

IN VITRO EVALUATION OF ANTIOXIDANT AND ANTICHOLINESTERASE ACTIVITIES OF *ROSA DAMASCENA* MILL. ESSENTIAL OIL AND ETHANOL EXTRACTS

ROSA DAMASCENA MİLL. UÇUCU YAĞI VE ETANOL EKSTRESİNİN ANTİOKSİDAN VE ANTİKOLİNESTERAZ AKTİVİTELERİNİN İN VİTRO OLARAK DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: Reduced acetylcholine levels, which play a critical role in learning and cholinergic neurotransmission, are closely associated with Alzheimer's disease. This study comparatively evaluated the in vitro antioxidant activity, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory effects, and neuroprotective potential of essential oil obtained from *Rosa damascena* Mill. grown in the Isparta-Gönen region and the ethanol extract prepared from its dried flowers.

Material and Method: Antioxidant activity was determined using DPPH and ABTS radical scavenging assays, while anticholinesterase activity was assessed by the Ellman method.

Results and Discussion: The essential oil exhibited strong antioxidant activity with IC₅₀ values of 0.9 µg/ml (DPPH) and 1.1 µg/ml (ABTS), whereas the ethanol extract showed IC₅₀ values of 2.6 µg/ml (DPPH) and 2.5 µg/ml (ABTS). The essential oil also demonstrated higher enzyme inhibition (AChE: 85.1%, BChE: 80.6%) compared to the ethanol extract (AChE: 74.5%, BChE: 69.9%). Overall, the essential oil showed superior antioxidant and anticholinesterase activities. These findings support the traditional use of rose oil for memory enhancement and suggest its potential as a natural source for pharmaceutical formulations targeting neurodegenerative disorders.

Keywords: Acetylcholinesterase, Alzheimer's disease, antioxidant activity, butyrylcholinesterase, *Rosa damascena* Mill.

ÖZ

Amaç: Öğrenme ve kolinerjik iletimde önemli rol oynayan asetilkolin düzeyindeki azalma, Alzheimer hastalığı ile yakından ilişkilidir. Bu çalışmada, Isparta-Gönen bölgesinde yetişen *Rosa damascena* Mill.'den elde edilen uçucu yağ ile kurutulmuş çiçeklerinden hazırlanan etanol ekstraktının in vitro antioksidan aktivitesi, asetilkolinesteraz (AChE) ve bütirikolinesteraz (BChE) inhibisyonu ile nöroprotektif potansiyeli karşılaştırmalı olarak incelenmiştir.

Gereç ve Yöntem: Antioksidan aktivite DPPH ve ABTS radikal giderim yöntemleriyle, antikolinesteraz aktivite ise Ellman yöntemi ile değerlendirilmiştir.

Sonuç ve Tartışma: Uçucu yağın IC₅₀ değerleri DPPH için 0.9 µg/ml ve ABTS için 1.1 µg/ml olarak

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belirlenirken, etanol ekstraktında bu değerler sırasıyla 2.6 ve 2.5 µg/ml bulunmuştur. Uçucu yağın enzim inhibisyon oranları AChE için %85.1 ve BChE için %80.6 iken, etanol ekstraktında AChE %74.5 ve BChE %69.9 olarak saptanmıştır. Sonuç olarak uçucu yağ, her iki parametrede de daha yüksek aktivite göstermiştir. Bulgular, geleneksel olarak hafıza güçlendirici olarak kullanılan gül yağının kolinesteraz inhibisyonu yoluyla nöroprotektif etki gösterebileceğini ve nörodejeneratif hastalıklara yönelik farmasötik ürünlerde potansiyel taşıdığını ortaya koymaktadır.

Anahtar Kelimeler: Alzheimer hastalığı, antioksidan aktivite, asetilkolinesteraz, butirilkolinesteraz, *Rosa damascena* Mill.

