

SOME BIOLOGICAL ACTIVITIES OF *ASPARAGUS ACUTIFOLIUS* L.

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Abstract

This study was aimed to determine the inhibition effect of wild asparagus extracts prepared with different solvents (water-ethanol-methanol) on the acetylcholinesterase (AChE) enzyme. In addition, antioxidant activities of plant extracts with DPPH and ABTS radical scavenging methods, and antibacterial activities against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Enterobacter faecalis bacteria by disc diffusion method were investigated. The half-maximal inhibitory concentration (IC₅₀) values on the AChE enzyme were determined between 0.93 – 5.46 mg/mL for all of the extracts. The most effective extract for AChE enzyme was determined as methanol extract. Moreover, all extracts of wild asparagus were found to scavenge both DPPH and ABTS radicals with quite strong activity. In addition, it has been observed that all extracts of wild asparagus have an effective antibacterial activity on all bacteria. It was also determined that their great potential as antibacterial agents and antioxidant molecules of wild asparagus make them important candidates for pharmaceutical applications like anticholinesterase drugs or starter compounds for synthesizing more effective AChE inhibitors.

Keywords: *Asparagus acutifolius* L., Acetylcholinesterase, Antibacterial, Antioxidant

1. INTRODUCTION

In recent years, interest in edible wild plants has increased to increase the diversity of the human diet due to their nutritional and medicinal values[1]. It is known that wild asparagus, which is used as an intestinal softener and diuretic among the Mediterranean folks, grows naturally in the Mediterranean basin, which belongs to the family of Asparagaceae. It is thought to be good for the intestinal system due to its rich flavonoid and essential oils contents like terpenes. In addition to these, wild asparagus is known to be rich in antioxidant phenolics, vitamin C, folates, ascorbic acid and dietary fiber etc[2]. The main compounds found among the phenolics are quercetin-3-orutinoside and isorhamnetin-3-O-rutinoside and to a lesser extent kaempferol-3-o-rutinoside [3]. Aqueous extracts of wild asparagus rich in these compounds also have important antiproliferative properties. It has been proven to show proapoptotic activity against bladder and lung cancer cell lines and significant antiproliferative activities against neuroblastoma, liver and cervical cancer cell lines[4-5]. The plant is also used for its medicinal properties related to the presence of different bioactive substances in various plant parts including phenolics, saponin and polysaccharides.

It is known that AChE enzyme activity is quite high in neurodegenerative diseases characterized by forgetfulness such as Alzheimer's disease. One of the most important symptoms in the definitive diagnosis of Alzheimer's is cholinergic loss. Many studies in which terpene derivatives play an active role in AChE inhibition have been observed in the literature [6-7]. Asparagaceae family is a very rich family in terms of diterpenes and triterpenes as well as flavonoids and phenolic compounds. In recent studies, it has been proven that terpenes and their derivatives performed reversible inhibition of the acetylcholinesterase (AChE) enzyme. AChE enzyme activity is quite high in dementia-type diseases. Control of excessive AChE activity leading to the symptom of forgetfulness is used as an alternative way to clear up this symptom. There is no study was found on the AChE enzyme activity of *Asparagus acutifolius* L. species in the literature. Therefore, a study was planned with water, ethanol and methanol extracts of wild asparagus. In addition, antioxidant activities were evaluated with DPPH and ABTS

methods. In addition, antibacterial activities of the plant sample against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* bacteria were investigated.

2. MATERIALS AND METHODS

2.1. Materials

Acetylthiocholine iodide, 5,5'-dithiobis[2-nitrobenzoic acid] (DTNB), galanthamine, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), gallic acid, methanol and ethanol (analytical grade) were purchased from SigmaAldrich. *Escherichia coli* (ATCC-8739) and *Staphylococcus aureus* (ATCC-6538) bacterial strains were purchased in the form of kwik-stik from Microbiologics of France. Mueller Hinton Agar and Mueller Hinton Broth were purchased for cultivation of bacteria and for determination of susceptibility of microorganisms to the plant extract from Biomark Laboratories of India.

2.2. Methods

2.2.1. Preparation of Plant Extract

Dried *Asparagus acutifolius* L. was powdered with a grinder. A 10 g of the sample was weighed, and 100 mL of solvent was added. The extract was left in a mixer at +4 °C overnight. It was centrifugated for 10 min at 4000 rpm, and the supernatant was removed. The pellet was re-extracted more two times with the same process. The supernatants were combined. The solvents were then removed by evaporation. The pellet samples were stored at -20 °C until used in experimental procedures.

2.2.2. Preparation of Acetylcholinesterase Homogenate

A portion of (2 g) bovine brain was homogenized by tissue homogenisator in 10mL of 50 mM phosphate buffer (pH 8.0) containing 300 mM sucrose. The homogenate was centrifuged at 2000 g for 10 min at 4 °C. The supernatant was separated as an enzyme source and used for experimental purposes[8].

