extract to a vezine cup. The extracts, transferred to a test tube, were diluted with an ethanol solution containing CH_3COOK and 10% $Al(NO_3)_3$ solutions. After vortex mixing and incubation at room temperature, the absorbance at 415 nm was recorded, with quercetin serving as the standard for determining the total flavonoid concentration (Kalın et al., 2015).

α- Glycosidase enzyme study

In the α-glycosidase enzyme study, the activity of α-glycosidase enzyme was determined using *p*-NPG as the substrate, following Tao et al.'s previous procedures (Tao, Zhang, Cheng, & Wang, 2013). Absorbances were measured spectrophotometrically at 405 nm, and IC_{50} values were calculated for each extract (Tao et al., 2013).

Acetylcholinesterase and butyrylcholinesterase enzymes studies

AChE, known for catalyzing the hydrolysis of acetylcholine and converting it into thiocholine and acetate, is a key enzyme in this process. In inhibition studies, DTNB is employed and reacts with thiocholine to produce a yellow compound, 5-thio-2-nitrobenzoic acid. The color intensity of this compound is measured at 412 nm. The absorbance at 412 nm for both blank and sample cuvettes is recorded after 5 minutes. Plant extracts' impact on acetylcholinesterase enzyme activity is examined, and the IC_{50} value is calculated based on the data obtained (Ellman, Courtney, Andres Jr, & Featherstone, 1961).

In the study of the butyrylcholinesterase (BChE) enzyme, the inhibition effect of plant extracts is investigated using a procedure similar to the previously described AChE method. However, instead of Acetylthiocholine iodate, which serves as a substrate in the AChE method, butyrylthiocholine iodate is used as the substrate for the BChE enzyme (Özler, Topal, Topal, & Öztürk Sarıkaya, 2023).

Antibacterial activity

The disc diffusion method was performed according to (Matuschek, Brown, & Kahlmeter, 2014). All test microorganisms were obtained from Gümüşhane University Central Research Laboratory and antibacterial tests were carried out in the Food Engineering Laboratory. In the study, antibacterial activities of different parts of *Angelica purpurascens* were measured by disk diffusion method (Matuschek et al. 2014). *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 9634, *Salmonella typhimurium* ATCC 23566, *Enterococcus faecalis* ATCC 29212, *Aeromonas hydrophila* ATCC 35654 and *Escherichia coli* ATCC 25922 were used as 6 type bacteria to determined the antibacterial activities of different part of *Angelica purpurascens*. 20 µL extract was injected into sterile discs placed on Nutrient agar, and then petri dishes were incubated at 36 °C for 24 hours. At the end of the incubation time, It was determined by measuring the transparent areas formed around the discs.

RESULTS AND DISCUSSION

In the reduce ferric ions employed for antioxidant investigations, the test solution undergoes a transformation from yellow to various shades of green. This change is attributed to the reducing actions of antioxidant substances within the experimental setting (İnan Ergün & Topal, 2023). The reducing capacity of the ethyl alcohol extracts of the branches (AB), leaves (AL) and flowers (AF) of the *A. purpurascens* plant used in the study increases in direct proportion to the increasing concentration (Figure 1).

After plotting the capacity to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) of the AB, AL and AF extracts (Figure 1), the absorbances corresponding to 20 µg/mL of each standard antioxidant and plant extracts were given in Table 1 and compared with each other. High absorbance values indicate high reducing capacity.

Table 1. Comparisons of the reducing capacities of ferric ions and cupric ions at 20 µg/mL concentration

Antioxidants	Fe^{3+} Reduction (700 nm)	CUPRAC Method (450 nm)
BHA	2.506	2.165
BHT	1.674	1.529
Tocopherol	0.724	0.867
Trolox	1.521	1.132
Ascorbic acid	2.353	1.030
AB	0.204	0.697
AL	0.252	0.705
AF	0.168	0.595

The comparison of the reducing forces of ferric ions with each other was determined as BHA > ascorbic acid > BHT > trolox > α-tocopherol > AL > AB > AF.

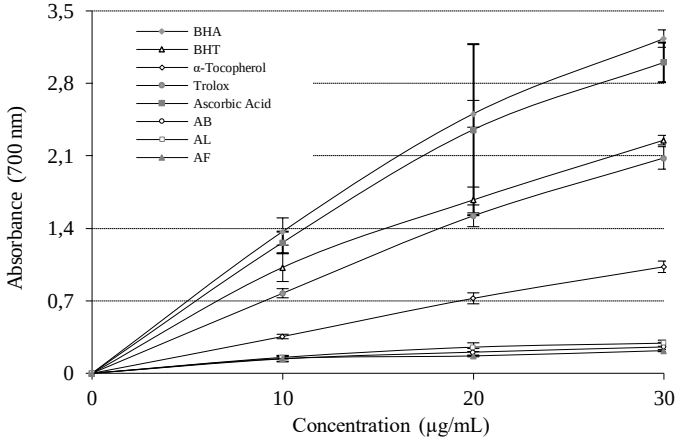

Figure 1. Comparison of Fe^{3+} reducing forces

It was found that the capacity to reduce cupric ions (Cu^{2+}) of ethyl alcohol extracts of branches, leaves and flowers of *A. purpurascens* plant increased in direct proportion to its concentration. After drawing the cupric ion reduction graph of *A. purpurascens* extracts and standard antioxidants (Figure 2), the absorbance values corresponding to 20 µg/mL for each standard antioxidant and *A. purpurascens* extracts were given in Table 1 and compared with each other.

