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Assessment of Antioxidant, Antityrosinase and Anticholinesterase Activities of *Ferulago asparagifolia* Boiss

Ferulago asparagifolia Boiss.'in Antioksidan, Antitirozinaz ve Antikolinesteraz Etkilerinin Değerlendirilmesi

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Abstract

Ferulago W. Koch is an important genus from the Apiaceae family, widely used in traditional medicine and rich in bioactive compounds. *Ferulago asparagifolia* Boiss. contains coumarins, terpenoids, flavonoids, and phenolic compounds. In this study, the antioxidant activities and total phenol/flavonoid content of *F. asparagifolia* fruit and leaf extracts were measured. The antioxidant activities were assessed using DPPH (1,1-Diphenyl-2-picrylhydrazyl), ABTS (2,2' azinobis (3-ethylbenzothiazoline 6-sulfonic acid)), and iron chelation methods spectrophotometrically. The ethanol extract of *F. asparagifolia* leaves showed 58.73% DPPH radical scavenging activity at 1 mg/mL concentration, while the fruit ethanol extract exhibited 53.13% activity. In the ABTS radical scavenging activity test, all extracts showed increased activity with increasing concentrations, with leaf and fruit ethanol extracts showing 85.85% and 85.70% activity at 1 mg/mL concentration, respectively. The highest phenol content (218.78 ± 4.55 mg gallic acid equivalent (GAE)/g) was found in the ethanol extract of *F. asparagifolia* leaves. The anti-acetylcholinesterase (Anti-AChE) and antibutyrylcholinesterase (Anti-BuChE) activity of *F. asparagifolia* extracts was investigated using the Ellman method, and the anti-tyrosinase (Anti-TYR) activity was examined using the dopachrome method in vitro. The hexane extract of *F. asparagifolia* fruit showed significant inhibitory activity against AChE (145.1 µg/mL). Additionally, the ethanol extract of *F. asparagifolia* leaves exhibited the highest anti-tyrosinase activity (2.42 mg/mL). In conclusion, while *F. asparagifolia* extracts possess significant antioxidant activities, they also demonstrate considerable antityrosinase activity, indicating their potential use in the treatment of neurodegenerative diseases and development of skin-whitening products. Therefore, further in-depth research is warranted for *F. asparagifolia* to be used in oxidative stress defense or in the development of products related to its anti-tyrosinase activity.

Keywords: Antioxidant activity, *Ferulago asparagifolia* Boiss., cholinesterase, tyrosinase.

Öz

Ferulago W. Koch Apiaceae familyasına ait geleneksel tıpta yaygın olarak kullanımı olan ve zengin biyoaktif içeriğe sahip önemli bir cinsdir. Bu çalışmada, *F. asparagifolia* meyve ve yaprak ekstrelerinin toplam fenol/flavonoit içeriği ölçülmesi yanı sıra, antioksidan aktiviteleri DPPH ((1.1-Difenil-2-pikrilhidrazil), ABTS (2.2'-azinobis(3-etilbenzotiyazolin-6-sülfonik asit) ve demir şelasyon metotları ile spektrofotometrik olarak ölçülmüştür. *F. asparagifolia* yaprak etanol ekstresi 1 mg/ml konsantrasyonunda %58.73, meyve etanol ekstresi ise %53.13 DPPH radikal süpürücü aktivite göstermiştir. ABTS radikal süpürücü aktivite testinde ise, tüm ekstreler konsantrasyon artışına bağlı olarak aktivitede artış göstermiş ve yaprak ve meyve etanol ekstreleri 1mg/ml konsantrasyonunda sırasıyla %85,85 ve %85.70 aktivite sergilemiştir. En yüksek fenol içeriği *F. asparagifolia* yapraklarının etanol ekstresinde (218.78 ± 4.55 mg Gallik aside eşdeğer/g) bulunmuştur. *F. asparagifolia* ekstrelerinin anti-asetilkolinesteraz ve anti-butirilkolinesteraz (Anti-AChE,

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Anti-BuChE) aktivitesi Ellman metodu ve antitirozinaz (Anti-TYR) aktivitesi ise dopakrom metodukullanılarak incelenmiştir. *F. asparagifolia* meyvesi hekzan ekstresi, AChE'ye (145.1 µg/mL) karşı anlamlı inhibitör aktivite göstermiştir. Ayrıca *F. asparagifolia* yaprak etanol ekstresi en yüksek Anti-TYR aktivite (2.42 mg/mL) sergilemiştir. Sonuç olarak *F. asparagifolia* ekstreleri önemli antioksidan ve dikkate değer antitirozinaz aktiviteye sahip olduğu gösterilmiştir. Sonuç olarak, *F. asparagifolia* bitkisi, vücudu oksidatif strese karşı korumada kullanılabilir veya cilt beyazlatıcı ürünlerin geliştirilmesinde etkili olabilecek antitirozinaz aktivitesiyle ilgili olarak daha fazla araştırılmaya ihtiyaç duymaktadır.

