South African Journal of Botany 147 (2022) 799–807

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Phytochemical screening and biological evaluation of *Salvia hydrangea* DC. ex Benth. growing in eastern Anatolia

Gizem Gülsoy Toplan[a,*], Mine Kürkçüoğlu[b], Fatih Göger[b], Turgut Taşkın[c], Ayşe Civaş[d], Gökalp İşcan[b], Gülay Ecevit-Genç[e], Afife Mat[f], K. Hüsnü Can Başer[g]

[a] Department of Pharmacognosy, Faculty of Pharmacy, Istinye University, Istanbul, Turkey
[b] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey
[c] Department of Pharmacognosy, Faculty of Pharmacy, Marmara University, Istanbul, Turkey
[d] Department of Pharmacy and Pharmaceutical Services, Tuzluca Vocational School, Igdır University, Igdir, Turkey
[e] Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey
[f] Department of Pharmacognosy, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey
[g] Department of Pharmacognosy, Faculty of Pharmacy, Near East University, Nicosia, Cyprus

A R T I C L E I N F O

Article History:
Received 13 November 2021
Revised 6 March 2022
Accepted 10 March 2022
Available online 19 March 2022

Edited by B. Venkidasamy

Keywords:
Salvia hydrangea
Volatile oil
Antimicrobial
GC-GC/MS
Antioxidant activity
LC-MS/MS

A B S T R A C T

Salvia species have lately gained significant interest as a result of their suitable utilization in various industries. In the current study, *S. hydrangea*, one of the most consumed sages in the eastern region of Turkey, has been evaluated for phytochemical composition as well as *in vitro* pharmacological potential comparatively for the first time. The phytochemical composition of *S.hydrangea* was investigated by LC-MS/MS, GC-FID, and GC/MS. To reveal its biological activities, antioxidant, antimicrobial, and also acetylcholinesterase activities of different solvent extracts such as water, *n*-hexane, chloroform, and methanol were determined. According to GC-GC/MS analysis, the primary components of the oil were identified as camphor (46.0%), 1,8-cineole (7.5%), camphene (6.8%), limonene (6.5%), β-pinene (6.11%) and α-pinene (5.6%). Additionally, in the infusion and methanol extract, rosmarinic acid and luteolin glycoside were detected as predominant phenolics by LC-MS/MS. In DPPH·, CUPRAC, and FRAP test results of the samples indicated strong to moderate antioxidant ability in all samples studied, additionally, among them, the infusion exhibited significant acetylcholine inhibition properties comparable with galanthamine. With regard to antimicrobial activity, all of the tested microorganisms had MIC values ranging from 15 to 2000 μg/mL. Based on these findings, *S. hydrangea* may have promising properties for a variety of industrial applications in the pharmaceutical, food, and cosmetic industries.

© 2022 SAAB. Published by Elsevier B.V. All rights reserved.

1. Introduction

Salvia is one of the largest and valuable genera in the family Lamiaceae, with over 1000 species found all over the world (Zare et al., 2021). Its species are commonly known as 'sage' and used for a wide variety of different purposes including treatment of cold, wounds, digestive and menstrual problems, skin infections, enhancement of the memory, flavoring foods, added to the formula of cosmetics and perfumes for the preservation of products (Demirci et al., 2003; Wu et al., 2012; Zengin et al., 2018; Poulios et al., 2020). *Salvia* species have gradually gained value as a result of their therapeutic applications in both the industry and folk medicine (Asadi et al., 2010). Furthermore, the economic significance is growing since some species are cultivated for use in pharmaceutical, food, and cosmetic industries (Sharma et al., 2019). Thus, the species of this genus have drawn the attention of researchers, and the number of studies increased in recent decades (Sharifi-Rad et al., 2018; Shojaeifard et al., 2021).

Several studies have been extensively conducted on their pharmacological activities as well as phytochemical composition. From a chemical viewpoint, *Salvia* species have been revealed to be abundant in several types of secondary metabolites, including mono, di- and triterpenes flavonoids, and phenolic acids (Sairafianpour et al., 2003; Asadi et al., 2010; Farimani et al., 2012; Kahnamoei et al., 2019). These species are important sources of essential oil and polyphenols (Bakkali et al., 2008). Investigations on the phenolic substances of the genus revealed the presence of carnosic acid, rosmarinic acid, and derivatives of these acids as major components (Toplan et al., 2017). The results of the investigations on the oil content of several *Salvia* species revealed a significant diversity including predominant compounds and their percentages (Baser 2002). The

* Corresponding author.
E-mail address: gizem.toplan@istinye.edu.tr (G.G. Toplan).

https://doi.org/10.1016/j.sajb.2022.03.021
0254-6299/© 2022 SAAB. Published by Elsevier B.V. All rights reserved.

major constituents of EOs have been identified as 1,8 cineole, α/β pinene, camphor, and thujone (Stešević et al., 2014; Sharifi-Rad et al., 2018; Ghavam et al., 2020). Previous reports indicated that the EOs and various extracts of *Salvia* species possess remarkable antioxidant, anti-inflammatory, antibacterial, antifungal, antitumoral, and neuroprotective properties (Bahadori and Mirzaei, 2015; Lopresti 2017; Xu et al., 2018; Poulios et al., 2020; Righi et al., 2021; Afonso et al., 2021; Askari et al., 2021). The members of the genus exhibited a broad variety of biological activity, which was linked to their unique chemical composition particularly rich in phenolic content (Lu and Foo, 2002; Wu et al., 2012). Due to the confirmation of its strong antioxidant and antimicrobial facilities, sages are used as natural preservatives to stabilize foods, cosmetics, and other products (Koşar et al., 2008; Poulios et al., 2020).