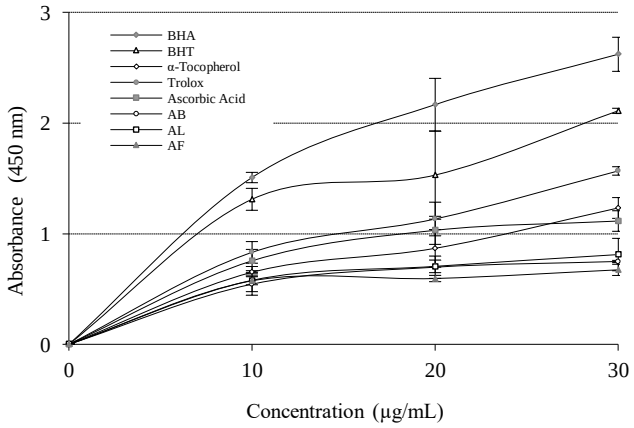

Figure 2. Comparison of cupric ions reducing activities

When the cupric ion reducing activities of *A. purpurascens* extracts and standard antioxidants at its concentration at 20 µg/mL are compared with each other: BHA > BHT > trolox > ascorbic acid > α-tocopherol > AL > AB > AF.

The DPPH free radical scavenging activity of AL, AB, AF and standard antioxidants increases in direct proportion to the concentration as seen in Figure 3.

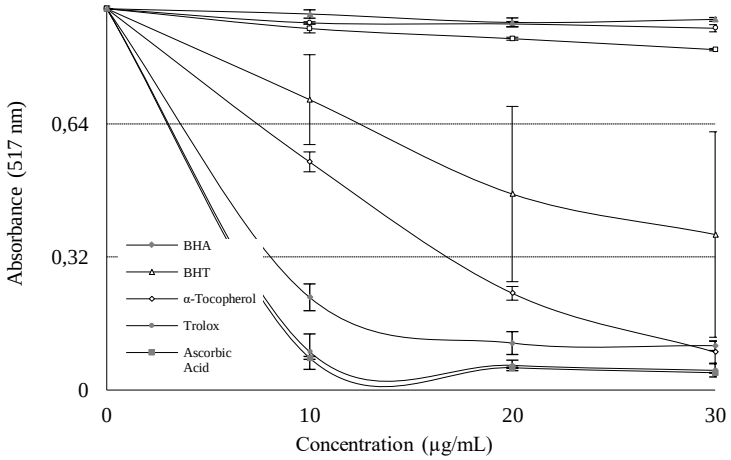

Figure 3. Comparison of DPPH free radical scavenging activities

A. purpurascens extracts and standard antioxidant molecules used showed DPPH free radical scavenging activity as follows, respectively: Ascorbic acid > Trolox > BHA > α-tocopherol > BHT > AL > AB > AF.

The % radical removal values of *A. purpurascens* extracts and each standard antioxidant were calculated and compared with three different methods at 20 µg/mL concentration (Table 2).

Table 2. Comparison of DPPH·, $ABTS^{\cdot+}$ and $DMPD^{\cdot+}$ % radical removal values

Antioxidants	**DPPH·**	**$ABTS^{\cdot+}$**	**$DMPD^{\cdot+}$**
BHA	87.68	99.48	35.98
BHT	48.60	99.45	*
Tocopherol	74.63	94.87	*
Trolox	93.53	99.61	69.51
Ascorbic acid	94.15	99.32	*
AB	4.00	38.72	5.36
AL	7.85	50.69	12.32
AF	3.60	24.30	7.93

* Both standard antioxidant compounds do not show activity in the $DMPD^{\cdot+}$ removal method (Gülçin, 2020).

$ABTS^{\cdot+}$ removal activities of *A. purpurascens* extracts and standard antioxidants are as follows, respectively; Trolox > BHA > BHT > ascorbic acid > α-tocopherol > AL > AB > AF (Figure 4).

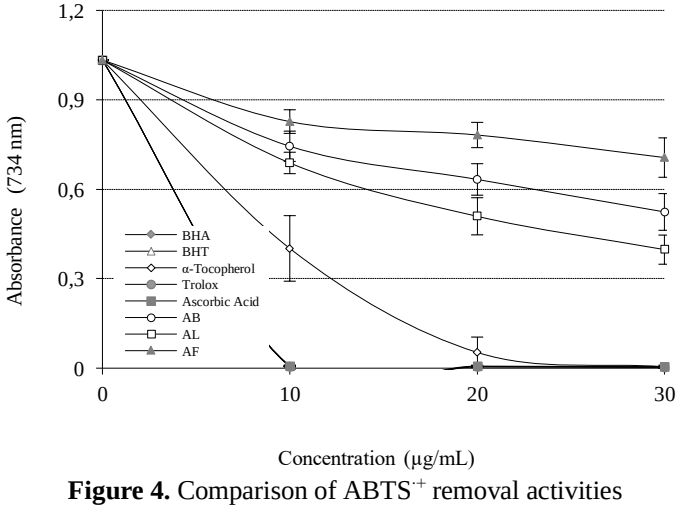

Figure 4. Comparison of $ABTS^{\cdot+}$ removal activities

The $DMPD^{\cdot+}$ removal activity *of A. purpurascens* extracts and the standard antioxidant molecules used are as follows; Trolox > BHA > AL > AF > AB.

