

Determination of Antioxidant Activity of *Salvia sclarea* L. and Its Inhibitory Effects on Acetylcholinesterase and Monoamine Oxidase A

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SUMMARY

Over the past two to three decades, there has been a significant increase in research focused on the treatment of neurodegenerative disorders. In this study, our aim was to determine some biological activities of the ethanolic and methanolic extracts of *Salvia sclarea* L. The extracts were first assessed for their capacity to scavenge DPPH radicals, then their total phenolic content (TPC) were determined. Afterward, the extracts were evaluated for their effects on acetylcholinesterase (AChE) and monoamine oxidase-A (MAO-A). These two enzymes play a crucial role in the treatment of neurodegenerative disorders. It has been found that, the DPPH activity of the methanolic extract was higher than that of ethanolic extracts; while TPC was higher for the ethanolic extract. For AChE, the IC₅₀ values for ethanolic extract and methanolic extract were 0,27±0,005 mg/mL and 1,19±0,037 mg/mL, respectively. And for MAO-A, the IC₅₀ values for ethanolic extract and methanolic extract were 6,53±0,72 µg/mL and 3,03±0,05 µg/mL, respectively. As the result of this study, the antioxidant property of *Salvia sclarea* was determined, and it was observed that this property changed in accordance with the total phenolic content of the plant. It has been shown that the extracts have inhibitory effects on both enzymes. This means, the obtained data are promising for further drug development studies.

Key Words: *Salvia sclarea* L., DPPH, total phenolic content, acetylcholinesterase, monoamine oxidase-A, neurodegenerative disorders.

***Salvia sclarea* L.'nin Antioksidan Aktivitesi ile Asetilkolinesteraz ve Monoamin Oksidaz-A Üzerindeki İnhibitör Etkilerinin Belirlenmesi**

ÖZ

Son yıllarda nörodejeneratif hastalıkların tedavisine odaklanan araştırmalarda önemli bir artış yaşanmıştır. Bu çalışmada amacımız, *Salvia sclarea* L.'nin etanolik ve metanolik ekstraktlarının bazı biyolojik aktivitelerini belirlemektir. Ekstreler elde edildikten sonra, öncelikle DPPH radikal süpürücü aktiviteleri test edilmiş ve sonra ekstraktların toplam fenolik içerikleri (TPC) tespit edilmiştir. Daha sonra ekstraktlar hem asetilkolinesteraz (AChE) hem de monoamin oksidaz-A (MAO-A) üzerindeki etkileri açısından değerlendirilmiştir. Bu iki enzim nörodejeneratif rahatsızlıkların tedavisinde çok önemli bir rol oynamaktadır. Metanolik ekstre nin DPPH aktivitesi etanolik ekstre ninkinden daha yüksek bulunmuştur; TPC ise etanolik ekstre için daha yüksek olarak tespit edilmiştir. AChE için etanolik ekstre ve metanolik ekstre için IC₅₀ değerleri sırasıyla; 0,27±0,005 mg/mL ve 1,19±0,037 mg/mL; MAO-A için ise etanolik ekstre ve metanolik ekstre için IC₅₀ değerleri sırasıyla; 6,53±0,72 µg/mL ve 3,03±0,05 µg/mL olarak hesaplanmıştır. Yapılan çalışmada sonucunda *Salvia sclarea*'nin antioksidan özelliği belirlenmiş; bu özelliğin total fenolik içerik ile uyumlu olarak değiştiği görülmüş; ekstraktların her iki enzim üzerine de inhibitör etkileri olduğu gösterilmiş ve bu elde edilen verilerin ilerideki ilaç geliştirme çalışmaları için ümit verici oldukları tespit edilmiştir.

Anahtar Kelimeler: *Salvia sclarea* L., DPPH, total fenolik içerik, asetilkolinesteraz, monoamin oksidaz-A, nörodejeneratif bozukluklar.