The human body is subject to several kinds of oxidative stress, resulting in many different degenerative illnesses, including skin diseases, various forms of cancer, cardiovascular disease, and neurological disorders (Bibi Sadeer et al., 2020). The past few decades have seen the prevalence of various diseases such as Alzheimer's disease (AD), Parkinson's diseases, (PD), and cancer increasing all over the world. These illnesses are seen as "global health" issues under this perspective. Antioxidants are assumed to slow or prevent oxidation, as well as the formation of oxidizing chain reactions, during the oxidation period of the chemical cycle (Shahidi, 2000). Numerous studies have established that a deficiency of antioxidant molecules plays a role in neurodegenerative diseases, and antioxidants may help prevent or delay neuronal cell death in idiopathic neurodegenerative disorders particularly Alzheimer's and Parkinson's diseases (Esposito et al., 2002; Lopresti 2017). However, the usage of synthetic antioxidants is limited due to their carcinogenic effects (Shahidi and Zhong, 2010). Hence, many studies have focused to discover natural and alternative antioxidant agents that have fewer adverse effects. Several studies have demonstrated that secondary metabolites of plants, especially EOs and phenolic compounds have been proven to decrease oxidative damage and prevent free radicals (Bursal et al., 2019; Firuzi et al., 2010; Poulios et al., 2020).

AD is a degenerative neurological disorder characterized by cognitive and behavioral abnormalities that have emerged as a serious public health concern, particularly in the industrialized world (Şenol et al., 2010). Acetylcholinesterase enzyme catalyzes the breakdown of acetylcholine, and its absence may result in neurodegenerative diseases such as AD (Akkol et al., 2012; Tundis et al., 2015). As a result, the developed methods for inhibiting this enzyme have emerged as a novel strategy for screening plant-based materials (Orhan et al., 2008). Researchers have focused their efforts on the enzyme-inhibiting properties of different plants and also identifying the responsible components in order to cure or prevent AD. Plenty of investigations showed promising anticholinesterase effects in different *Salvia* species studied (Orhan et al., 2012; Şenol et al., 2010).

Another significant health problem is the increased antibiotic resistance all over the world (Gibbons 2005). Only a few new antimicrobial medications have been introduced over the years, and developing new drugs to combat resistant bacteria has become a top priority nowadays (Kwapong et al., 2019). There have been several studies to prove that plant extracts and also their secondary metabolites are effective against bacterial and fungal infections. Among them, *Salvia* species are quite popular throughout the world and many of them have been used for centuries to treat infections and to protect against microbial contamination due to their antimicrobial facilities (Al-Bakri et al., 2010; Firuzi et al., 2013; Delamare et al., 2007; Gvaham et al., 2020; Askari et al., 2021).

The genus *Salvia* is represented by approximately 100 species in Turkey, almost half of which are endemic (Guner et al., 2012). In the Anatolian traditional medicine, *Salvia* species have long been used against wounds, pharyngitis, stomatitis, stomachache, headache, common cold, memory problems, and galactorrhoea (Gürdal and Kültür, 2013). There are several *Salvia* species that are frequently utilized in Anatolia, the most consumed species is *S. triloba* L. which is commercially available in many herbal stores (Baytop 1999). In Ağrı, in the eastern part of Turkey, an infusion of *Salvia hydrangea* has been used by local people for the treatment of several diseases as herbal tea. As far as we know, despite a few studies which have been published on the essential oil composition of *S. hydrangea,* there is no comprehensive study on the biological activities of different extracts as well as the phenolic composition of the plant (Kotan et al., 2008; Kahnamoei et al., 2019; Ghavam et al., 2020; Zare et al., 2021). The goal of the present study is to evaluate the phytochemical composition of *S. hydrangea* with *in vitro* biological potential.

2. Material and methods

2.1. Plant material

The aerial parts of *S. hydrangea* were collected in Ağrı (Doğubeyazıt), 2017, in the easternmost region of Turkey, during the flowering stage. Plant materials are stored at the Herbarium of the Pharmacy Faculty of Istanbul University (ISTE Number: 116568). The aerial parts were dried at room temperature and kept in a dark place.

2.2. Preparation of extracts

The essential oil (EO) was extracted from aerial parts of the plant by hydrodistillation for 3 h with a Clevenger-type apparatus. The essential oil was kept at +4 °C in an amber-colored vial until analysis.

The aerial parts of *S. hydrangea* were powdered using a mill and then extracted with different solvents in the order of *n*-hexane, chloroform, and methanol using a Soxhlet apparatus. Thereafter, extracts were filtered through a Whatman paper and evaporated to dryness under reduced pressure at a temperature below 40 °C. Additionally, an infusion extract of the plant was also prepared via the maceration procedure. 10 g of air-dried *S. hydrangea* was macerated by shaking using 100 mL hot water twice and then lyophilized and stored at −20 °C until analysis.

2.3. Chemical analyses of essential oil

An Agilent 6890 N GC system was used in the GC analysis. The EO of *S. hydrangea* was examined by capillary Gas Chromatography (GC-FID) and Gas Chromatography-Mass Chromatography (GC/MS) simultaneously using an Agilent GC−MSD system. The GC/MS analysis was performed with an Agilent 5975 GC/MSD system (Agilent Technologies Inc., Santa Clara, CA). To obtain the same elution order with GC/MS, the simultaneous injection was accomplished by using the same column and operational parameters. In the analysis, the Innowax FSC column (HP, U.S.A.) (60 *m* × 0.25 mm; film thickness 0.25 m (microliter) was used, and the FID temperature was set at 300 °C. Helium at a flow rate of 0.8 mL/min was used as carrier gas. The temperature of the GC oven was maintained at 60 °C for 10 min and increased to 220 °C at a rate of 4 °C/min, and kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1 °C/min. The split ratio was adjusted 40:1. The injector temperature was at 250 °C. Mass spectra was taken at 70 eV. Mass range was from *m/z* 35−450.

The constituents of EO were identified by comparison of their mass spectra with those in the Adams Library, Baser Library of Essential oil Constituents, Wiley GC/MS Library, and MassFinder Library confirmed by comparing their retention indices. The essential oil sample was analyzed three times.