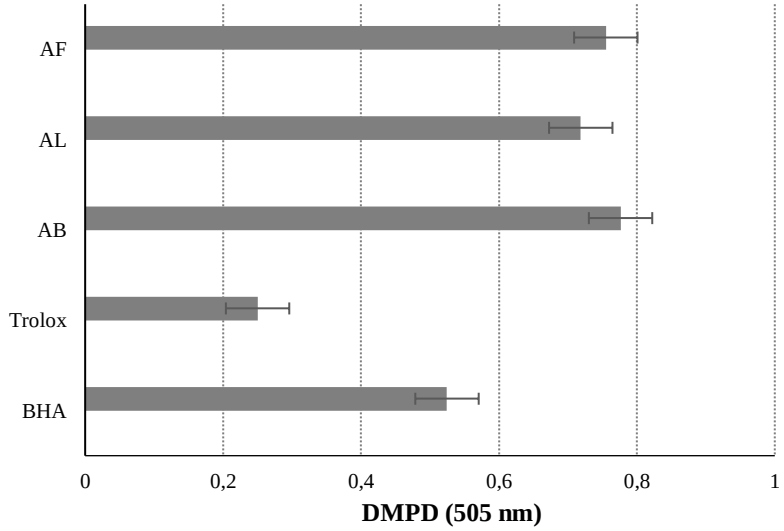

Figure 5. Comparison of absorbances of $DMPD^{\cdot+}$ removal activities at 20 µg/mL concentration

Gallic acid was used as the standard phenolic compound for the determination of the total amount of phenolic compounds in *A. purpurascens* extracts. A standard graph was obtained. The total amount of phenolic compounds found in the ethyl alcohol extracts of the branches and leaves of the *A. purpurascens* plant was calculated as gallic acid equivalent (GAE) (r^2: 0.9939. The standard gallic acid graph prepared for this purpose is given in Figure 6 (A)

Quercetin was used as a standard to determine the total amount of flavonoid compounds in *A. purpurascens* extracts. With the help of the equation obtained from the standard graph, the total flavonoid amount found for AL, AB and AF was calculated as quercetin equivalent (QE) (r^2: 0.9898). The standard graphic prepared for this purpose is given in Figure 6 (B).

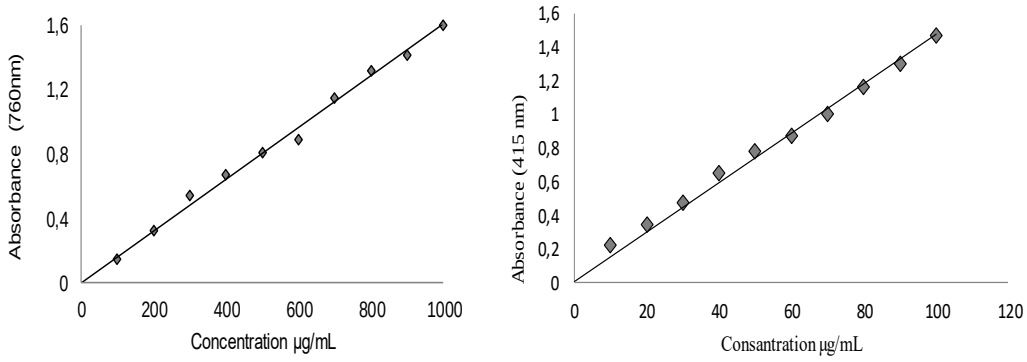

Figure. 6. A) The standard graph prepared for the determination of the total amount of phenolic compounds amount **B)** The standart graph prepared for the determination of total flavonoid

Using the equation found, the total flavonoid and phenolic compounds in the extracts were calculated. The results are given in Table 3.

Table 3. The amount of total phenolic and total flavonoid compounds found in *A. purpurascens* plant extracts

Extract	Total Phenolic (µg GAE/mg extract)	Total Flavonoid (µg QE/mg extract)
AB	26.25	28.96
AL	28.96	45.22
AF	19.79	17.14

The effect of *A. purpurascens* plant extracts for the AChE enzyme was examined. Activity (%) was plotted for AB, AL and AF. The mean IC_{50} value and mean r^2 values were calculated (Table 4).

Table 4. IC_{50} (mg/mL) and r^2 values of *A. purpurascens* plant extracts for the enzymes

Enzymes	AChE		BChE		α-Glycosidase	
İnhibitors	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2
AB	37.26	0.9978	9.08	0.9918	165.00	0.9809
AL	92.40	0.9917	22.87	0.9911	73.72	0.9459
AF	73.72	0.9899	9.97	0.9942	67.28	0.9796

The effect of *A. purpurascens* plant extracts for the BChE enzyme was examined. Activity (%) was plotted for AB, AL and AF. The mean IC_{50} value and mean r^2 values were calculated (Table 4).