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INTRODUCTION

A significant rise in the number of non-contagious diseases, which now account for nearly 64% of global deaths, has been seen in recent years (Institute for Health Metrics and Evaluation, 2019). Alzheimer's disease, a non-contagious condition characterized in late adulthood, is defined by progressive memory loss and cognitive impairment (Alzheimer's Association, 2017). Acetylcholine is a neurotransmitter released into the interneuron space and is shown to be one of the causes of the illness by the decline in its levels (Martorana, 2010). It has also been suggested that some diseases of the central and peripheral nervous systems may be linked to alterations in monoamine oxidase (MAO) activity (Riederer, 2004). On the other hand, oxidative stress brought on by reactive oxygen species (ROS) is known to damage cells by causing cellular and biomolecule oxidation. In addition, oxidative stress is implicated in the development of several neurological illnesses, such as Alzheimer's and Parkinson's (Bastianetto, 2002; Viña, 2004). By stopping or postponing free radicals from oxidizing other molecules, antioxidants reduce the harm they cause in the body (Namiki, 1990). It is seen that the consumption of antioxidant-containing plants as food or food additives not only has a therapeutic but also a preventive effect. According to the World Health Organization (WHO), there is a considerable demand for alternative or complementary medicine practices and practitioners globally (WHO, 2013). Numerous studies have examined the pharmacological uses of phytochemicals and found that they have beneficial effects on human health (Fernando, 2017).

Salvia sclarea L. (Clary sage) has been used from ancient times that belongs to the Lamiaceae family. The Lamiaceae family is of great importance in the fields of medicine, food, and cosmetics because it is rich in essential oils, aromatic compounds, and secondary metabolites, and the members of this family are frequently used in ethnobotanical practices (Baser, 1992; Matkowski, 2008). *Salvia* genus has 100 species in Türkiye and 1049 species in the world. Fif-

ty-eight of the *Salvia* taxa found in Türkiye are endemic (Celep, 2012; World Flora Online). It is known in Aladağlar, Niğde (Türkiye) as "Misk adaçayı, Yağlı kara"; flowers are used as a digestive, branches and leaves are used as a sedative and for diarrhea in tea preparation (Özdemir, 2015). In Antalya, its infusion is used for throat ache (Fakir, 2016). The leaf of *Salvia sclarea*, which is known as "Dağ çayı" is used for cold as a tea in the East Anatolia (Altundag, 2011). *Salvia* species have been shown to contain considerable amounts of flavonoid derivatives and have significant bioactivities. According to a study conducted in 2017, ethanolic extract from *Salvia sclarea*'s aerial part was characterized by HPLC, and the active ingredients found in the result were phenolic acids (rosmarinic acid 165.30 µg/mg, caffeic acid 0.95 µg/mg), flavonoid aglycones (luteolin 0.50 µg/mg, apigenin 0.22 µg/mg), flavonoid glycosides (luteolin-7-O-glucoside 5.55 µg/mg, apigenin-7-O-glucoside 8.5 µg/mg) (Kostić, 2017). Acacetin, one of the major components studied in *Salvia* species, has been identified as a powerful compound with substantial anti-inflammatory and anti-cancer activity. However, further research is required to understand these benefits fully (Singh, 2020). Lutein, a derivative of flavones, has beneficial antioxidant, anti-cancer, anti-inflammatory, and neuroprotective properties (Nabavi, 2015). Apigenin has comparatively potent therapeutic effects in boosting health (Salehi, 2019).

In this study, *Salvia sclarea* was chosen to be tested for some of its biological activities. We aimed to show its DPPH activity and the correlation between this activity and its phenolic content. We also wanted to see the effects of its extracts on acetylcholinesterase (AChE) and MAO-A; those are two important enzymes in the focus of science due to their roles in the treatment of neurodegenerative disorders.

MATERIAL AND METHODS

Plant Materials

The *Salvia sclarea* was collected from Gemlik (Bursa, Türkiye) in the June of 2021. The plant sample

was identified by Asst. Prof. Dr. Ebru Özdemir Nath from the Department of Pharmaceutical Botany, Faculty of Pharmacy, Altınbaş University, İstanbul/Türkiye, and the herbarium specimen is deposited at the Herbarium of Altınbaş University Faculty of Pharmacy (HERA) with the HERA1037 herbarium number.

Extraction

The plant parts were air-dried in a dark, shaded area at room temperature, pounded into a fine powder using a mechanic's grinder, and weighed roughly using a digital balance. The aerial parts of *Salvia sclarea* were weighed and powdered. The plant samples were extracted via maceration, occasionally shaking for 24 hours using ethanol and methanol with the volume-to-mass ratio of the solvent to the sample was 10/1 (v/w). The solvent evaporated to dryness under a rotary evaporator (Heidolph Hei-VAP Advantage Rotary Evaporator) and 2.8 g ethanolic extract and 7.84 g methanolic extract was obtained from 100 g plant. The plant extract of *Salvia sclarea* was stored at +4 °C until biological activity studies.