INTRODUCTION

Rosa damascena Mill., belonging to the Rosaceae family, is a perennial shrub that grows in European and Middle Eastern countries. Many countries cultivate this plant to produce rose oil, a high-value commercial product. In Türkiye, it is widely cultivated in the Lakes Region, particularly in Isparta, Burdur, and Afyonkarahisar [1,2]. The flowers of *R. damascena* are rich in terpenes, terpene glycosides, flavonoids, flavone glycosides, and anthocyanins, while its essential oil primarily contains citronellol, geraniol, methyl eugenol, linalool, and nerol [2,3]. Traditionally, *R. damascena* flowers have been used as a laxative, diuretic, anti-fatigue remedy, and for treating colds, coughs, flu, abdominal pain, and gallstones. *R. damascena* holds significant value in traditional medicine [1]. Studies have demonstrated its antibacterial, antioxidant, anti-inflammatory, anticancer, antidepressant, analgesic, and relaxant activities [4]. Today, over 100 different commercial products, including rose water, rose oil, rose lotion, rose cream, rose-flavored Turkish delight, rose jam, rose syrup, and rose soap, are produced from *R. damascena* [5,6].

Since ancient times, plants have been used for the treatment of diseases due to their bioactive compounds. The combination of this historical knowledge with modern technology has inspired scientists to discover new drugs beneficial to human health and further improve existing ones [7].

When the activity of free radicals in the body exceeds the capacity of the body's defense mechanisms, oxidative stress occurs. The imbalance between the production and neutralization of free radicals by the antioxidant system causes oxidative stress, which causes irreversible cellular harm and contributes to various diseases, including cardiovascular diseases, cancer, accelerated aging, and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases [8].

Alzheimer's disease (AD) is a progressive neurological condition primarily impacting older adults, commonly presenting with memory deterioration and impaired cognitive functions. Although the exact causes of the disease remain unclear, several hypotheses have been proposed. One such hypothesis is the oxidative stress hypothesis, which suggests that oxidative stress-induced free radical damage in the brain is directly linked to the pathogenesis of AD. Another key hypothesis is the cholinergic hypothesis, which postulates that a decrease in acetylcholine, a neurotransmitter essential for learning and cholinergic activity in the nervous system, leads to AD. Acetylcholine is hydrolyzed in the nervous system by the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) [9]. Studies have shown that AD is characterized by a rapid decline in AChE levels. While AChE activity is prominent in the early stages of the disease, its levels increase as the disease progresses [10,11]. AChE inhibitors, which are frequently used in the treatment of AD, enhance cholinergic neurotransmission by increasing acetylcholine levels at cholinergic synapses [12]. Today, AChE inhibitors are the most widely and effectively used drug group in Alzheimer's disease. In addition, recent studies have shown that BChE levels are increased in the brains of Alzheimer's patients, and inhibition of this enzyme has become an important target in the treatment process [13].

Given this, recent studies have focused on investigating the inhibitory effects of phytochemicals in plants on AChE and BChE to determine whether they exhibit anticholinergic activity, which is implicated in the pathogenesis of degenerative neurological conditions like Alzheimer's disease and dementia. A curative agent for AD should ideally function as both an AChE/BChE inhibitor and an antioxidant, making dual-function compounds particularly valuable for treatment [14].

In the present study, the essential oil obtained from *Rosa damascena* flowers grown in Isparta-Gönen region by the producer company was analyzed by GC/MS to determine its chemical composition.

Additionally, an ethanol extract was prepared from dried flowers. The antioxidant, AChE, and BChE enzyme inhibition activities of the essential oil and extract were comparatively evaluated.

MATERIAL AND METHOD

Plant Material

The essential oil and dried flower buds of *R. damascena* used in this study were obtained from the producer company "Only Rose," operating in Gönen-Isparta.

Chemicals and Reagents

All chemicals and solvents used in the study were of high purity, at least analytical grade, and were purchased from Sigma Aldrich or Merck.