The effect of *A. purpurascens* plant extracts for the α-glycosidase enzyme was examined. Activity (%) was plotted for AB, AL and AF. The mean IC_{50} value and mean r^2 values were calculated (Table 4).

Table 5. The antibacterial activity of part of Part of A. Purpurascens (zone mm in diameter)

Bacteria	Flower	Leaf	Branch	*Anova (p)*
Pseudomonas aeruginosa ATCC 27853	$14.93\pm1.37^{B,b}$	$12.63\pm1.10^{B,a}$	$12.13\pm1.05^{A,a}$	*0.002*
Bacillus cereus ATCC 9634	$12.75\pm0.52^{A,a}$	$12.88\pm0.90^{B,a}$	$11.95\pm1.11^{A,a}$	*0.172*
Eschericha coli ATCC 25922	$17.63\pm1.04^{C,b}$	$12.50\pm1.18^{B,a}$	$12.97\pm0.95^{A,a}$	*0.000*
Salmonella typhimurium ATCC 23566	$12.93\pm1.21^{A,b}$	$10.13\pm0.57^{A,a}$	$13.38\pm1.36^{A,b}$	*0.000*
Aeromonas hydrophila ATCC 35654	$15.62\pm1.67^{B,b}$	$12.42\pm0.80^{B,a}$	$12.77\pm2.12^{A,a}$	*0.007*
Enterococcus faecalis ATCC 29212	$16.35\pm1.48^{BC.b}$	$14.58\pm1.78^{C,ab}$	$12.67\pm2.09^{A,a}$	*0.010*
Anova (p)	*0.000*	*0.000*	*0.608*	

Samples showing capital letters (among bacteria type) and lower letters (among part of *A. purpurascens*) do differ significantly.

The results of part of *A. purpurascens* showing antimicrobial activity are given in Table 5. The antibacterial activities were determined mainly *A. purpurascens* flower. The flower of A. Purpurascens has the highest antibacterial activity against the *Eschericha coli* ATCC 25922 ($p<0.001$). The branch of *A. purpurascens* was showed antibacterial activity against the bacteria type, but this property was observed statistical insignificant.

Maintaining a delicate equilibrium between reactive oxygen species (ROS) production and antioxidant defenses is crucial *in vivo*. Disrupting this balance in favor of ROS can lead to oxidative stress, triggered by factors such as inadequate antioxidant diets, insufficient synthesis of metal ion-binding proteins from dietary proteins, excessive production of $O_2^{\bullet}$ and H_2O_2 in metabolism, exposure to drugs or toxins metabolizing into free radicals, and overactivation of radical-producing systems like phagocytes in chronic inflammatory diseases (Gülçin et al., 2012; Halliwell, 1996).

Fats, particularly lipid-based foods, are susceptible to degradation through heating and prolonged storage, resulting in diminished nutritional value and sensory quality. The prevention of these oxidation processes is paramount throughout the food chain, from producers to consumers. Effective mechanisms include restricting oxygen access, utilizing lower temperatures, inactivating oxidation-catalyzing enzymes, reducing oxygen pressure, and employing suitable packaging. Chemical protection against

oxidation involves the use of antioxidants, additives that hinder the formation of lipid free radicals or stabilize lipid hydroperoxides. Some substances known as synergists enhance the activity of antioxidants, reducing free radical content (Yen & Chen, 1995).

While cells can generally handle mild oxidative stress by adjusting antioxidant defense systems, severe oxidative stress leads to DNA damage, elevated intracellular free Ca^{2+} and iron, and protein damage, ultimately causing cell injury and death (Halliwell, 1996). Oxidative stress is often a secondary factor in human diseases but remains significant; for instance, it contributes to atherosclerosis development and is linked to conditions like rheumatoid arthritis, inflammatory bowel diseases, and Parkinson's disease (Göçer et al., 2013; Gülçin & Alwasel, 2023).

Studies highlight the preventive potential of dietary changes, such as reducing fat intake and increasing fruit, grain, and vegetable consumption, against cardiovascular disease and cancer. Since endogenous antioxidant defenses aren't entirely efficient, dietary natural antioxidants play a crucial role in mitigating cumulative oxidative damage over a human lifespan. DNA damage by reactive nitrogen species (RNS) and ROS contributes to age-related cancer development, emphasizing the importance of dietary natural antioxidants. Herbal antioxidants, observed in both *in vivo* and *in vitro* studies as anticancer agents, gained renewed interest due to concerns about toxic effects associated with synthetic antioxidants (Durmaz et al., 2022; M. Topal & Gulcin, 2022).

Methods to measure oxidative damage to DNA, proteins, and lipids in the human body provide insights into optimal nutrient intake. Antioxidants, obtained through diet or metabolic synthesis, have the potential to delay major cancer and vascular diseases, offering significant social and economic benefits (Durmaz et al., 2022).

Antioxidants contribute to an improved quality of life by reducing cell destruction and tumor formation. Various food categories, including fruits, spices, vegetables, proteins, herbs, teas, oils, seeds, and grains, contain antioxidants. As interest in natural antioxidants rises, herbal antioxidants also gain prominence (Gülçin, 2020).