2.4. Determination of phenolics using LC-MS/MS

Phenolic compounds were determined with a Shimadzu HPLC 20A system attached to an Applied Biosystems Q-Trap 3200 LC-MS/

MS system. Mass spectrum analyses were performed in the negative ionization mode at a mass range of 150–800 amu. 250 × 4.6 mm, 5 μm, ODS analytical column was chosen at 40 °C for the chromatographic analysis. UV Chromatograms were taken at 280 and 320 nm. $CH_3OH:H_2O:CH_2O_2$ (10:89:1, v/v/v) (solvent A) and CH_3OH : H_2O : CH_2O_2 (89:10:1, v/v/v) (solvent B) were used for the gradient analysis at flow rate 1 ml/min. The composition of B was increased from 15% to 100% in 40 min.

2.5. Total phenolic contents of the samples

The Folin–Ciocalteu reagent (FCR) method was used to determine the total phenolic content of four different extracts from the aerial parts of the plant. Briefly, 5 μL extract (5 mg/mL- 0.5 mg/mL) was taken and 225 μL of water was added to the tube. Then 5 μL of Folin-Ciocalteu reagent (diluted 1/3 with distilled water) and 15 μL of 2% sodium carbonate solution were added to the combination. Afterward, the combination was allowed to rest at room temperature for two hours before the absorbance at 760 nm was recorded against the standard reference. The total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE)/g extract (Ozsoy et al., 2008).

2.6. Antioxidant activity of the samples

The antioxidant capacity and free radical scavenging activity of extracts were measured by CUPRAC, DPPH·, and FRAP assays.

The antioxidant capacity of the samples was determined using the CUPRAC method. A plate was combined with 1 mL of Cu (II) (1.10−2 M), neocuproine ethanolic solution (7.3.10−3 M), and 1 M NH_4Ac buffer solution. Extracts 1 mL and 0.1 mL pure EtOH were added to the initial mixture to make the final volume: 4.1 mL. After ten seconds of vortexing, the absorbance of the solution was measured at 450 nm against a reagent blank. Samples of CUPRAC measurements have been demonstrated as equivalents for Trolox (mM Trolox/mg extract) (Apak et al., 2004).

The DPPH· method was used to test the capacity of free radical scavenging in four distinct extracts. To summarize, 240 μL of DPPH· solution (0.1 mM) was mixed with 10 μL of extracts prepared at various concentrations (5 mg/mL-0.5 mg/mL). Then, the mixture was kept for a further 30 min at room temperature before its usage. The absorbance of the combination was determined in comparison to a standard using a microplate reader set at 517 nm. The experiment was replicated three times and the outcomes were given as ascorbic acid equivalent (mg AAE/g extract) (Wei et al., 2010).

The FRAP method has been studied for evaluating the ability of ferric reducing of different extracts (5 mg/mL-0.5 mg/mL). In brief, the FRAP reagent (3.8 mL) was mixed with samples (0.2 mL) and 4 min later, the absorbance of the mixture was measured against the reference at 593 nm. The standard curve was prepared using $FeSO_4$ and FRAP values of the samples were expressed as an mM Fe^{2+}/mg extract (Benzie and Strain, 1996).

2.7. Anticholinesterase activity of the samples

The inhibition of cholinesterase enzymes of the samples was determined using a 96-well microplate reader previously described by Ellman et al. (1961), with some changes. Firstly, 50 mM Tris–HCl buffer (pH 8.0) was used to prepare all reagent solutions (daily). Shortly, the AChE solution and each sample were mixed in 20 μl concentration with 40 μl of Tris–HCl buffer. This mixed solution has been stood at 25 °C for 10 min. Then, the reaction was started adding 20 μl of ATCI (50 mM) into the combination and the total solution was incubated for 5 min. at room temperature. After all, 20 mM DTNB (100 μl, containing 1 M NaCl and 0.2 M $MgCl_2 \cdot 6H_2O$) was added to the combination and its absorbance was read at 412 nm against the reference. Each experiment was conducted in triplicate. As a reference compound, galanthamine was used (Ellman et al., 1961).

2.8. Antimicrobial activities of the samples

Anticandidal and antibacterial tests were performed according to partly modified CLSI M27-A2 and M7-A7 reference protocols. Amphotericin-B and Ketoconazole (Sigma-Aldrich) were used as standard antifungal agents while Chloramphenicol and Ampicillin (Sigma-Aldrich) were used as antibacterial. *Candida albicans* ATCC 10231, *Candida tropicalis* NRRL Y-12968, *Candida albicans* ATCC 90028, *Candida tropicalis* ATCC 750, *Candida utilis* NRRL Y-900, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258 were used as test strains for anticandidal assay. *Escherichia coli* NRRL B-3008, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 7853, *Salmonella typhimurium* ATCC 13311, *Serratia marcescens* NRRL B-2544, *Klebsiella pneumoniae* NCTC 9633 were used as test microorganisms in antibacterial activity method.

Different from the standard protocol, EO and extracts of *S. hydrangea* were diluted between the concentrations of 2 mg/mL to 0.004 mg/mL where the standard antifungals were diluted following CLSI methods (CLSI (NCCLS) M27-A2 2002; CLSI (NCCLS) M7-A7 2006). Stored yeast strains were refreshed onto Potato Dextrose Agar (PDA, Fluka) while bacteria were inoculated onto Mueller Hinton Agar (MHA, Fluka) for checking purity. All tests were achieved by using sterile 96 U-shaped multi-well plates (Brand). Antimicrobial test results were screened after the incubation period at 35±2 °C, 16–20 h. The MIC (minimal inhibitory concertation) is defined as the lowest concentration in which an optically clear well is observed. Furthermore, according to the M27-A2 method, recommended MIC limits of two quality control strains [*C. krusei* (ATCC® 6258) and *C. parapsilosis* (ATCC® 22,019)] against Amphotericin-B and Ketoconazole were considered for the precision and accuracy of the assay.