Extraction Method

In this study, dried flower buds of *R. damascena* were obtained from the producer company and ground into a fine powder. A total of 100 grams of the powdered material was weighed, and 1000 mL of ethanol was added. The mixture was kept at room temperature in a shaker at 150 rpm for 24 hours. After the extraction process, the extract was filtered through filter paper, and the solvent was removed using a rotary evaporator at 40°C. The obtained extract was stored in a dark glass bottle at -20°C for further experimental procedures [15].

GC/MS Analysis

The essential oil composition of *R. damascena* flowers were analyzed as a service at Bezmialem Vakıf University Phytotherapy Education, Research, and Application Center (BİTEM). Gas Chromatography-Mass Spectrometry (GC/MS) was used for the identification of essential oil components, while Gas Chromatography-Flame Ionization Detector (GC-FID) was employed to determine relative percentages of the components.

Antioxidant Activities

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Antioxidant Activity Assay

The total antioxidant capacity of *R. damascena* ethanol extract was determined using the DPPH• method, as described by Blois et al. [16]. The reaction mixture contained 100 µM DPPH• in methanol along with the sample formulations. After 30 minutes, the absorbance was measured at 517 nm using a UV spectrophotometer (UV-1800, Shimadzu, Japan) at 25±2°C [17]. Ascorbic acid was used as a positive control. All experiments were performed in triplicate, and results were expressed as IC₅₀ (µg/ml).

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Antioxidant Activity Assay

The antioxidant capacity of *R. damascena* ethanol extract was measured using the ABTS• radical cation decolorization assay described by Re et al. [18]. To generate ABTS• radicals, 7 mM ABTS• dissolved in water was mixed with 2.45 mM potassium persulfate. The mixture was incubated in the dark at 25°C for 16 hours before use. Ethanol was added to the reaction, and the absorbance was measured at 734 nm at 25°C [17]. Trolox was used as a positive control. All experiments were conducted in triplicate, and results were expressed as IC₅₀ (µg/ml).

AChE and BChE Inhibition

Cholinesterase inhibition was determined spectrophotometrically using a modified Ellman method. Galantamine was used as the positive control, while ethanol served as the negative control. The reaction mixture included sodium phosphate buffer, AChE enzyme stock solution, and *R. damascena* ethanol extract at a concentration of 20 µg/ml, followed by a 30-minute incubation. The reaction was initiated by adding Ellman's reagent (5,5'-dithio-bis-(2-nitrobenzoic acid), DTNB) and acetylthiocholine. For BChE enzyme testing under the same conditions, butyrylthiocholine chloride was

used as the substrate. The reaction was monitored at 412 nm by measuring the formation of the yellow-colored 5-thio-2-nitrobenzoate anion, resulting from the interaction between DTNB and thiocholine. All experiments were conducted in triplicate, and results were expressed as % inhibition [19].

Statistical Analysis

All tests were performed in triplicate, and results were expressed as mean $\pm$ standard deviation (SD).

RESULT AND DISCUSSION

Chemical Composition of *Rosa damascena* Essential Oil

The composition of the essential oil was analyzed using Gas Chromatography-Mass Spectrometry (GC/MS) for component identification and Gas Chromatography-Flame Ionization Detector (GC-FID) for the determination of relative percentages. A total of twenty-seven compounds were identified, representing 100% of the essential oil composition. The major compound identified was citronellol (33.197%), making it the predominant component of the oil. Other major constituents included geraniol (17.395%), nonadecane (16.044%), nerol (8.755%), eugenol (6.130%), Z-5-nonadecane (2.822%), heptadecane (1.920%), and alpha-pinene (1.362%) (Table 1). According to the literature, GC/MS results from the study by Loghmani-Khouzani et al. determined the primary constituents of *R. damascena* essential oil as citronellol (14.5–47.5%), nonadecane (10.5–40.5%), geraniol (5.5–18%), and heneicosane (7–14%) [20]. Similarly, a 2023 study by Kumar et al. analyzed the essential oil profile of *R. damascena* using GC/MS and found citronellol (20.35–44.75%) and geraniol (15.63–27.76%) to be the most abundant components, followed by nonadecane (13.02–28.78%) [21]. The main component of the commercial oil we used in our study is citronellol. These results showed us that the plant-based chemical constituents of *R. damascena* essential oil is consistent with the studies [22–25] reported in the literature.