Methods such as DPPH, $ABTS^{\bullet+}$, and $DMPD^{\bullet+}$ serve as effective ways to assess the antioxidant capacity of food products (Öztürk Sarıkaya, 2015). In antioxidant studies in plants, they react with DPPH radicals and donate protons. The results are calculated graphically by measuring the absorbance value in the color change. In the study, plant extracts showed lower DPPH removal activity than antioxidants used as standard. According to this; Ascorbic acid > Trolox > BHA > α-tocopherol > BHT > AL > AB > AF.

The % DPPH radical scavenging activities were calculated as 11.6% and 32.7%, respectively, in the study performed on both water and methanol extract of Achillea schischkinii plant, which was determined as an antioxidant plant. ABTS radical removal activity of the same plant was calculated as 25.1% for water extract and 28.4% for methanol extract (Türkan, Atalar, Aras, Gülçin, & Bursal, 2020). In this study, ABTS radical removal activity % was calculated for AB (38.72%), AL (50.69%) and AF (24.30%). Achillea schischkinii plant extracts were found to have more effective removal activity than plant extracts. ABTS radical scavenging activity is more preferred for herbal antioxidant molecules. It is widely used because it can be dissolved in both polar and nonpolar solvents, is not affected by ionic strength, and can be worked in a wide pH range (Gülçin et al., 2012). ABTS radical scavenging activity is applied in a short time, and it exhibits activity with electron transfer as well as hydrogen transfer are among the advantages of the method (M. Topal & Gulcin, 2022).

The disadvantage of the $DMPD^{\bullet+}$ removal activity method is that it cannot be applied to hydrophobic antioxidants. For this reason, not all standard antioxidants are used. The % DMPD radical removal activities are listed as Trolox > BHA > ascorbic acid > AL > AB.

AB, AF and AL extracts, whose total phenolic and total flavonoid compound amounts were determined, showed higher results especially AL than the others. Especially with the total flavonoid content of 45.22 µg QE/mg extract, a significantly higher amount was obtained than the other extracts. It was shown by the absorbances that AL had higher reduction in the reducing capacities of the ferric ions (0.252) and cupric ions (0.705) produced than the other extracts.

Flavonoids contain a group of polyphenolic compounds in plants. Antioxidant effects are at the forefront of the biological properties of flavonoids. Flavonoids are antioxidant molecules with metal chelating, free radical scavenging and lipid peroxidation inhibition properties. Flavonoid compounds also have a protective effect against oxidation of low density lipoproteins (LDL) and coronary heart diseases (Kızıltaş et al., 2021; Saija et al., 1998).

AD, the leading cause of dementia, is a fatal, debilitating, neurodegenerative disease that is pathologically characterized by progressive neuronal loss and biochemical abnormalities including extracellular amyloid beta (Aβ) plaque accumulation, hyperphosphorylated tau, and mitochondrial dysfunction. Clinically, AD is characterized by memory and cognitive deficits that impair a person's ability to independently perform activities of daily living. AD, which has only limited symptomatic treatment options for which there is no cure, affects many people rapidly and continues to impose a significant burden on our society with its high care costs (Ashleigh, Swerdlow, & Beal, 2023).

During the life cycle of mitochondria, it plays an important role in meeting the physiological needs of eukaryotic cells, maintaining mitochondrial biogenesis and mitochondrial homeostasis. In AD, decreased enzymatic activity of the Krebs cycle, ATP production, respiratory chain activity, and increased levels of free radicals and ROS affect decreased mitochondrial bioenergetic function (Maruszak & Żekanowski, 2011).

This suggests that mitochondrial dysfunction accompanies aging and age-related neurodegenerative disorders. Numerous studies show defects in mitochondrial function as an early event in the course of AD. The brain is particularly vulnerable to free radical attack for several reasons. When the level of free radicals exceeds the cellular antioxidant defense system, oxidative stress occurs. It has relatively low antioxidant protection when exposed to high oxygen concentrations, as well as high levels of membrane polyunsaturated fatty acids, high concentration of iron and ascorbate (Moreira et al., 2005).

On the other hand, DM is associated with the development of cognitive impairment due to its vascular and neurodegenerative effects. AD, the most common neurodegenerative disorder, is characterized by the presence of various pathological signs, including neuronal loss, the formation of senile plaques in the brain consisting of extracellular amyloid beta (Abeta) deposits. Insulin resistance, one of the main components of type 2 diabetes, is a known risk factor for AD (Li, Cesari, Liu, Dong, & Vellas, 2017; Türkeş et al., 2021).

The genetic structures of AChE and BChE enzymes are common enzymes, with an average of 54% similar amino acid sequences. There are significant differences in active regions. Therefore, they may exhibit different inhibition effects. While AChE has 14 aromatic amino acid residues, BChE has 8 aromatic and 6 aliphatic residues (M. Topal, 2019). In the study, it was observed that the IC_{50} values of the BChE enzyme had lower values than the AChE enzyme. It is thought that the ratios in the IC_{50} values in between are due to the active difference of both enzymes.