Biological Activities

DPPH Radical Scavenging Activity

The capacity to scavenge the stable free radical (DPPH) was evaluated according to the method of Brand-Williams (Brand-Williams, 1995). 10 µL of plant extracts (10, 5, 1, and 0.5 mg plant extracts dissolved in 1 mL DMSO) were mixed with 240 µL of 1 mM DPPH radical containing methanolic solution. The well plates were incubated at room temperature for 10 minutes in the dark. As a standard, quercetin from Sigma-Aldrich (Germany) was employed. The following formula (1) was used to calculate the radical scavenging activity (Inh %) as a proportion of DPPH discoloration:

$$\text{Inh \%} = [1 - (\text{Abs}_{\text{extract}} / \text{Abs}_{\text{DPPH}})] \times 100 \quad (1)$$

Total Phenolic Content

A modified Folin Ciocalteu method was used to quantify the extracts' total phenolic content spectrophotometrically (Slinkard, 1977). At 760 nm, the re-

agent's reduction, which produced the development of blue color, was seen. The plant extracts (10, 5, 1, and 0.5 mg plant extracts dissolved in 1 mL DMSO) were mixed with 225 µL of distilled water, then 5 µL of Folin Ciocalteu reagent was added. The mixture was incubated at room temperature. After 3 min, 15 µL of 2% Na₂CO₃ was added. A multimode microplate reader, BioTek Synergy H1 (Agilent), was used to detect the absorbance at 760 nm following a two-hour incubation period at room temperature in a dark area. The standard solution was gallic acid from Sigma-Aldrich (Germany). The average values were used to calculate equivalent gallic acid amounts in the extracts.

Acetylcholinesterase (AChE) Inhibitory Activity

The effects of the extracts on acetylcholinesterase activity were carried out spectrophotometrically. Briefly, the activity of the AChE of *Electrophorus electricus* (electric eel) was determined by the Ellman method (Ellman, 1961). Acetylthiocholine iodide (ATC) was used as the substrate in the study. AChE activity was determined in 0.4 mM ATC and 0.125 mM 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB), 100 mM Tris HCl (pH 8.0) buffer at 25 °C. AChE was added in amounts of ~0.05 U/mL to start the reactions. The hydrolysis of acetylthiocholine was monitored over the increase in absorbance at 412 nm using a spectrophotometer (Cary 60 Single Beam Spectrophotometer, Agilent Technologies, USA). Enzyme activity was calculated using the linear portions of the absorbance-time curve over the first 60 seconds. Runs for each ligand were repeated at least three times.

Monoamine oxidase A (MAO-A) Inhibitory Activity

According to a previously published protocol, monoamine oxidase-A (MAO-A) inhibition assays were performed (Krajl, 1965; Urban, 1991). Monoamine oxidase-A activities of the extracts were determined spectrofluorometrically and carried in triplicate. 100 mM at a pH of 7.4 potassium phosphate buffer was used to carry out each reaction. Kynuramine, a suitable non-fluorescent substrate that is converted

into the fluorescent metabolite 4-Hydroxyquinoline (4-HQ) during the process, was used as substrate in a method to examine the test inhibitors' ability to inhibit MAO-A. Before testing, all extracts dissolved in their organic solvents. Buffer, substrate, and extracts were preincubated for 10 min at 37 °C. Reactions were started by adding the enzyme and the reactions were then kept at 37 °C for another 20 minutes. Following the addition of NaOH (2 N) to stop the reactions, 1000 mL of distilled water were added. Using a multimode microplate reader, BioTek Synergy H1 (Agilent) with an excitation wavelength of 310 nm and an emission wavelength of 400 nm, the amount of fluorescence was measured. The associated IC₅₀ values were to express the inhibitory potencies of the extracts.

Table 1. DPPH radical scavenging activity and TFC of the methanolic and ethanolic extracts of *Salvia sclarea*.