Antioxidant Activities of Oil and Extracts

DPPH Antioxidant Activity Assay

In this study, all samples exhibited a high radical-scavenging ability against DPPH•. The highest antioxidant activity was observed with the lowest IC₅₀ value. The antioxidant capacities of *R. damascena* essential oil and ethanol extract, measured using the DPPH• assay (with ascorbic acid as the positive control), are presented in Table 2. Based on the DPPH test results, the IC₅₀ value of the essential oil was calculated as 0.9 µg/ml, while that of the ethanol extract was 2.6 µg/ml. These findings indicate that *R. damascena* essential oil exhibits higher antioxidant capacity compared to its ethanol extract.

ABTS Antioxidant Activity Assay

All tested samples demonstrated a strong ability to neutralize ABTS• radicals. The highest antioxidant capacity was associated with the lowest IC₅₀ value. The antioxidant capacities of *R. damascena* essential oil and ethanol extract, measured using the ABTS• assay (with Trolox as the positive control), are presented in Table 2. Based on the ABTS• assay results, the IC₅₀ value of the essential oil was determined to be 1.1 µg/ml, while that of the ethanol extract was 2.5 µg/ml. These findings indicate that *R. damascena* essential oil exhibits higher antioxidant capacity than its ethanol extract.

In a study on the antioxidant activity of *R. damascena* essential oil [26], the DPPH IC₅₀ value was determined to be 3.54 $\pm$ 1.22 µg/ml. When compared, the essential oil used in our study was found to have a considerably high antioxidant capacity. In another study measuring the antioxidant capacity of the aqueous ethanol extract of *R. damascene*, the DPPH IC₅₀ value was reported as 0.27 $\pm$ 0.01 mg/ml, and the ABTS IC₅₀ value was found to be 1.98 $\pm$ 0.01 mM Trolox/gE. Similarly, in another study, the DPPH IC₅₀ value for the ethanol extract was determined to be 2.24 $\pm$ 0.98 µg/ml. The studies coincide with our results [27].

Table 1. Chemical composition of *Rosa damascena* essential oil

Compounds	%
Citronellol	33.197
Geraniol	17.395
Nonadecane	16.044
Nerol	8.755
Heneicosane	6.130
Z-5-Nonadecane	2.822
Heptadecane	1.920
Eicosane	1.650
α pinene	1.362
Phenethyl alcohol	1.157
Methyleugenol	1.082
Germacrene D	0.995
Dotriacontane	0.936
Tricosane	0.896
Geranyl acetate	0.754
Menthol acetate	0.650
Caryophyllene	0.633
β mycrene	0.540
Neryl acetate	0.447
Citronellol acetate	0.446
Humulene	0.420
Linalool	0.395
Azulene+ α guaiene	0.376
Eugenol	0.295
4-Terpineol	0.290
β pinene	0.278
Trans rose oxide	0.136
Total	99.976

Table 2. Antioxidant capacities of *Rosa damascena* essential oil and ethanol extract

	DPPH•	ABTS•
	IC ₅₀ (µg/ml)*	IC ₅₀ (µg/ml)*
Essential Oil	0.9	1.1
Etoh Extract	2.6	2.5
Ascorbic Acid	0.002	-
Trolox	-	0.01

* 50% Inhibitory concentration of DPPH and ABTS (average of three experiments $\pm$ SD)