In the study performed on water and methanol extracts of *Salvia eriophora* plant, IC_{50} values for acetylcholinesterase enzyme were calculated as 15.06 and 9.91 µg/mL, respectively. The IC_{50} values obtained for the BChE enzyme of the same plant were 10.82 µg/mL for the water extract, while the methanol extract was calculated as 5.17 µg/mL. The *α*-Glycosidase enzyme was calculated as 5.54 µg/mL in water extract and 2.94 µg/mL in methanol extract (Bursal et al., 2019). As seen in the literature, multicomplex interactions are observed by evaluating enzyme inhibitions on different extracts of plants.

The most effective IC_{50} value for AChE enzyme was obtained in AB extract (37.26 µg/mL), and for BChE enzyme, the most effective IC_{50} value was obtained in AB (9.08 µg/mL) extract. The results are given in Table 4.

However, when the α-Glycosidase enzyme activity was examined, the IC_{50} value was obtained in AF (67.28 µg/mL) extract, unlike cholinesterases. This shows that it is important to examine the whole plant while observing the multicomplex effects of natural products.

Excessive production of free radicals leads to the depletion of the body's antioxidant system. It reveals a reduction in the antioxidant enzymatic barrier, which is reflected by the reduction of the specific activity of the main antioxidant enzymes. It has been found that there is an increase in ROS production in AD, which causes depletion of too many antioxidants. The ability of antioxidant systems is reduced to protect the body against oxidative attack (Bhatia & Sharma, 2021).

As the result of the antibacterial activities, it was determined flower, leaf and branch of *A. purpurascens* show antibacterial against the gram-positive and gram-negative bacteria. In this study, it was determined that the antibacterial effect of the flower part was higher than the other parts. As a matter of fact, similar results have been found by some researchers. (Park et al., 2022)reported that the *A. gigas* flower had an antibacterial effect on *Pseudomonas aeruginosa*, *Eschericha coli* and *Staphylococcus aureus*. However, in other studies, it has been stated that different species of the *Angelica* plant have antibacterial effects on pathogenic bacteria (Aćimović et al., 2017; Chunchao Han & Guo, 2012; Krishnaraj, Young, & Yun, 2022).

In this case, natural antioxidant intake becomes important to protect the body. In this study, it was presented that plant extracts of *A. purpurascens*, an endemic species, are also effective on natural antioxidant and metabolic enzymes.

CONCLUSION

Oxidative stress and mitochondrial dysfunction are evident during aging and in conditions like Alzheimer's disease (AD). Various age- and lifestyle-related factors such as hypertension, traumatic brain injury, diabetes mellitus (DM), hypercholesterolemia, hyperhomocysteinemia, high calorie intake, smoking, and lack of exercise contribute to increased oxidative stress and AD risk. Conversely, factors that support the neuronal system, including recognized free radical scavengers such as vitamin C and E, estrogen, non-steroidal anti-inflammatory drugs, statins, and calorie restriction, offer protective effects against AD. Natural antioxidant compounds also play a role in this protective system (Sharma & Mehdi, 2023; Singh, Mahajan, Kumar, Singh, & Chowdhary, 2023).

Angelica purpurascens, specifically its flower part, exhibits significant antibacterial activity. Future research should explore flowers of different Angelica species for their antibacterial properties. Our study on the endemic species *A. purpurascens* reveals natural antioxidant properties and effective inhibition of enzymes like AChE, BChE, and *α*-Glycosidase. This plant shows promise in pharmacology, alternative medicine, and the pharmaceutical industry.

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Conflict of Interest

The authors declare no conflict of interest.

Author's Contributions

The authors declare that they have contributed equally to the article.

REFERENCES

Aćimović, M. G., Pavlović, S. Đ., Varga, A. O., Filipović, V. M., Cvetković, M. T., Stanković, J. M., & Čabarkapa, a. I. S. (2017). Chemical Composition and Antibacterial Activity of Angelica archangelica Root Essential Oil. *Natural Product Communications, 12*(2), 205-206.

Apak, R., Güçlü, K., Özyürek, M., Esin Karademir, S., & Erçağ, E. (2006). The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. *International Journal of Food Sciences and Nutrition, 57*(5-6), 292-304.

Ashleigh, T., Swerdlow, R. H., & Beal, M. F. (2023). The role of mitochondrial dysfunction in Alzheimer's disease pathogenesis. *Alzheimer's & Dementia, 19*(1), 333-342.

Bhatia, V., & Sharma, S. (2021). Role of mitochondrial dysfunction, oxidative stress and autophagy in progression of Alzheimer's disease. *Journal of the Neurological Sciences, 421*, 117253.

Bingöl, Z., Kızıltaş, H., Gören, A. C., Kose, L. P., Topal, M., Durmaz, L., . . . Gulcin, I. (2021). Antidiabetic, anticholinergic and antioxidant activities of aerial parts of shaggy bindweed (Convulvulus betonicifolia Miller subsp.)-profiling of phenolic compounds by LC-HRMS. *Heliyon, 7*(5), e06986.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 181*(4617), 1199-1200.