2.9. Statistical analysis

Results were expressed as mean ± standard deviations (SD) of 3 parallel and independent analyses. After performing ANOVA tests, significant differences between means were identified by performing a Tukey Multiple Comparison test.

3. Results and discussion

3.1. Fraction yields and total phenolic content

Using the hydrodistillation method, the essential oil of *S. hydrangea* was obtained in 1.7% yield from its aerial parts as a yellow oil with a characteristic fragrance. The fraction yields of *S. hyrangea* obtained by using nonpolar to polar ranged solvents are as follows: *n*-hexane extract (0.379 g), chloroform extract (0.097 g), methanol extract (1.63 g), and the infusion (1.44 g), by expressing extractable compounds as (EC)/gram of dry weight (DW).

3.2. Determination of phytochemical composition

The EO composition of *S. hydrangea* was characterized by GC-FID and GC/MS analyses, in triplicate. A total of 66 constituents, representing 98.8% of the total components in the EO of *S. hydrangea* have been described. The retention indices and percentage composition of substances are presented in Table 1.

The major compounds of EO of *S. hydrangea* were camphor (46.0%) followed by 1,8-cineole (7.5%), camphene (6.8%), limonene (6.5%), α-pinene (5.6%), and β-pinene (6.1%). None of the remaining components were detected in concentration higher than 10%. Sabinene (1.0%), terpinen-4-ol (1.1%), α-humulene (1.9%), and *trans*-

Table 1
The essential oil composition of the aerial parts from *S. hydrangea*.

RRI	Compounds	%	Identification Metod
1014	Tricyclene	0.3	MS
1032	α-Pinene	5.6	t_R, MS
1035	α-Thujene	0.2	MS
1076	Camphene	6.8	t_R, MS
1118	β-Pinene	6.1	t_R, MS
1132	Sabinene	1.0	t_R, MS
1138	Thuja-2,4 (10)-diene	0.1	MS
1174	Myrcene	0.6	t_R, MS
1188	α-Terpinene	0.1	t_R, MS
1203	Limonene	6.5	t_R, MS
1213	1,8-Cineole	7.5	t_R, MS
1246	(*Z*)-β-Ocimene	0.3	t_R, MS
1255	ϒ-Terpinene	0.3	t_R, MS
1266	(*E*)-β-Ocimene	0.1	t_R, MS
1267	3-Octanone	0.1	t_R, MS
1280	*p*-Cymene	0.4	t_R, MS
1290	Terpinolene	0.3	t_R, MS
1468	*trans*-1,2-Limonene epoxide	tr	MS
1473	Camphenilone	tr	MS
1474	*trans*-Sabinene hydrate	tr	t_R, MS
1479	δ-Elemene	0.2	MS
1497	α-Copaene	0.1	MS
1499	α-Campholene aldehyde	0.3	MS
1532	Camphor	46.0	t_R, MS
1547	Dihydroachillene	tr	MS
1550	*cis*-α-Bergamotene	tr	MS
1553	Linalool	0.3	t_R, MS
1556	*cis*-Sabinene hydrate	tr	t_R, MS
1586	Pinocarvone	0.4	MS
1590	Bornyl acetate	0.2	t_R, MS
1600	β-Elemene	tr	MS
1601	Nopinone	tr	MS
1610	Calarene	0.3	t_R, MS
1611	Terpinen-4-ol	1.1	t_R, MS
1612	β-Caryophyllene	tr	t_R, MS
1648	Myrtenal	0.6	MS
1664	*trans*-Pinocarveol	0.6	t_R, MS
1671	(*Z*)-β-Farnesene	0.3	MS
1687	α-Humulene	1.9	t_R, MS
1690	*trans*-Verbenol	0.6	MS
1707	α-Terpinyl acetate	0.5	t_R, MS
1719	Borneol	0.3	t_R, MS
1726	Germacrene D	0.5	MS
1727	Verbenone	0.3	t_R, MS
1741	α-Bisabolene	0.4	t_R, MS
1751	Bicyclogermacrene	0.5	t_R, MS
1754	β-Curcumene	0.3	MS
1755	Carvone	0.8	t_R, MS
1765	Geranyl acetate	0.5	t_R, MS
1773	δ-Cadinene	tr	t_R, MS
1783	β-Sesquiphellandrene	tr	MS
1786	*ar*-Curcumene	0.2	MS
1797	Myrtenol	0.5	MS
1845	(*E*) −Anethole	0.9	MS
1849	*trans*-Carveol	0.5	t_R, MS
1864	*p*-Cymen-8-ol	0.2	t_R, MS
2000	*trans*-Sesquisabinene hydrate	1.3	MS
2008	Caryophyllene oxide	tr	t_R, MS
2012	Maaliol	0.6	MS
2071	Humulene epoxide-II	0.7	MS
2144	Spathulenol	0.4	t_R, MS
2161	β-Bisabolol	0.8	MS
2162	αAcorenol	tr	MS
2232	α-Bisabolol	0.2	t_R, MS
2255	β-Eudesmol	0.3	MS
2260	Alismol	0.4	MS
	Monoterpene hydrocarbons	28.7	
	Oxygenated monoterpenes	59.8	
	Sesquiterpene hydrocarbons	4.3	
	Oxygenated sesquiterpenes	4.7	
	Others	1.3	
	Identified compound	66	
	Total%	98.8	

RRI; Relative retention indices calculated against *n*-alkanes.%; calculated from the FID chromatograms. tr: trace (<0.1 %); IM: Identification Method; tR, identification based on comparison with co-injected with standards on a HP Innowax column; MS, identified on the basis of the computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder, and Wiley libraries.

sesquisabinene hydrate (1.3%) were also identified in lower percentages in the oil. The EO had a high concentration of oxygenated monoterpenes (59.8%).