Anahtar Kelimeler: Antioksidan aktivite, *Ferulago asparagifolia* Boiss., kolinesteraz, tirozinaz.

1. Introduction

The Apiaceae, or Umbelliferae, is one of the largest plant families globally, known for its aromatic plants, typically with hollow stems. Many species in this family are valued for their use as vegetables, in cooking, or for medicinal purposes. The genus *Ferulago* W. Koch, part of the Apiaceae family, includes 49 species and is primarily found across Asia, Europe, and Africa. In Türkiye, it is commonly known as "Çakşır" or "Çağşır," with 34 species, 18 of which are endemic to the region (Kızılarslan 2013, Rahimpour et al. 2021). Türkiye is regarded as the biodiversity center for *Ferulago* and is likely the species' main area of origin and primary center of diversity (Uruşak and Kizilarslan 2013). The *Ferulago* genus exhibits a broad range of biological activities, such as antimicrobial, antioxidant, acetylcholinesterase inhibitory, anticoagulant, and anti-inflammatory effects, along with cytotoxic and hepatoprotective properties (Badalamenti et al. 2021, Süzgeç-Selçuk et al. 2021). Although *Ferulago* species possess medicinal properties, their use requires caution due to potential toxicity, particularly from coumarins and other bioactive compounds. A study on *Ferulago trifida* identified the presence of coumarins, which are linked to beneficial anticoagulant effects but can also cause toxicity, such as liver damage and photosensitivity (Tavakoli et al. 2017).

Studies on the anti-cholinesterase activity of various *Ferulago* species have been conducted, including *F. cassia* Boiss. and its bioactive components (Karakaya et al. 2019), *F. angulate* (Schltdl.) Boiss., *F. subvelutina* Rech. (Bagci et al. 2016), *F. isaurica* Peşmen, *F. syriaca* Boiss. (Karakaya et al. 2018a), *F. pauciradiata* Boiss. & Heldr. (Karakaya et al. 2018b), *F. stellata* Boiss. (Rahimpour et al. 2022), *F. trifida* Boiss. essential oil (Tavakoli et al. 2017), *F. carduchorum* Boiss. & Hausskn and its coumarins (Golfakhrabadi et al. 2016), and *F. campestris* (Besser) Grecescu (Dall'Acqua et al. 2010). These studies demonstrate that *Ferulago* species possess strong cholinesterase inhibitory activities. Additionally, research has shown that species such as *F. longistylis* Boiss. (Kürkçüoğlu et al. 2022), *F. trachycarpa* (Fenzl) Boiss. (Ahmed et al. 2024), *F. lutea* (Poir.) as well as essential oil of *F. lutea* (Poir.) Grande (Alves-Silva et al. 2023) exhibit depigmenting properties.

Ferulago is a genus that has been widely used in folk medicine throughout history. It has been used in the treatment of hemorrhoids, intestinal worms, peptic, sedative, carminative, snakebite, wound skin infections, headaches, and gastrointestinal tract diseases (Rahimpour et al. 2021). In Turkish traditional medicine, *Ferulago* are used as an immunostimulatory, tonic, sedative, digestive, anti-bronchitis, flavoring, vermicidal, anthelmintic, and antipeptic. One of these species, *F. sylvatica* (Besser) Reichb. root has a long history of medicinal use treating skin diseases in Türkiye (Bulut et al. 2014, Rahimpour et al. 2021). In phytochemical studies, it was found that *Ferulago* species mainly contain non-volatile compounds such as coumarin, flavonoid and terpenic compounds, volatile oil and fixed oil components (Badalamenti et al. 2021). Ethnobotanical studies on *F. asparagifolia* indicate that not only its fruit but also its leaves and aerial parts have been traditionally used for various purposes. In regions like Turkey and Iran, these parts of the plant are utilized in folk medicine for digestive issues, sedative effects, and antimicrobial properties. Both fruits and leaves are significant due to their essential oil content, which has demonstrated a range of biological activities, such as antioxidant and antimicrobial effects. Research has shown that these different plant parts contribute to the plant's medicinal value, which justifies studying them together (Karakaya 2016, Badalamenti 2021). In your study, both ethanol and hexane extracts were prepared due to their complementary extraction capabilities for different types of compounds. Ethanol is a widely used solvent in phytochemical studies because of its ability to extract a broad range of secondary metabolites, including flavonoids, alkaloids, and phenolic compounds. This makes it a versatile solvent for accessing various bioactive compounds present in plants. Hexane, on the other hand, was selected because the fruit of *F. asparagifolia* contains a significant amount of fixed oils. Hexane is particularly effective in extracting non-polar compounds such as lipids and fatty acids, which are abundant in the oily components of fruits. By using hexane, the study could

target these lipid-rich components, which might not be as effectively extracted using more polar solvents like ethanol.