Bursal, E., Aras, A., Kılıç, Ö., Taslimi, P., Gören, A. C., & Gülçin, İ. (2019). Phytochemical content, antioxidant activity, and enzyme inhibition effect of Salvia eriophora Boiss. & Kotschy against acetylcholinesterase, α-amylase, butyrylcholinesterase, and α-glycosidase enzymes. *Journal of Food Biochemistry, 43*(3), e12776.

Chunchao Han, C., & Guo, J. (2012). Antibacterial and Anti-inflammatory Activity of Traditional Chinese Herb Pairs, Angelica sinensis and Sophora flavescens. *Inflammation, 35*(3).

Demir, Z., & Türkan, F. (2022). Asetilkolinesteraz ve Bütirilkolinesteraz Enzimlerinin Alzheimer Hastalığı ile İlişkisi. *Journal of the Institute of Science and Technology, 12*(4), 2386-2395.

Durmaz, L., Kiziltas, H., Guven, L., Karagecili, H., Alwasel, S., & Gulcin, İ. (2022). Antioxidant, antidiabetic, anticholinergic, and antiglaucoma effects of magnofluorine. *Molecules, 27*(18), 5902.

Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology, 7*(2), 88-95.

Fogliano, V., Verde, V., Randazzo, G., & Ritieni, A. (1999). Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *Journal of Agricultural and Food Chemistry, 47*(3), 1035-1040.

Göçer, H., Akıncıoğlu, A., Öztaşkın, N., Göksu, S., & Gülçin, İ. (2013). Synthesis, Antioxidant, and Antiacetylcholinesterase Activities of Sulfonamide Derivatives of Dopamine-Related Compounds. *Archiv der pharmazie, 346*(11), 783-792.

Gülcin, İ. (2006). Antioxidant and antiradical activities of L-carnitine. *Life Sciences, 78*(8), 803-811.

Gülçin, İ. (2009). Antioxidant activity of L-adrenaline: A structure-activity insight. *Chemico-Biological Interactions, 179*(2-3), 71-80.

Gülçin, İ. (2012). Antioxidant activity of food constituents: an overview. *Archives of Toxicology, 86*(3), 345-391.

Gülçin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of Toxicology, 94*(3), 651-715.

Gülçin, İ., & Alwasel, S. H. (2023). DPPH radical scavenging assay. *Processes, 11*(8), 2248.

Gülçin, İ., Beydemir, Ş., Topal, F., Gagua, N., Bakuridze, A., Bayram, R., & Gepdiremen, A. (2012). Apoptotic, antioxidant and antiradical effects of majdine and isomajdine from Vinca herbacea Waldst. and kit. *Journal of Enzyme Inhibition and Medicinal Chemistry, 27*(4), 587-594.

Gülçin, İ., Topal, F., Çakmakçı, R., Bilsel, M., Gören, A. C., & Erdogan, U. (2011). Pomological features, nutritional quality, polyphenol content analysis, and antioxidant properties of domesticated and 3 wild ecotype forms of raspberries (Rubus idaeus L.). *Journal of Food Science, 76*(4), C585-C593.

Halliwell, B. (1996). Antioxidants in human health and disease. *Annual Review of Nutrition, 16*(1), 33-50.

İnan Ergün, A., & Topal, M. (2023). Determination of the Antioxidant Capacity of Puerarin and Its Effect on Cholinesterases by *in vitro* and *in silico* Methods. *ChemistrySelect, 8*(41), e202302751.

Kalın, P., Gülçin, İ., & Gören, A. C. (2015). Antioxidant activity and polyphenol content of cranberries (Vaccinium macrocarpon). *Records of Natural Products, 9*(4), 496.

Kim, K., Lim, C. E., & Lee, B. Y. (2020). The complete chloroplast genome sequence of Peucedanum chujaense (Apiaceae), an endemic species to Korea. *Mitochondrial DNA Part B, 5*(2), 1937-1938.

Kızıltaş, H., Bingol, Z., Gören, A. C., Kose, L. P., Durmaz, L., Topal, F., . . . Gülçin, İ. (2021). LC-HRMS profiling and antidiabetic, anticholinergic, and antioxidant activities of aerial parts of kınkor (Ferulago stellata). *Molecules, 26*(9), 2469.

Koçyiğit, Ü. M. (2018). Investigation of Inhibition Effect of Oxytocin on Carbonic Anhydrase and Acetylcholinesterase Enzymes in the Heart Tissues of Rats. *Journal of the Institute of Science & Technology/Fen Bilimleri Estitüsü Dergisi, 8*(1).

Krishnaraj, C., Young, G. M., & Yun, S. (2022). In vitro embryotoxicity and mode of antibacterial mechanistic study of gold and copper nanoparticles synthesized from Angelica keiskei (Miq.) Koidz. leaves extract. *Saudi Journal of Biological Sciences, 29*, 2552-2563.

Li, J., Cesari, M., Liu, F., Dong, B., & Vellas, B. (2017). Effects of diabetes mellitus on cognitive decline in patients with Alzheimer disease: a systematic review. *Canadian Journal of Diabetes, 41*(1), 114-119.

Maruszak, A., & Żekanowski, C. (2011). Mitochondrial dysfunction and Alzheimer's disease. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 35*(2), 320-330.