Plenty of studies demonstrated variations in the EO of composition of several *Salvia* species. Başer reported a wide range of chemical variety among essential oils derived from *Salvia* species in Turkey (Başer, 2002). The oils of *Salvia* species were categorized according to their primary components. There are three major categories of commercial oils that have been identified: the 1,8-Cineole/Camphor (CiCa) group, the thujone group, and the pinene group (Başer, 2002). The EO of *S. hydrangea* can be classified in Camphor/1,8-Cineole (CaCi) group. Additionally, several studies have demonstrated that genetic and environmental variables have a significant impact on the production of essential oil from *Salvia* species (Barazandeh, 2004; Başer, 2002; Ghavam et al., 2020).

There are a few studies on the EO composition of *S. hydrangea* growing in Turkey. Kotan et al. have examined the EO composition of the *S. hydrangea* collected from Iğdır (Kotan et al., 2008), and found camphor (54.2%) and α-humulene (4.0%) as main constituents. The main constituents of the EO of *S. hydrangea*, collected from Kars, were identified as 1,8-cineole (7.4%), camphene (9.4%), and camphor (46.9%). Both locations are quite near each other and in the eastern part of Turkey. Our results were found to be comparable with those studies except for the percentage amounts of the constituents. The essential oil composition of *S. hydrangea* from several locations around Iran (Isfahan region) has been previously recorded and β-caryophyllene (33.4%) and caryophyllene oxide (25.4%) were found to be predominant compounds. In another study from the Daran region of Iran, the essential oil compositions of leaf and flower parts of *S. hydrangea* were investigated with their antimicrobial activities. The major compounds found in the leaf EO were spathulenol (16.07%), 1,8-cineole (13.96%), *trans*-caryophyllene (9.58%), β-pinene (8.91%), and β-eudesmol (5.33%) and those found in flower EO were caryophyllene oxide (35.47%), 1,8-cineole (9.54%), *trans*-caryophyllene (6.36%), β-eudesmol (4.11%), caryophyllenol-II (3.46%), and camphor (3.33%). Gvaham et al., classified the EO of *S. hydrangea* comparing the previous reports as naphthalene, 1,8-cineole, camphor, and α-terpineol are the major bioactive found in plants over 2000 m, whereas 1,8-cineole, camphor, β-pinene, naphthalene, and α-amorphene are found in plants above 1100 m (Gvaham, et al., 2020). Our investigation established a qualitative and quantitative difference from the previously studied *S. hydrangea* in terms of essential oil composition. Many previous studies have approved that the chemical composition of *Salvia* species varied depending on the collecting period, the place of collection, distilled parts, genetic diversity, and also changes in biosynthetic pathways (Temel et al., 2016).

The phenolic contents of the infusion and methanolic extract obtained from the aerial parts of *S. hydrangea* were determined by LC−MS/MS. The detailed phenolic contents are demonstrated with retention time, UV_{max} spectra, and all MS data for each compound in Table 2. The chromatograms of the infusion and methanol extract are presented in Figs. 1 and 2, respectively.

As a consequence of the analysis, luteolin glycoside, rosmarinic acid, caffeoyl glucose, danshensu, coumaric acid, mediaresinol, diosmetin rutinoside, apigenin glycoside, luteolin acetyglycoside, and apigenin acetylglycoside, were identified by comparison of the retention times and mass spectra of references. The major compounds were detected in the methanol extract as rosmarinic acid while luteolin glycoside was the predominant phenolic constituent in the infusion. Both samples contained mediaresinol, luteolin glycoside, rosmarinic acid. However, caffeoyl glucose, danshensu, diosmetin rutinoside, and apigenin glycoside were only observed in the methanol extract. On the other hand, coumaric acid, luteolin acetylglycoside, and apigenin acetylglycoside were found in the infusion of *S. hydrangea*. Additionally, the flavonoids identified in both samples are in glycosidic form. It is well-known that the glycoside link in the

Table 2
The phenolic compositions of infusion and methanol extract obtained from *S. hydrangea*.

RT	$[M-H]^-$	MS^2	Compound	Extract	Ref.
2,9	341	179,161,143	Caffeoyl glycoside	M	Chen et al. (2011)
5,1	197	179,135,123	Danshensu	M	Dong et al. (2013)
6,2	163	119	Coumaric acid	I	Xie et al. (2014)
6,7	387	207, 163	Mediaresinol	I,M	Hossain et al. (2010)
10,0	447	285	Luteolin glycoside	I, M	Cvetkovikj et al. (2013)
11,2	607	299,284	Smilar to Diosmetin rutinoside	M	Roowi and Crozier (2011), Al-Qudah et al. (2014)
11,7	431	268,241, 171	Apigenin glycoside	M	Cvetkovikj et al. (2013)
12,2	489	285	Luteolin acetylglycoside	I	Lin and Harnly (2010)
12,9	359	197, 179, 161, 135	Rosmarinic acid	I, M	Kontogianni et al. (2013)
13,9	473	269	Apigenin acetylglycoside	I	Kontogianni et al. (2013)

*R_t: Retention time; I: infusion, M: methanol extract.