Alzheimer's disease (AD) is a multifactorial and complex chronic neurological disorder that starts with memory loss, behavioral changes, and cognitive decline, ultimately leading to death. According to the cholinergic hypothesis, one of the main theories for treating AD, the disease is caused by a deficiency in the neurotransmitter acetylcholine (ACh) (Gupta et al. 2023). Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are involved in the hydrolysis of Ach/Buch. Therefore, if the hydrolysis of ACh by AChE is inhibited in the AD patient's brain, it is expected that the amount of ACh in the synapse will increase significantly and the neurotransmission mechanism will be more fluent (Tamfu et al. 2021). For many years, the European Medicines Agency and the Food and Drug Administration approved only four medications for the treatment of Alzheimer's disease: galantamine, donepezil, and rivastigmine, all of which were developed based on the cholinergic hypothesis; and memantine, an N-methyl-D-aspartate (NMDA) receptor antagonist, which was developed later (Martins et al. 2023). For this reason, the discovery of new cholinesterase inhibitors in treatment has always been the focus of interest.

The tyrosinase (TYR) enzyme, present in a wide range of microorganisms, animals, and plants, is crucial to melanogenesis. It also affects antibiotic resistance, the production of skin pigment, the sclerotization of the cuticle, neurodegeneration, and the unfavorable browning of fruits and vegetables (Mermer and Demirci 2023). TYR inhibitors are substances that suppress the activity of the enzyme TYR, which is involved in the production of melanin. There has been an increase in studies on the whitening and blemish removal properties of TYR inhibitors, particularly in light of the public idea of beauty. Naturally produced TYR inhibitors are less irritating, safer, more stable, and have long-term skin whitening potential than chemically synthesized TYR inhibitors. Since traditional Chinese medicines (TCMs) are the primary source of natural products, screening for excellent TRY inhibitors has become a top focus. TCMs have been found to have TRY inhibitory activity. Of these, flavonoids account for the majority and are followed by lignans, terpenoids, simple phenylpropanoids, and stilbenes. These natural inhibitors are often used in skincare products aimed at reducing hyperpigmentation, evening out skin tone, and promoting a brighter complexion (Li et al. 2023).

Free radicals (reactive oxygen species (ROS)/ reactive nitrogen species) are structures that contain at least one unpaired electron in the outer shell, giving them generally high reactivity. When the oxidant/antioxidant balance in the body is disrupted, free radicals, reactive species cause damage to DNA, proteins, and membranes. This situation triggers the pathogenesis of many chronic diseases (Alzheimer, Parkinson, diabetes etc.) and aging (Jomova et al. 2023). Oxidative stress, which occurs with the excessive accumulation of free radicals, is one of the causes of the pathogenesis of AD, where neural cycloskeleton is modified irreversibly causing its dysfunction and ultimately neuronal death. For this reason, to control the oxidative stress by restricting the quantity of ROS produced in the patient with AD, antioxidants are used as a major component in Alzheimer's therapy (Dubey and Singh 2023). On the relationship between antioxidant and skin whitening activity, ROS increase on the skin with exposure to ultraviolet rays. ROS activates the transcription of the TYR gene, which synthesizes TYR and facilitates melanogenesis. For this reason, antioxidants have been used to treat skin hyperpigmentation (D'Angelo Costa and Maia Campos 2021).

Natural chemicals that are inexpensive, straightforward, easily obtainable, and natural make up a significant portion of the global search for medications with novel enzyme inhibitory activities (Rauf and Jehan, 2017). This study aimed to assess, for the first time, the antioxidant, anti-cholinesterase, and anti-TRY properties of ethanol and hexane extracts derived from the fruits and leaves of *F. asparagifolia*.

In this study, the anticholinesterase activity of ethanol and hexane extracts of *F. asparagifolia* fruits and leaves was evaluated, since other *Ferulago* species have high anti-cholinesterase activity. This genus is known for its medicinal properties, including antimicrobial, anti-inflammatory, and antioxidant activities. Studies have demonstrated that the essential oils and extracts from some *Ferulago* species such as *Ferulago asparagifolia* Boiss., *F. galbanifera* (Miller) W. Koch, *F. humilis* Boiss. (Endemic) and *F. trachycarpa* Boiss. exhibit significant biological activities, which support its traditional uses (Demirci et al. 2000).The antioxidant activity of the plant extract contributes positively to skin whitening and anti-Alzheimer's effects (Dubey and Singh 2023, D'Angelo Costa and Maia Campos 2021), so the aim of our study was to investigate the antioxidant, anti-cholinesterase, and anti-TRY properties of ethanol and hexane extracts derived from the fruits and leaves of *F. asparagifolia*.