Previous studies have demonstrated the antioxidant activities of rose extract due to its high content of polyphenols, proanthocyanidins, flavonoids, and carotenoids [28]. The antioxidant activity of *Rosa damascena* essential oil was investigated by radical scavenging (DPPH) and ferric reducing antioxidant power (FRAP) analyses and DPPH=5.72% inhibition, FRAP=0.132% inhibition values were reached. When these results were evaluated; it was determined that it showed very low activity compared to reference substances [29]. In a study conducted with three different rose species, the antioxidant activity of methanol extracts obtained from fresh flowers was investigated by the DPPH free radical method. The maximum free radical scavenging activity was shown by *R. brunonii* (IC₅₀=35.2 $\pm$ 0.69), followed by *R. bourboniana* (IC₅₀=25.0 $\pm$ 0.71) and *R. damascena* (IC₅₀=21.4 $\pm$ 0.52) [30].

AChE and BChE Inhibition

The essential oil and ethanol extract of *R. damascena* were evaluated for their inhibition of AChE and BChE, which are enzymes associated with AD. The results of the AChE and BChE activity assays

for the essential oil and extract are presented in Table 3, with galantamine used as the positive control. The essential oil inhibited AChE by 85.1% and BChE by 80.6%. It was observed that the essential oil exhibited inhibition levels comparable to those of galantamine, the positive control. When comparing AChE and BChE inhibition, the essential oil of *R. damascena* was found to inhibit AChE to a greater extent than BChE. The ethanol extract inhibited AChE by 74.5% and BChE by 69.9%. It was observed that the extract inhibited both enzymes at a good level, although not higher than galantamine used as a positive control. When evaluated in terms of comparing the inhibition of AChE and BChE; It was concluded that the inhibition of AChE enzyme by *R. damascena* ethanol extract was higher than the inhibition of BChE enzyme.

Table 3. Inhibition of cholinesterase enzyme by *Rosa damascena* essential oil and ethanol extract

	AChE % Inhibition*	BChE % Inhibition
Essential Oil	85.1%	80.6%
Etoh Extract	74.5%	69.9%
Galantamine	99.2%	86.9%

* The % inhibition of AChE and BChE (average of three experiments $\pm$ SD)

A prominent alteration in the brains of Alzheimer's patients is the reduction in acetylcholine levels within the cerebral cortex. Therefore, the inhibition of AChE and BChE can help restore acetylcholine levels in the brain [31]. Most drugs used in the treatment of AD are designed as enzyme inhibitors, such as galantamine [32]. The inhibition of AChE and BChE, which are the key enzymes that hydrolyze acetylcholine (ACh) and butyrylcholine (BCh), has become an important therapeutic strategy against AD [33]. Based on this information, our study was initiated to investigate the inhibition of these key enzymes involved in the pathogenesis of AD.

In the study conducted by Senol et al.; AChE and BChE enzyme inhibition of *Rosa damascena* essential oil was evaluated by Ellman's spectrophotometric method. At the highest concentration of 1000 $\mu\text{g/ml}$, *Rosa damascena* essential oil showed $60.86 \pm 1.99\%$ inhibition on AChE and $51.08 \pm 1.70\%$ on BChE. Therefore, it was determined that it could help Alzheimer's disease in preventing memory loss [29]. Although the essential oil concentration in our study was tested quite low compared to the reference study (20 $\mu\text{g/ml}$), the % inhibition rates were significantly higher.

Phenylethyl alcohol, a characteristic compound of rose oil and rose aroma, has been found to inhibit AChE and BChE enzymes, suggesting its potential role in preventing memory loss and aiding in the treatment of AD [29]. In another study examining the inhibitory effects of rose water on AChE and BChE enzymes, the IC_{50} values were determined as 0.6227 $\mu\text{g/ml}$ for AChE and 0.1956 $\mu\text{g/ml}$ for BChE [34].