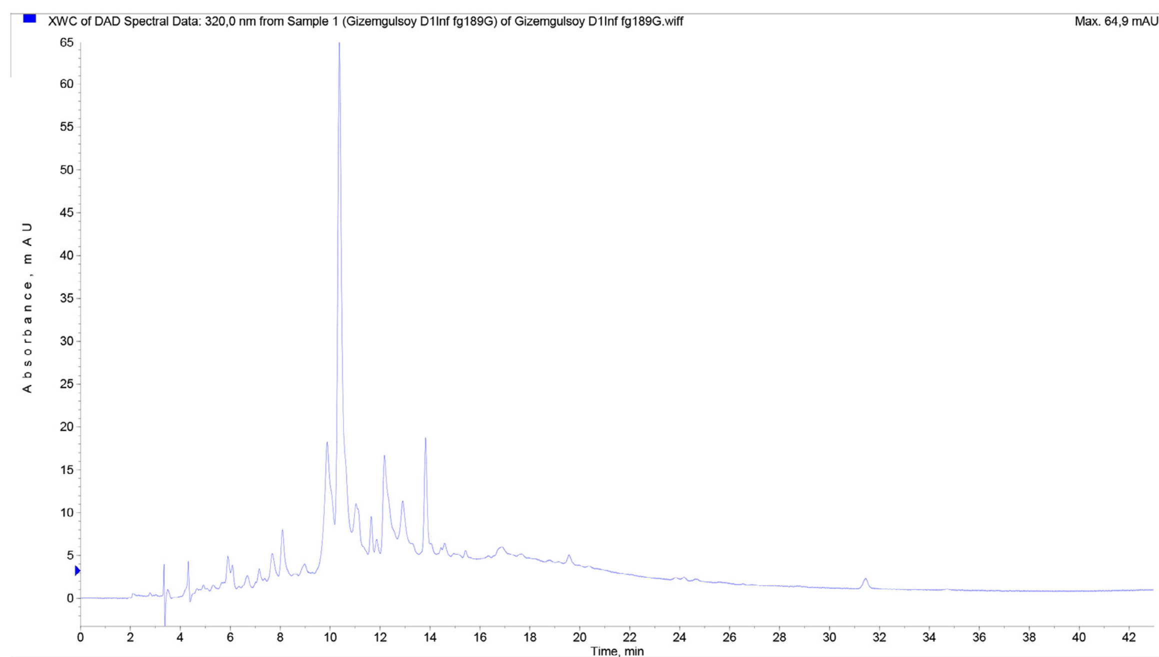

Fig. 1. LC chromatogram of infusion from aerial parts of *S. hydrangea*.

structure increases the solubility of flavonoids, hence it is reasonable to detect these flavonoids in the infusion of *S. hydrangea*.

The total phenolic content of samples was measured spectrophotometrically, using the Folin–Ciocalteau method, as reported previously (Ozsoy et al., 2008). The total phenolic contents of each sample are demonstrated in Table 3. According to the results, the infusion and methanol extract of *S. hydrangea* contained almost similar amount of phenolics with the highest values among the investigated extracts.

Previous studies have described phenolic compositions of the many different *Salvia* species. These reports indicated that *Salvia* species are rich in phenolics and also show variability in terms of the phenolic groups. Among these substances, rosmarinic acid is a chief component when comparing many *Salvia* species. The phenolic compounds isolated from several sages have demonstrated remarkable pharmacological activity and also potential as great resources for different industrial purposes (Li et al., 2015).

3.3. Antioxidant activity

To examine the antioxidant capacity of the samples, it is preferred to employ more than one assay to explain the action of different pathways and to provide a more comprehensive evaluation of their antioxidant properties. In the current study, the antioxidant potential of *S. hydrangea* was evaluated using three complementary biochemical methods, namely DPPH· free radical scavenging, FRAP, and CUPRAC activity methods. The results are demonstrated in Table 3.

An antioxidant relationship can be described by the action of free radical scavenging (DPPH•), which is one of the numerous methods used to determine antioxidant activity (Maltaş et al., 2010). In order to assess the free radical scavenging effects of different solvent extracts, DPPH• radical assay, the most frequently used method was applied. Among the samples studied, methanol (60.6 ± 0.8 mg AAE/g extract) and infusion (49.9 ± 2.97 mg AAE/g extract) extracts showed the strongest free radical scavenging activity in the DPPH· test compared to other extracts. The DPPH· method is very important in interpreting the role of hydrophilic antioxidants (Büyüktuncel, 2013). Therefore, it is thought that infusion and methanol extracts, which have polar compounds compared to other extracts, exhibit strong free radical scavenging activity. The weakest effects were observed in the chloroform extracts.

The reducing power potential of the extracts obtained from the aerial parts of *S. hydrangea* have been carried out using CUPRAC and FRAP methods. The CUPRAC test was used to reveal the cupric reducing antioxidant capacity of the infusion, *n*-hexane, chloroform, and methanol extracts. In the current investigation, the infusion,