2. Material and Methods

2.1. Plant Materials and Extraction

Plant material was collected and identified by botanist Dr. Yavuz Bağcı. (C4: Antalya; between Gazipaşa -Alanya 10. km, ca. 50 m, 20.07.2021, Bağcı 4206) and the voucher specimens are deposited in Herbarium of Selcuk University Herbarium (KNYA), Konya, Turkiye. *F. asparagifolia* leaves and fruit parts were dried in the shade at room temperature. Then, their dry plant materials (20 g) were weighed and left to macerate with 200 mL of hexane / ethanol at room temperature. The extracts were filtered through Whatman filter paper, and the filtrate was evaporated to dryness in a Rotary evaporator at 40˚C (Büchi, Sweden). It was kept in the refrigerator at -20˚C until biological activity studies. The yield of hexane and ethanol extracts of *F. asparagifolia* leaves and fruits were 3.2% (hexane extract of *F. asparagifolia* leaves: FALH), 16.02% (ethanol extract of *F. asparagifolia* leaves: FALE), 6.90% (hexane extract of *F. asparagifolia* fruits: FAFH) and 2.92% (ethanol extract of *F. asparagifolia* fruits: FAFE), respectively.

2.2. Chemicals

The chemicals such as DPPH, ABTS, ferrozine, and other reagents used in this study were of analytical grade and were purchased from Sigma Aldrich, Germany. Folin & Ciocalteu's phenol reagent was provided by Merck Supelco, Germany.

2.3. Total Phenol/ Total Flavonoid Content (TPC/TFC)

Total phenol content was estimated using the Folin-Ciocalteu reagent (Clarke et al. 2013), and total flavonoid content was determined using the aluminum chloride colorimetric test (Yang et al. 2011). For TPC, extracts were mixed with 100 µL of F-C reagent. 100 µL of 7.5% Na_2CO_3 solution was added to the plates and the absorbance was measured at 650 nm after 60 minutes. For TFC, 150 µL of leaf/ fruit extract and 150 µL of 2% $AlCl_3$ solution were added to the plates. After 15 minutes of incubation, absorbances were read at 435 nm using a Elisa reader (Multiscan SKY, USA). Analyzes were replicated four parallel runs of the samples. TPC of the extracts were calculated as mg gallic acid equivalents per gram extract (mg GAE/g extract) (Singleton et al. 1999) ($y = 0.0011x + 0.1459$, $r^2 = 0.9951$). TFC of the extracts were calculated as mg quercetin equivalents per gram extract (mg QE/g extract) ($y = 0.0059x + 0.0739$, $r^2 = 0.9971$).

2.4. Free Radical Scavenging Activity Assays

The radical scavenging activities of ethanol and hexane extracts of *F. asparagifolia* leaves and fruits were tested using validated techniques, such as the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay (Clarke et al. 2013) and ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] assay (Re et al. 1999). 20 µL of leaf and fruit extracts or quercetin was mixed with 180 µL of DPPH solution in a 96-well plate and incubated for 15 min in the dark. Absorbances were read at 540 nm using a Elisa reader (Multiscan SKY, USA).

$ABTS^+$ radical was prepared by reacting 15 mL of 7 mM ABTS with 264 µL of 140 mM potassium persulfate solution by keeping it in the dark at room temperature for 16 h before the experiment (stock solution). The ABTS working solution was adjusted to give an absorbance value of 0.70 ± 0.02 at 734 nm by first diluting the prepared stock solution with methanol. 50 µL of leaf/fruit extract was added with 100 µL of ABTS working solution. After incubating for 10 minutes, absorbance was measured at 734 nm using Elisa reader (Multiscan SKY, USA). Quercetin and butylated hydroxytoluene (BHT) were used as positive control in the DPPH and ABTS method, respectively. Samples were processed in triplicate.

2.5. Iron-Chelating Activity Assay

The way the extracts interacted with the ferrozine Fe^{2+} complex formation contributed to establishing their iron chelating ability (Chai et al. 2014). To summarize, a reaction was conducted at room temperature using a combination consisting of 0.4 mL of 0.2 mM ferrozine, 0.2 mL of 0.1 mM $FeSO_4$, and 0.2 mL of extract. After incubating for 10 minutes, the absorbance at 562 nm was measured using Elisa reader. EDTA was used as positive control. Samples were processed in triplicate.