2.2.3. Inhibition of Acetylcholinesterase

Inhibition of AChE activity was performed using Ellman's colorimetric method[8]. Enzyme inhibition analyzes, test mixtures (200 µL total volume) into a microplate were prepared with 0.1 mM phosphate buffer (pH 8.0, 120-155 µL of 0.1mM), 20 µL of 10mM DTNB, 25 µL the inhibitor solution (0.1, 0.25, 0.5, 1 or 2 mg/mL) and 20 µL enzymatic extract solutions. The test solutions, after the incubated for 15 min at 37 °C, 10 µL of 10 mM substrate solution were added to initiate of reaction. Absorbance was measured spectrophotometrically at 412 nm. The blank (reference) sample contained all of the components except the enzyme. The IC₅₀ values were determined for all extracts in this way.

2.2.4. Antioxidant Activity

The antioxidant capacities of the extracts were performed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity [9]. A 0.024 g portion of DPPH was dissolved in 100 mL MeOH. Then, 1-50 µL of plant extract, 200 µL of DPPH solution were added to a 96-well microplate and the total volumes were completed 250 µL with MeOH. The well plate was kept in the dark for 1 h. For the control, MeOH was used instead of a sample. Spectrophotometric measurements were done at 517 nm.

ABTS radical scavenging activity of the samples was determined by the method of Re et al.[10]. ABTS⁺ radical solution was prepared using equal volumes of 7 mM ABTS salt and 2.4 mM ammonium persulphate and kept in dark overnight. After then, the solution was diluted with MeOH until an absorbance of 1.50±0.01 at 734 nm was obtained. For the sample analysis, 200 µL of the ABTS⁺ solution and 1 - 50 µL of the extract were added to a 96-well microplate. Measurements were done at 734 nm by a UV-Visible Spectrophotometer. The measurements were performed in triplicate for each extract. The radical scavenging activity of the extracts was calculated as IC₅₀.

2.2.5. Antibacterial Activity

Antibacterial analyzes of the *Asparagus* extracts were analyzed against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* by disc diffusion method. For this aim, the concentration of fresh bacterial cultures was lined up as 10^5 CFU/mL. The bacteria were inoculated in Muller Hinton Agar (MHA) medium. Then, discs (6 mm diameter) soaked with plant extract overnight were placed on an MHA medium. The petri dishes were incubated at 37 °C for 24 h. Finally, the diameters of the inhibition zones forming around the discs were evaluated for antibacterial activities of the extracts.

3. RESULTS

Table 1 shows the inhibition of the AChE enzyme and antioxidant activity against DPPH and ABTS radicals of plant extracts. Among the results calculated as IC_{50} values, the most effective inhibitor on AChE is methanol extract (IC_{50} : 0.93 ± 0.01 mg/mL). This result was followed by water extract (IC_{50} : 2.42 ± 0.02 mg/mL) and ethanol extract (IC_{50} : 5.46 ± 0.01 mg/mL), respectively. It was determined that the inhibition activity of the methanol extract from the raw *Asparagus* extracts was quite effective when compared to the positive control.

Table 1. AChE inhibition activity and antioxidant activity of the extracts of *Asparagus acutifolius* L. (IC_{50} (mg/mL)).

Extract	AChE inhibition	DPPH radical scavenging activity	ABTS radical scavenging activity
Water	2.42 ± 0.02	0.583 ± 0.021	0.091 ± 0.006
Ethanol	5.46 ± 0.01	0.185 ± 0.016	0.084 ± 0.011
Methanol	0.93 ± 0.01	0.254 ± 0.016	0.139 ± 0.001
*Gallic acid	-	0.005 ± 0.001	0.001 ± 0.000
**Galanthamine	0.09 ± 0.00	-	-

*Gallic acid was used as a positive control for antioxidant activity against DPPH and ABTS radicals,
**Galanthamine was used as a positive control for AChE inhibitory.

The antioxidant activity of *Asparagus* extracts was investigated with radical scavenging properties, and their IC_{50} values were calculated and given in Table 1. It was observed that all extracts showed potent activities against both radicals. Ethanol extract had the highest DPPH radical scavenging activity, followed by methanol and ethanol extract, respectively. ABTS radical scavenging activity showed the highest activity in ethanol, water and methanol, respectively. It was determined that all extracts showed a better scavenging effect of ABTS radical compared to DPPH radical.

Table 2. Antibacterial activity results of *Asparagus acutifolius* L. performed by disc diffusion method

Bacteria strains	Inhibition zone (mm)
<i>Staphylococcus aureus</i>	10.58 ± 0.11
<i>Escherichia coli</i>	12.11 ± 0.29
<i>Enterococcus faecalis</i>	10.43 ± 0.22
<i>Pseudomonas aeruginosa</i>	13.05 ± 0.15

Antibacterial activity of plant extracts was investigated against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* bacteria by disk diffusion method. The antibacterial activity results of the plant are shown in Table 2, and the zone diameters are shown in Figure 1. It was observed that the plant showed antibacterial activity with zone diameters varying between 10-13 mm.