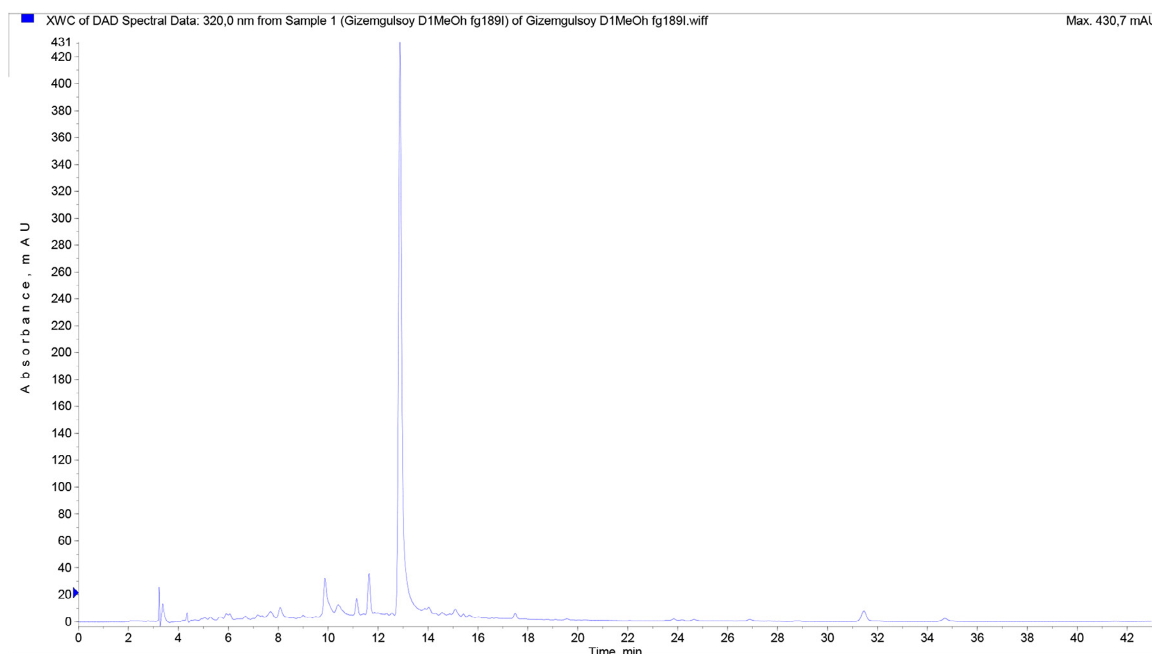

Fig. 2. LC chromatogram of methanol extract from aerial parts of *S. hydrangea*.

Table 3
Antioxidant activity of samples from *S. hydrangea*.

Samples	Phenolics (mg GAE/g extract)	DPPH• (mg AaE/g extract)	CUPRAC(mMtrolox/mg extract)	FRAP assay (mM Fe^{2+}/mg extract)
n-Hexane	41 ± 2.0	47.7 ± 2.5	0.094 ± 0.004	0.214 ± 0.047
Chloroform	12 ± 0.6	33.2 ± 2.2	0.101 ± 0.001	0.109 ± 0.007
Methanol	122 ± 1.1	60.6 ± 0.8	0.104 ± 0.001	0.025 ± 0.001
Infusion	121 ± 0.9	49.9 ± 2.97	0.108 ± 0.002	0.050 ± 0.001
BHT				1.1 ± 0.12
BHA			1.622 ± 0.12	

Values are mean of triplicate determination (*n* = 3) ± standard deviation; a *P* <0.05 compared with the positive control, bP <0.01 compared with positive control, cP <0.001 compared with positive control.

chloroform, and methanol extracts had almost equal cupric reducing properties. The highest copper (II) reducing antioxidant capacity was detected in the infusion while the lowest effects were observed in the *n*-hexane extract. On the other hand, *n*-hexane extract exhibited the strongest reducing power ability in FRAP assay compared to the other studied samples. Unfortunately, none of the investigated samples were found to be as powerful as the reference compounds. The CUPRAC and FRAP methods are very suitable for the determination of hydrophilic and lipophilic antioxidants. The FRAP method is non-specific and any compound with a redox potential of less than 0.70 V, which does not show antioxidant properties *in vivo*, can reduce iron. In addition, thiol antioxidants such as glutathione cannot be measured by the FRAP method. The CUPRAC method is fast enough to oxidize thiol-type antioxidants (Büyüktuncel, 2013). In this study, it is thought that the different CUPRAC and FRAP activity results of the extracts are due to these advantages and disadvantages of the methods.

Various antioxidants are found in aromatic and medicinal plants. Among them, flavonoids and phenolic acids possess strong antioxidant properties as confirmed by the literature. Due to the hydrogen-donating characteristic of these compounds, a broad range of antioxidant effects was observed depending on a variety of their chemical structure. Numerous studies revealed that a large number of phenolic chemical components in *Salvia* species was most likely responsible for the plant's considerable antioxidant effect (Koşar et al., 2011; Firuzi et al., 2013; Al-Qudah et al., 2014; Bahadori and Mirzaei, 2015; Zengin et al., 2018). Rosmarinic acid is the most prevalent phenolic acid found in *Salvia* species, and several studies have demonstrated its high antioxidant potential (Petersen and Simmonds, 2003). Furthermore, the current investigation demonstrates that the existence of rosmarinic acid in the infusion and methanolic extract of *S. hydrangea* has a significant potential for antioxidant effect comparing the other extracts. However, the relevance of strong antioxidant effect is not only due to the existence of rosmarinic acid but also the occurrence of synergistic effect with other phenolic substances.

3.4. Anticholinesterase activity

Water, *n*-hexane, chloroform, and methanol extracts were screened for their acetylcholinesterase enzyme inhibition potential compared to galantamine, as a reference compound. The results of anticholinesterase activities are given in Table 4. According to the applied Ellman method, the infusion showed excellent cholinesterase inhibitor effects (92.825±0.582 μg/mL) which was almost equal to standard (94.52±0.14 μg/mL). Furthermore, methanol extract (89.460±3.257 μg/mL) also showed notable inhibition effects on acetylcholinesterase enzyme. Notwithstanding, the chloroform extract exhibit poor effects to inhibit the enzyme while no anticholinesterase activity was measured in the *n*-hexane extract.

In a recent study, Şenol et al. (2010) investigated the anticholinesterase and antioxidant potential of 55 taxa of Turkish sages including the *S. hydrangea*. The dichloromethane, ethyl acetate, and methanol extracts were prepared from aerial parts and were scanned for their enzyme inhibitor potential. Unfourtanetly, only dichloromethane

Table 4
Anticholinesterase activity of the extracts from *S. hydrangea*.