2.6. Acetylcholinesterase (AChE)/Butyrylcholinesterase (BuChE) Enzyme Inhibition Assay

The assay was performed as follows in accordance with the Ellman method (Ellman et al. 1961). The mixture was prepared as follows: 20 µL of the enzyme (0.22 Unit/mL for acetylcholinesterase/0.1 U/mL for butyrylcholinesterase) prepared in phosphate buffer; 140 µL of 0.1 mM phosphate buffer (pH 6.8); 10 µL of 3 mM 5,5'-dithio-bisnitrobenzoic acid (DTNB); and 20 µL of test sample/reference standard of various concentrations. The reaction was initiated by adding 10 µL of the substrate (0.71 mM acetylthiocholine

iodide/0.2 mM butyrylthiocholine chloride in phosphate buffer). Absorbance was measured at 412 nm Elisa reader (Multiscan SKY, Epoch, USA). Galantamine served as the positive reference, while buffer used as control instead of extract. Samples were processed in triplicate. IC_{50} values were calculated with GraphPad Prism 8.0.1 by subtracting the concentration.

2.7. Tyrosinase (TRY) Enzyme Inhibition Assay

With a few minor adjustments, the previously published method for measuring TRY inhibitory activity was followed (Yang et al. 2012). In short, a 96-well plate was filled with 20 µL of the leaf or fruit extract, 20 µL of the enzyme solution (made from 250 U/mL of mushroom tyrosinase in phosphate buffer), and 100 µL of phosphate buffer (0.1 M, pH 6.8). Subsequently, 20 µL of 3 mM L-tyrosine solution made in phosphate buffer was added as the substrate, and the mixture was incubated for 30 minutes at 25°C. The absorbance was measured with a Elisa reader (Multiscan SKY, USA) at 492 nm. Kojic acid was used as the standard. Samples were processed in triplicate. IC_{50} values were calculated with GraphPad Prism 8.0.1 by subtracting the concentration.

In all biological activity studies, % inhibition values were calculated according to the formula below (Atere et al. 2018).

% inhibition = (Acontrol-ATest) / Acontrol x 100

2.8. Statistical Analysis

The biological activity results were statistically evaluated using GraphPad Prism 9.0 software. To determine whether there were significant differences between the groups, a one-way analysis of variance (ANOVA) was employed. Following this, the Tukey post-hoc test was applied to identify the specific differences between the groups.

3. Results and Discussion

F. asparagifolia total phenol/flavonoid contents were found to be higher in ethanol extracts than in hexane extracts (Figure 1). While *F. asparagifolia* leaf ethanol extract has the highest TPC content (218.78 ± 4.55 mg GAE/g extract), *F. asparagifolia* fruit ethanol extract has the highest TFC content (115.25 ± 7.27 mg QE/g extract). TPCs and TFCs of *F. asparagifolia* fruit (21.99 ± 5.51 mg GAE /g extract; 39.55 ± 1.12 mg QE/g extract) and leaf (20.02 ± 2.68 mg GAE/g extract; 49.17 ± 4.21 mg QE/g extract) hexane extracts were found to be lower.

It is known that many phenolic compounds have strong antioxidant activity, and their physiological and pharmacological properties are related to their antioxidant activity. Antioxidant activities of phenolic compounds are determined by the number and position of hydroxyl groups, relevant glycosylation and other substituents (Cai et al. 2006). Phenolic compounds can show antioxidant activity through multiple mechanisms such as chain breaking, free radical scavenging, and metal chelation. Many previous studies have found a positive correlation between total phenol and flavonoid content of medicinal plants and its free radical scavenging activity ability (Leja et al. 2013). In the DPPH and ABTS radical scavenging activity results, *F. asparagifolia* fruit and leaf ethanol extracts were found to have high antioxidants. Hexane extracts gave similar inhibition activity results. While *F. asparagifolia* leaf ethanol extract had high radical scavenging activity in both methods (Figure 2 and Figure 3), it had the lowest iron chelation activity (Figure 4). *F. asparagifolia* fruit

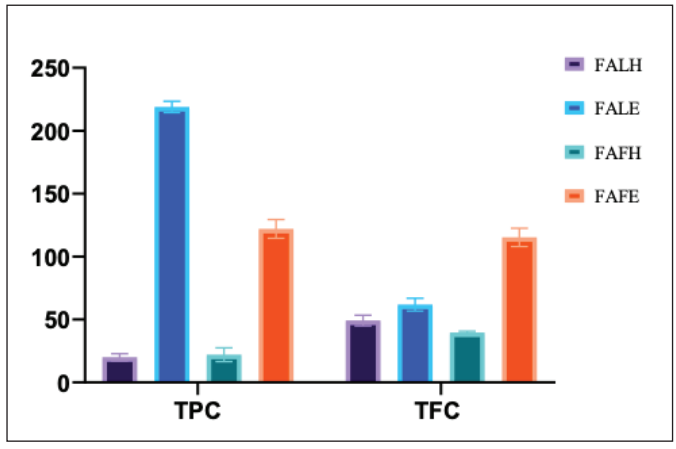

Figure 1. Total phenol and total flavonoid content of *F. asparagifolia* leaf and fruit extracts.