Esfandiary et al reported that *Rosa damascena* can reverse amyloid- β -induced behavioral disorders and may be a potential agent in the prevention and management of cognitive impairments associated with Alzheimer's disease [35]. Karadağ et al. measured the AChE and BChE enzyme activities of *R. damascena* essential oil using the modified Ellman method. *R. damascena* oil was determined to have an IC_{50} value of approximately 69.39 ± 2.07 $\mu\text{g/ml}$ for AChE and 55.13 ± 1.62 $\mu\text{g/ml}$ for BChE. These values indicate that the oil inhibits BChE slightly more strongly than AChE [2]. In our study, it was determined that AChE enzyme inhibition of *Rosa damascena* essential oil was higher compared to BChE enzyme inhibition. This variation might stem from alterations in the constituent makeup of the essential oils and the experimental parameters used. Findings in the literature show that the type of plant, chemotype and geographical region where it grows significantly affect the variety and amount of phytochemical compounds it contains. The amount and variety of these compounds play a decisive role on the biological activity profile of the plant.

There are many studies investigating the antioxidant activity and neuroprotective effects of the essential oil, fresh and dried flower extracts, absolute and aromatic waters of *Rosa damascena* plant with different methods. However, despite literature searches, no study or report has been found comparing the essential oil obtained from *Rosa damascena* from the company from which the product support is received and the ethanol extract prepared from dried flowers in terms of both antioxidant

potential and neurodegenerative damage reduction capacity. In this respect, our study aims to make an original contribution to show the capacity neuroprotective effects of *Rosa damascena*.

Ethanol extract of *Rosa damascena* flowers has been reported to contain a wide range of bioactive compounds, including flavonoids (quercetin, kaempferol, rutin, apigenin), phenolic acids (gallic acid, caffeic acid, chlorogenic acid, ellagic acid), tannins and other polyphenolic antioxidants, as well as organic acids such as malic and citric acid, and various sugar derivatives. These phytoconstituents contribute to the extract's notable antioxidant, anti-inflammatory, and antibacterial activities, supporting its traditional and potential therapeutic applications [25,36].

In this study, the essential oil and ethanol extract of *Rosa damascena* flowers were investigated for their antioxidant capacity and effects on AChE and BChE enzymes. The collected experimental data confirmed the antioxidant potential of the plant's essential oil and ethanol extract, with the essential oil exhibiting particularly high antioxidant capacity. Enzyme inhibition studies demonstrated that both the essential oil and ethanol extract strongly inhibited AChE and BChE enzymes, with the essential oil exhibiting a notably high level of inhibition. This study confirms that rose oil, traditionally used for memory enhancement, may have a neuroprotective effect through cholinesterase inhibition. The major components found in the essential oil of *Rosa damascena* (such as citronellol, geraniol, nonadecane, and nerol) could serve as potential candidates for identifying new AChE and BChE inhibitors for Alzheimer's disease treatment. It is anticipated that our findings will contribute to the literature as a preliminary study for the development of a new phytotherapeutic agent for Alzheimer's disease treatment. Further *in vivo* and *in vitro* studies are recommended to support these findings in future research. To the best of our knowledge this study is the first to compare essential oil and ethanol extract. In future studies, we aim to reveal which enzyme this compound is more selective against by conducting molecular modeling studies on the compound that shows the highest inhibition among the main components of the essential oil.

AUTHOR CONTRIBUTIONS

Concept: E.S.G.; Design: E.S.G.; Control: E.S.G., A.E.K., A.A.; Sources: E.S.G., A.E.K., A.A.; Materials: E.S.G., A.E.K., A.A.; Data Collection and/or Processing: E.S.G., A.E.K., A.A.; Analysis and/or Interpretation: E.S.G., A.E.K., A.A.; Literature Review: E.S.G., A.E.K., A.A.; Manuscript Writing: E.S.G., A.E.K., A.A.; Critical Review: E.S.G., A.E.K., A.A.; Other:-

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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