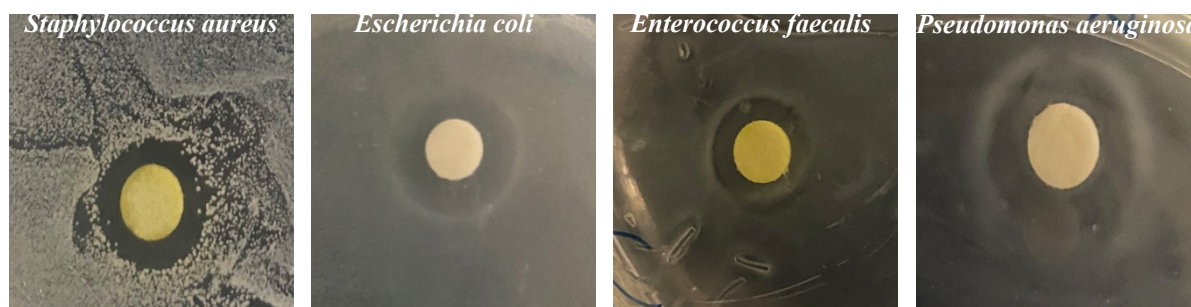


Figure 1. Antibacterial activity of *Asparagus acutifolius* L.

4. DISCUSSION

In this study, AChE enzyme inhibition of *A. acutifolius* was studied for the first time, and it was found that the methanol extract showed a strong inhibitory effect on AChE. In the literature, studies were found in which methanolic extracts of various plants showed strong inhibition effects on the AChE enzyme. It was found that the methanol extract of *Cassia timorensis* L. plant exhibited 94-97% inhibition on AChE [11]. In another study, it was proved that as the concentration of methanolic extract of *Macrosphyra longistyla* L. plant increases, its inhibitory activity on AChE also increases. When the plant concentration was 5 mg/mL, it showed 80% inhibition effect on AChE [12]. In another study; Methanol extracts of *Levisticum officinale* L., *Bergeris integrima* L. and *Rheum ribes* L.[13], methanolic extract of *Sideritis perfoliata* L.[14] and *Piper capense* L., *Ziziphus mucronata* L. and *Crinum bulbispermum* L.[15] has been proven that have an inhibitory effect of more than 50% on the AChE enzyme. The fact that all these plants are rich in flavonoids, phenolics, essential compounds etc and that show an inhibitory effect on AChE is common with our study.

On the other hand, a critical discussion involving neurological disorders is the ability of drug candidates to cross the blood-brain barrier. The ability of a compound to cross the blood-brain barrier depends on its lipophilicity. Less polar compounds are more lipophilic and have blood-brain barrier permeability compared to more polar flavonoids. therefore, extracts containing flavonoids can cross the blood-brain barrier more easily and react with AChE[1]. Uriarte-Pueyo and Calvo reported the ability of 128 flavonoid compounds, including subgroup classes such as flavones, flavanones, flavonols, isoflavones, and chalcones, as AChE inhibitors. They reported that the inhibitory activity of flavones is effective on the AChE enzyme, followed by flavanones[16].

Most of the studies related to the antioxidant activity of *A. acutifolius* extracts were performed with extracts obtained from the edible part of the plant, which is in common with this study. [1-4] In this study, as a result of antioxidant activity, ABTS radical scavenging activity was 0.084-0.139 mg/mL, while DPPH radical scavenging activity was in the range of 0.185-0.583 mg/mL. In other studies, Ferrara et al. (2011) [17], Morales et al. (2012) [18] and Martins et al. (2011) [19] measured antioxidant activity by DPPH radical scavenging of *A. acutifolius*, the values IC_{50} 72.4 μ g/mL, 4.87 ± 0.38 mg/mL and 423 ± 24 μ g/mL, respectively. Hamdi et al. (2021) reported that ABTS radical scavenging activities of all *A. acutifolius* extracts in their study were much more effective compared to DPPH and FRAP analyses [1]. As seen in Table 1, it is noteworthy that the results of this study are compatible with the literature.

The antibacterial activity of *A. acutifolius* extracts prepared as 25 mg/mL was measured in the range of 10-13 mm against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and

Enterococcus faecalis. In a similar study, the zone diameters of the extracts (100 mg/mL) prepared with different parts of *A. acutifolius*, measured against the same bacteria, were recorded as 16-18 mm. It is seen that the difference between both studies is entirely due to the concentration difference [19].

5. CONCLUSION

In this study, the inhibition property of the extracts of *Asparagus acutifolius* L. plant on the AChE enzyme was investigated for the first time. All of the extracts prepared with water, ethanol and methanol showed strong inhibitory properties on the AChE enzyme, and methanol-extract was the most effective among the extracts. In addition, it was determined that all extracts showed strong antioxidant activity against DPPH and ABTS radicals. The effectiveness of antioxidants, it can be interpreted as a good result to minimize the destructive effects of increasing oxidants in dementia types as well as in every disease. In addition, it has a strong antibacterial activity with its activity against four pathogenic bacteria. Considering all of the findings, it has been shown that the edible wild plant *Asparagus acutifolius* L. species can be used in pharmaceutical applications as a precursor molecule in the treatment of neurodegenerative diseases such as Alzheimer's, diseases caused by oxidants or in the treatment of infectious diseases.

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