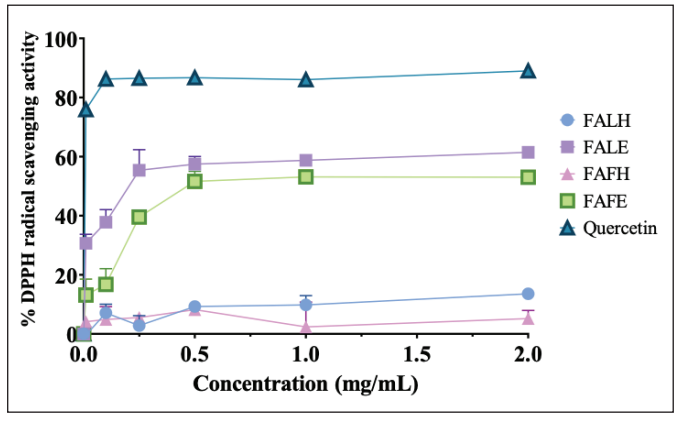

Figure 2. DPPH radical scavenging activity of *F. asparagifolia* leaf and fruit extracts.

Table 1. Enzyme inhibition activities of extracts.

	Plant parts	Solvents	AChE IC_{50} values (µg/mL)	BuChE IC_{50} values (µg/mL)	TYR IC_{50} values (mg/mL)
F. asparagifolia	Leaf	Hexane	395.5 ± 0.83	267.4 ± 0.63	4.609 ± 0.94
		Ethanol	590.5 ± 1.84	1047 ± 3.94	2.42 ± 0.55
	Fruit	Hexane	145.1 ± 2.35	873.6 ± 2.29	2.66 ± 0.54
		Ethanol	451.6 ± 1.66	232.1 ± 0.86	3.61 ± 1.07
References			32.80 ± 0.79[a]	24.98 ± 1.13[a]	0.132 ± 0.89[b]

Values are expressed as mean ± standard deviation (S.D).[a]Galantamine; [b] Kojic acid

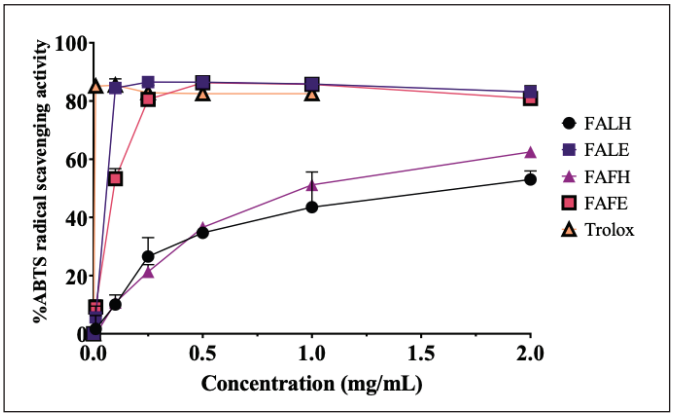

Figure 3. ABTS radical scavenging activity of *F. asparagifolia* leaf and fruit extracts.

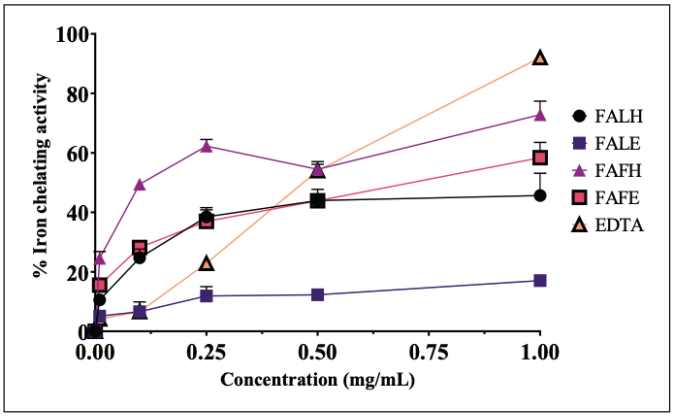

Figure 4. Iron chelating activity of *F. asparagifolia* leaf and fruit extracts.

hexane extract was found to have the highest iron chelation activity while having low radical scavenging activity. It is observed that concentration-dependent % inhibition in *F. asparagifolia* extracts increase from low to high.