Matuschek, E., Brown, D. F. J., & Kahlmeter, G. (2014). Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clinical Microbiology and Infection, 20*, 255-266.

Moreira, P. I., Smith, M. A., Zhu, X., Nunomura, A., Castellani, R. J., & Perry, G. (2005). Oxidative stress and neurodegeneration. *Annals of the New York Academy of Sciences, 1043*(1), 545-552.

Oyaizu, M. (1986). Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition and Dietetics, 44*(6), 307-315.

Özler, E., Topal, F., Topal, M., & Öztürk Sarıkaya, S. B. (2023). LC-HRMS Profiling and Phenolic Content, Cholinesterase, and Antioxidant Activities of Terminalia citrina. *Chemistry & Biodiversity*, e202201250.

Öztürk Sarıkaya, S. B. (2015). Acethylcholinesterase inhibitory potential and antioxidant properties of pyrogallol. *Journal of Enzyme Inhibition and Medicinal Chemistry, 30*(5), 761-766.

Park, C. H., Park, H. W., Yeo, H. J., Jung, D. H., Jeon, K. S., Kim, T. J., Park, S. U. (2022). Combined transcriptome and metabolome analysis and evaluation of antioxidant and antibacterial activities in white, pink, and violet flowers of *Angelica gigas*. *Industrial Crops & Products, 188*, 115605.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9-10), 1231-1237.

Saija, A., Trombetta, D., Tomaino, A., Cascio, R. L., Princi, P., Uccella, N., . . . Castelli, F. (1998). In vitro'evaluation of the antioxidant activity and biomembrane interaction of the plant phenols oleuropein and hydroxytyrosol. *International Journal of Pharmaceutics, 166*(2), 123-133.

Sharma, V., & Mehdi, M. M. (2023). Oxidative stress, inflammation and hormesis: The role of dietary and lifestyle modifications on aging. *Neurochemistry International*, 105490.

Singh, S., Mahajan, M., Kumar, D., Singh, K., & Chowdhary, M. (2023). An inclusive study of recent advancements in Alzheimer's disease: A comprehensive review. *Neuropeptides*, 102369.

Tao, Y., Zhang, Y., Cheng, Y., & Wang, Y. (2013). Rapid screening and identification of α-glucosidase inhibitors from mulberry leaves using enzyme-immobilized magnetic beads coupled with HPLC/MS and NMR. *Biomedical Chromatography, 27*(2), 148-155.

Topal, F. (2019a). Anticholinergic and antidiabetic effects of isoeugenol from clove (Eugenia caryophylata) oil. *International Journal of Food Properties, 22*(1), 583-592.

Topal, F. (2019b). Determination of antioxidant capacity of 2, 6-quinolinediol. *Journal of the Institute of Science and Technology, 9*(3), 1520-1527.

Topal, F. (2019c). Inhibition profiles of Voriconazole against acetylcholinesterase, α-glycosidase, and human carbonic anhydrase I and II isoenzymes. *Journal of Biochemical and Molecular Toxicology, 33*(10), e22385.

Topal, F., Gulcin, I., Dastan, A., & Guney, M. (2017). Novel eugenol derivatives: Potent acetylcholinesterase and carbonic anhydrase inhibitors. *International Journal of Biological Macromolecules, 94*, 845-851.

Topal, M. (2019). The inhibition profile of sesamol against α-glycosidase and acetylcholinesterase enzymes. *International Journal of Food Properties, 22*(1), 1527-1535.

Topal, M. (2020). Secondary metabolites of ethanol extracts of pinus sylvestris cones from eastern anatolia and their antioxidant, cholinesterase and α-glucosidase activities. *Records of Natural Products, 14*, 129-138.

Topal, M., & Gulcin, İ. (2022). Evaluation of the in vitro antioxidant, antidiabetic and anticholinergic properties of rosmarinic acid from rosemary (*Rosmarinus officinalis* L.). *Biocatalysis and Agricultural Biotechnology, 43*, 102417.

Topal, M., Ozturk Sarıkaya, S. B., & Topal, F. (2021). Determination of Angelica archangelica's Antioxidant Capacity and Mineral Content. *ChemistrySelect, 6*(31), 7976-7980.

Türkan, F., Atalar, M. N., Aras, A., Gülçin, İ., & Bursal, E. (2020). ICP-MS and HPLC analyses, enzyme inhibition and antioxidant potential of *Achillea schischkinii* Sosn. *Bioorganic Chemistry, 94*, 103333.

Türkeş, C., Akocak, S., Işık, M., Lolak, N., Taslimi, P., Durgun, M., . . . Beydemir, Ş. (2021). Novel inhibitors with sulfamethazine backbone: synthesis and biological study of multi-target cholinesterases and α-glucosidase inhibitors. *Journal of Biomolecular Structure and Dynamics*, 1-13.

Türkuçar, S. A., Karaçelik, A. A., & Karaköse, M. (2021). Phenolic compounds, essential oil composition, and antioxidant activity of *Angelica purpurascens* (Avé-Lall.) Gill. *Turkish Journal of Chemistry, 45*(3), 956-966.

Yen, G.-C., & Chen, H.-Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry, 43*(1), 27-32.