Extract	Concentration. (mg/mL)	DPPH. Equivalent Quercetin (mg/mL)	DPPH. %	TFC. Equivalent Gallic Acid (mg/mL)
Ethanolic Extracts	10	0.24±0.02	82.21±1.79	0.87±0.005
	5	0.16±0.01	73.86±1.51	0.42±0.35
	1	0.05±0.001	50.55±1.18	0.09±0.003
	0.5	0.03±0.0004	38.34±0.72	0.04±0.00
Methanolic Extracts	10	0.33±0.11	88.49±2.63	0.81±0.041
	5	0.20±0.006	78.30±0.98	0.40±0.017
	1	0.06±0.004	53.80±2.59	0.09±0.0016
	0.5	0.03±0.001	39.45±2.47	0.04±0.0016

Antioxidants are molecules that often include a phenolic group and work to prevent the development of free radicals or harm to the cell caused by radicals. They do this by removing radicals. In general, it is known that even in extremely low concentrations, antioxidants are capable of protecting biomolecules in their surroundings from oxidative damage that can be caused by free radicals (Halliwell, 1986). Because of this, it is vital to investigate the antioxidant capabilities and the phenolic content of potential therapeutic plants like *Salvia*. There are a variety of other procedures that may be used to determine the antioxidant

RESULTS AND DISCUSSION

DPPH radical scavenging activity and total phenolic content (TPC)

The results of the DPPH radical scavenging activity and total phenolic content assays of methanolic and ethanolic extracts of *Salvia sclarea* are given in Table 1. Different concentrations of ethanolic and methanolic *Salvia sclarea* extracts were tested at various doses. It has been found that the total amount of quercetin equivalent to the methanolic extract was quite higher than that of the ethanolic extract. At low doses, there does not appear to be any significant distinction between the effects of the different percentages of radical scavenging of both extracts. At low concentrations, the gallic acid equivalent TPCs were also similar, which is parallel to the radical scavenging effect, as well.

capabilities of plant extracts. Still DPPH is selected as a method that is commonly used with its advantages like its simple application, high accuracy, and speedy response (Buyuktuncel, 2013). In the DPPH technique, typically, violet-colored DPPH radical is changed into yellow DPPH-H form by the antioxidants (compounds with phenolic groups) in the extract. In other words, the DPPH radical becomes less reactive. This color shift can be evaluated spectrophotometrically at 517 nm, at which point one additionally can determine the number of radicals that have been reduced (Brand-Williams, 1995).

In research with essential oils and extracts from *Salvia officinalis* L., it was shown that plant oil had a low antioxidant content, while methanolic extracts had the highest antioxidant content (DPPH IC_{50} =37.29 g/g) (Abdelkader, 2015). There hasn't been a lot of research done on *Salvia sclarea* L., another significant species in the *Salvia* genus. Both chloroform and acetone extracts of the plant were investigated, and the results showed that 100 mg of chloroform extract had a greater effect on scavenging radicals than acetone extract; nevertheless, this effect was less potent than the other standard compounds that were investigated (Gülçin, 2004). The TPC of plant extracts containing 1000 mg of dried extracts was also tested in the same study. The results showed that the chloroform extract had 28.91 μ g of pyrocatechol equivalent phenolic compound, while the acetone extract contained 35.24 μ g of this molecule. In a study carried out in Turkey using six distinct species of *Salvia*, non-polar subfractions of methanol extracts of *Salvia sclarea* were analyzed, and the IC_{50} value for DPPH was found to be 23.4 mg/mL. DPPH radical scavenging activity was found to be 79.48% in another study that used 10 mL of methanol extract of 1 gram of dry *Salvia sclarea* plant material, while in our study, a similar radical scavenging activity was observed in the extract sample that was prepared at a dose of 5 mg/mL (Pop (Cuceu), 2016). In a different investigation, methanolic extract of *Salvia sclarea* was employed, and BHT was used as the standard. The quantity of IC_{50} was calculated to be 58.20 g/mL, and the TPC was 24.38 mg

per gram of dry weight. Comparing the levels of TPC and IC_{50} DPPH in plants that grew in environments with varying concentrations of salt was the purpose of that investigation. According to the findings of the research carried out by Taarit, DPPH activity and the overall quantity of phenolic compounds were proportionate to one another (Taarit, 2012). In our research, just as in the study conducted by Taarit, it was demonstrated that DPPH activity increased with increasing concentrations of phenolic content. In another study comparing the methanolic contents of *Salvia sclarea* samples collected at different times of the day, it was found that plant collection time had no effect on phenolic content and DPPH radical scavenging activity (Tulukcu, 2009). The HPLC method was used to assess the TPC of aqueous extracts of different *Salvia* species that were collected in the region of Salento in Southern Italy. According to the findings of the study, *Salvia sclarea* has phenolic content of 55.60 mg/g DW (Vergine, 2019).