The enzyme inhibitory activity results are shown in Table 1, representing the IC_{50} values for the four extracts prepared. The lowest IC_{50} AChE inhibitory activity was found for *F.*

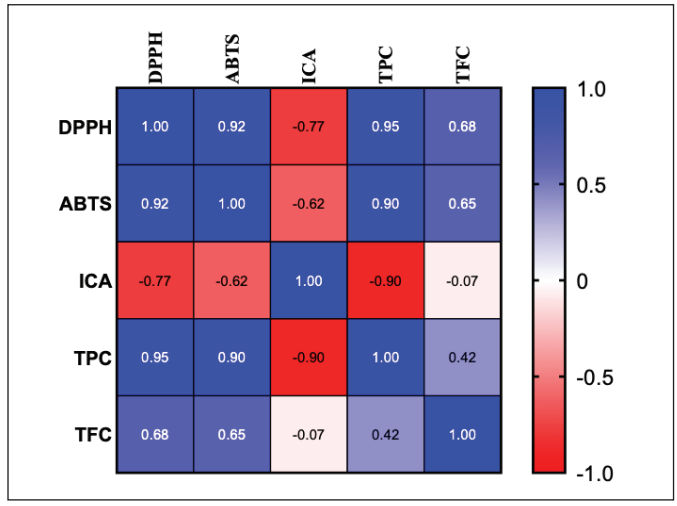

Figure 5. Correlation between phytochemical contents and antioxidant activities of extracts.

asparagifolia fruit hexane extract (145.1 ± 2.35 µg/mL) followed by leaf hexane extract > fruit ethanol extract> leaf ethanol extract displayed values of 395.5 ± 0.83, 451.6 ± 1.66 and 590.5 ± 1.84 µg/mL, respectively. Galantamine served as the reference standard for AChE inhibition, demonstrating an IC_{50} value of 32.80 ± 0.79 µg/mL. *F. asparagifolia* leaf ethanol and hexane, and *F. asparagifolia* fruit ethanol extracts have moderate anti-acetylcholinesterase and anti-butyrylcholinesterase activity. The extract with the highest anti-TYR activity was found to be *F. asparagifolia* leaf ethanol extract (2.42 mg/mL).

In the correlation study between total phenol/flavonoid and antioxidant activity studies of all extracts (Figure 5), DPPH and ABTS radical scavenging activities were found to be significant in both TPC (r=0.95; r=0.90, respectively) and TFC (r=0.68; r=0.65, respectively) are seen to be positively correlated with their contents. This suggests that the ability of the extracts to scavenge DPPH and ABTS radicals is to some extent more attributable to their total phenol content.

In our study, DPPH, ABTS, and TPC/TFC contents were found to have a positive correlation, while no correlation was found with iron chelating capacity (Figure 5). In a similar study, extracts prepared from seaweeds had high iron ion chelating activity, but no correlation was found with their TPC (Wang et al. 2009). The reasons for this may be the importance of functional groups for the Fe^{+2} binding activities of phenolic constituents. For example; ortho-dihydroxyl groups, the presence of 5-OH and/or 3-OH together with a C4 keto group or large number of OH groups contribute to iron chelating activity (Khokhar and Apenten 2003). It is predicted that the absence of these groups in the flavonoids in the components of *F. asparagifolia* extracts affects the correlation and the iron chelating activities of the extracts. *F. asparagifolia* ethanol extracts were found to have higher TPC/TFC content than hexane extracts. The reason for this may be that the solubility of phenolic components is better in solvents with higher polarity (Tomsone et al. 2012).

In biological activity studies conducted on other *Ferulago sp,* the highest TPC was found in the ethylacetate fraction of *F. syriaca* root (1156.01 ± 4.92 mg/g). *F. syriaca* and *F. isaurica* extracts showed antioxidant activity by DPPH test, and the highest activities were shown in the chloroform fractions of *F. syriaca* and *F. isaurica* roots. (9.99 and 8.78 µg/mL, respectively). In the same study, the chloroform fraction of *F. isaurica* root was found to have strong inhibition against AChE (46.99%) and BuChE (88.56%) at 20 µg/mL (Karakaya et al. 2018a). When the antioxidant and anti-cholinesterase activities of *F. stellata* aerial parts were evaluated, its antioxidant activity was found to be high. *F. stellata* ethanol extract showed strong inhibitory activity against acetylcholinesterase (IC_{50}=1.772 µg/mL) (Kızıltaş et al. 2021). It was determined that *F. angulata* and *F. subvelutina* extracts showed weak/moderate inhibitory activity against acetylcholinesterase (Hajimehdipoor et al. 2014). In another study, while *F. cassia* roots and fruits had the highest total phenolic content, DPPH analysis results showed the highest antioxidant activity in roots and fruits. Similar to our study, there is a positive correlation between TPC and DPPH radical scavenging activity. In the same study showed that *F. cassia* root and fruit dichloromethane extracts have strong inhibition against BuChE (96.56±2.98% and 82.33±2.69%, respectively), while *F. cassia* root and flower dichloromethane fractions have significant inhibition against AChE (53.24±1.22% and 31.38±5.41% respectively) (Karakaya et al. 2019).

There is only one study on depigmenting effect of *Ferulago* species. In this study, it was reported that *F. lutea* essential oil exhibited a depigmenting effect on B16V melanocytes. This study focused on a number of functions of the essential oil of *F. lutea*, especially found as significant natural source, when considering skin diseases and aging (Alves-Silva et al. 2023).

In the phytochemical study, it was determined that *F. asparagifolia* contains secondary metabolites including umbelliferone, aegelinol, grandivittin, agasyllin, asparagifolin, xanthotoxin, rutarin, osthenol, prantschimgin prantschimgin, (-)-angelicoidenol-2-*O*-β-apiofuranosyl-(1→6)-β-glucopyranoside, chlorogenic acid, 3,5-di-(E,E)-caffeoylquinic acid, apigenin, kaempferol, and luteolin (Alkhatib et al. 2009). The main components of the fruit essential oil of *F. asparagifolia* were found to be 2,3,6-trimethyl benzaldehyde (38.9%) and myrcene (18.2%) (Baser et al. 2001). *F. asparagifolia* seeds were found to contain oleic acid (48.5), linoleic acid (30.8), and palmitic acid (8.3) (Ghafoor et al. 2019). In our study, *F. asparagifolia* fruit and leaf extracts were investigated on their total phenol and flavonoid contents. *F. asparagifolia* leaf ethanol extract was found the highest TPC, while fruit ethanol extract was detected the highest TFC. In accordance with the literature, the phytochemical constituents of this genus that may be responsible for the biological activities (Tomsone et al. 2012, Karakaya et al. 2018a, Karakaya et al. 2019).

In many studies conducted on the *Ferulago* genus, antioxidant and anticholinesterase activities were found to be remarkable. *Ferulago* species are rich in phenolic compounds, which are well-known for their strong antioxidant properties. These compounds include flavonoids, tannins, and phenolic acids such as ferulic acid (Azarbani et al. 2014). The essential oils extracted from *Ferulago* species also contribute to their antioxidant activity. These oils contain various terpenoids and other volatile compounds with antioxidant potential (Ebadi et al. 2019). Antioxidants in *Ferulago* act by scavenging free radicals, chelating metal ions, and inhibiting oxidative enzymes. This helps to protect cells from oxidative stress and damage. For instance, the methanolic extract of *Ferulago angulata* showed significant free radical scavenging activity in DPPH (2.2-diphenyl-1-picrylhydrazyl) assays. Another study on *Ferulago capillaris* reported high total phenolic and flavonoid contents, correlating with strong antioxidant effects in various in vitro models. Research on *Ferulago* species, such as *Ferulago campestris*, has revealed significant anticholinesterase activity (Öztürk et al. 2011).

Extracts of this species inhibited AChE and BChE in vitro, suggesting potential benefits in managing Alzheimer's disease. Another study on *Ferulago nodosa* identified several coumarin derivatives with strong anticholinesterase effects. These compounds showed promise as lead structures for the development of new therapeutic agents. Similar to previous studies, in this study, both antioxidant, anti-cholinesterase and anti-tyrosinase activities of some *F. asparagifolia* extracts were found to be significantly activity.

4. Conclusion

This study provides important information for the phenolic content and biological activity of *F. asparagifolia*. The analyses of *F. asparagifolia* extract, particularly the ethanol extract reveals significant antioxidant potential, highlighting its role in neutralizing free radicals and protecting cells from the oxidative stress. This suggests a promising natural source of antioxidants with potential uses for human health. When evaluating at the literature, it seems that there are not enough studies on *F. asparagifolia*. It is thought that the anti-Alzheimer and antioxidant activity of *F. asparagifolia* may provide potential health advantages, but further research is considered to determine the specific mechanisms and components responsible for the effect.

Author contribution:

Yavuz Bagcı: planned and designed the study, Tugsen Buyukyildirim: Gathered and analyzed data about the study, Fatma Ayaz: review & editing, Nuraniye Eruygur: Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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