AChE and MAO-A Inhibitory Activity

The results of the inhibitory effects of both ethanolic and methanolic extracts of *Salvia sclarea* on acetylcholinesterase and MAO-A are given in Table 2. A wide range of concentrations of the extracts were tested with different doses, and for both AChE and MAO-A, IC_{50} values were calculated. While the effect of the ethanolic extract of *Salvia sclarea* on AChE was nearly four times higher, on the contrary, the effect of the methanolic extract of *Salvia sclarea* on MAO-A was found higher.

Table 2. AChE and MAO-A inhibitory activity of the methanolic and ethanolic extracts of *Salvia sclarea*.

Extracts	IC_{50} AChE (mg/mL)	IC_{50} MAO-A (mg/mL)
Ethanolic Extracts	0.27 ± 0.005	6.53 ± 0.72
Methanolic Extracts	1.19 ± 0.037	3.03 ± 0.05

A deficiency of the neurotransmitter acetylcholine, which is essential for cognitive functioning, is thought to be a sign of Alzheimer's disease (AD), an irreversible neurodegenerative condition. Cholinesterase in-

hibitors are now the medications used to treat AD the most frequently. *Salvia* species are used in traditional European medicine for their memory-enhancing properties (Bahadori, 2017; Orhan, 2013). Because

of this, we have determined the effects of the extracts of *Salvia sclarea* on AChE. In a study, the IC_{50} value of the ethanolic extracts of the leaves of *S. fruticosa* and *S. officinalis* were found to be 287.02 and 268.45 $\mu\text{g/mL}$, respectively (Mervić, 2022). These are nearly the same results for our extracts. In another study, the IC_{50} value of AChE for acetone extract obtained from the roots of *S. syriaca* was found as 500 $\mu\text{g/mL}$ (Bahadori, 2016). In one of the most comprehensive studies about *Salvia* ethyl acetate, dichloromethane, and methanol extracts were studied and, dichloromethane extract of *S. fruticosa* was shown to have the highest inhibitory effect on AChE compared with the others in the same study (Senol, 2010). Also, essential oil of *Salvia sclarea* has been studied and found to have a very low inhibitory effect on AChE (Orhan, 2008).

Due to its crucial function, MAO inhibitors serve as an effective therapeutic option for several mental and neurological conditions and have become the focus of scientists (Rudorfer, 1989; Gökhan-Kelekçi, 2007; Gökhan-Kelekçi, 2009). On the other hand, some medicinal plants utilized in traditional medicine have been used as a significant source for the treatment of depression, Parkinson's disease, and other neuropsychiatric and neurological illnesses (Akhondzadeh, 2003, Saki, 2014). In a study, *S. miltiorrhiza* Bunge has been shown to have some inhibitory effects on MAO-A (Dittmann, 2004). According to our searches, the effects of other types of *Salvia* species on monoamine oxidases have not been studied yet.

Here in this study, we have determined the effects of the ethanolic and methanolic extracts of *Salvia sclarea* on AChE and MAO-A. Although the IC_{50} values were higher when compared to their specific inhibitors, galantamine and clorgiline, the results were encouraging in comparison to other plant species.

CONCLUSION

Here in this study, we have determined some of the biological activities of ethanolic and methanolic extracts of *Salvia sclarea*. Although various studies have explored the properties of *Salvia* species, our

research is the first to demonstrate the impact of the methanolic extract of *Salvia sclarea* on monoamine oxidase-A. Additionally, ethanolic extract of *Salvia sclarea* has been evaluated for its DPPH, TPC, AChE, and MAO-A activities for the first time. The data obtained is quite encouraging and promising. Further studies can be done to use *Salvia sclarea* as a natural source to cure for many neurodegenerative disorders.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

Developing the hypothesis, literature research, writing the original draft (YYY, EON), collection, identification, and extraction of the plant material (EON), analysis of the biological activities and interpretation of the data (YYY).

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