Samples	Enzyme inhibition (%) (500 μg/mL)
n-Hexane	–
Chloroform	32.889±0.940
Methanol	89.460±3.257
Infusion	92.825±0.582
Galanthamine	94.52±0.14

Values are mean of triplicate determination (n = 3) ±standard deviation; * P <0.05 compared with the positive control, ** P <0.01 compared with positive control.

and ethyl acetate extracts of *S. fruticosa*, and ethyl acetate extract of *S. pomifera* exhibited moderate inhibition. Nevertheless, none of the methanol extracts showed any anticholinesterase effects. In another study, several extracts such as dichloromethane, methanol, and water from *S. euphratica* Montbret and Aucher ex Bentham var. *leiocalycina* (Rech. fil.) Hedge, *S. verticillata* subsp. *amasica* Freyn. & Bornm. and *S. blepharochlaena* Hedge and Hub. were evaluated for their anticholinesterase activity as well as antioxidant capacity. Among the studied samples, water extracts were found to be rich in phenolics and also possessed strong antioxidant properties. The dichloromethane extracts of three *Salvia* species investigated showed the highest effects (Zengin et al., 2018). Extraction methods/solvents are known to be an important factor in the extraction of compounds from medicinal plants (Zhang et al., 2018). In this study conducted by Zengin et al., dichloromethane, methanol and water extracts were prepared separately by maceration method. In another study, each plant was extracted with dichloromethane, ethyl acetate followed by methanol, using the maceration method, respectively. In our study, unlike other studies, sequential extraction was performed with the Soxhlet method and the compounds were classified according to their polarity. The reason why the findings obtained in our study are different from previous studies suggests that it is due to the extraction method/solvents of plants.

Many studies have revealed that phenolic compounds have varying antioxidant, antimicrobial, and antitumor bioactivities according to their functional groups (Asadi et al., 2010; Şenol et al., al.,2010; Orhan et al., 2012). These functional groups determine/contribute their antioxidant properties and protect cellular biomolecules against oxidative damage, reducing the risk of degenerative disease due to oxidative stress (Zeb et al., 2014; Bibi Sadeer et al., 2020). In Anatolian folk medicine, *Salvia* species have been used to enhance cognitive ability (Baytop, 1999). Thus, the members of the genus have been investigated by several researchers particularly for *in vitro* anticholinesterase capability of the responsible component/s (Perry et al., 1996; Şenol et al., 2010). According to previous reports, the remarkable inhibitory effects can be attributed to a wide range of different chemical components, particularly phenolic substances.

3.5. Antibacterial and antifungal screening of the samples

To compare samples, different solvent extracts prepared from aerial parts of *S. hydrangea* were evaluated using the broth microdilution method on a panel of six pathogenic bacteria, and seven yeasts using the CLSI guidelines. The antibacterial and anticandidal effects of the samples are given in Tables 5 and 6, respectively. In comparison with reference substances, the samples demonstrated strong to modest antimicrobial activity against the tested pathogenic bacteria and the yeasts.

The EO and methanol extract of *S. hydrangea* obtained from the aerial parts of the plant exhibited considerable inhibitor effects with 125 (μg/mL) MIC values against *S. aureus*. This Gram-positive bacteria was detected as the most susceptible strain against the samples. Among the samples studied, only the essential oil was effective on *E. coli* at a dose of 500 μg/mL, while none of the other samples were found active against these resistant bacteria panels. *P. aeruginosa* was the most resistant bacterial strain against the screened samples, none of the extracts showed inhibitory properties.

As to the anticandidal activity, *n*-hexane and chloroform extracts expressed remarkable inhibitor potential particularly, against *C. tropicalis, C. utilis*, and *C. krusei* ranging from 15 μg/mL to 62 μg/mL MIC values. Additionally, *C. utilis* was the most susceptible yeast strain to *n*-hexane extract with 15 μg/mL MIC values. The EO of *S. hydrangea* showed moderate to low anticandidal effects between the concentrations of 125 μg/mL to 500 μg/mL against all the studied *Candida* yeasts. Infusion and methanol extract of *S. hydrangea* exhibited antifungal properties with the same concentrations range against only four investigated *Candida* strains.

Table 5
Antibacterial effects of *S. hydrangea* extracts (MIC, mg/mL).

Bacteria panel	Strain no	*n*-Hexane	Chloroform	Methanol	Infusion	EO	St-1 (μg/mL)	St-2 (μg/mL)
E. coli	NRRL B-3008	>2	>2	>2	>2	0.5	2	1
Staphylococcus aureus	ATCC 6538	0.5	0.25	0.125	1	0.125	0.1	0.5
Pseudomonas aeruginosa	ATCC 27853	>2	>2	>2	>2	>2	64	32
S. typhimurium	ATCC 13311	0.5	1	0.25	>2	1	1	1
Serratia marcescens	NRRL B-2544	0.5	1	0.5	>2	1	32	8
Klebsiella pneumoniae	NCTC 9633	>2	>2	0.5	1	0.5	0.5	2

St-1: ampicillin; St-2: chloramphenicol.

Table 6
Anticandidal effects of *S. hydrangea* extracts (MIC, mg/mL).

Candida panel	Strain no	*n*-Hexane	Chloroform	Methanol	Infusion	EO	St-3 (μg/mL)	Std-4 (μg/mL)
Candida albicans	ATCC 10231	0.25	0.25	>2	>2	0.25	0.25	0.06
Candida albicans	ATCC 90028	0,5	0.25	>2	>2	0.25	0.5	0.03
Candida tropicalis	NRRL Y-12968	0.125	0.25	>2	>2	0.5	0.25	0.03
Candida tropicalis	ATCC 750	0.062	0.062	0.25	0.5	0.125	0.25	0.03
Candida utilis	NRRL Y-900	0.015	0.031	0.125	0.5	0.125	0.06	0.06
Candida parapsilosis	ATCC 22019	0.125	0.125	0.25	0.5	0.125	0.25	0.03
Candida krusei	ATCC 6258	0.062	0.125	0.25	0.5	0.125	0.5	0.06

St-3: amphotericin-B; St-4: ketoconazole.

4. Conclusion

Natural products have always provided a significant contribution to the development of modern medicine, and still continue to play a crucial role in the discovery of new medications today. As the oxidative stress is an important milestone in neurodegenerative disease pathogenesis, sages have been shown to be beneficial for therapeutic purposes due to their notably antioxidant properties as well as strong inhibitor effects on cholinesterase enzymes. *S. hydrangea* may be of interest to the pharmaceutical industries and the current study has highlighted the biological potential as well as phytochemical characterization. In the present work, the main compound in the EO of *S. hydrangea* was determined as camphor. Furthermore, the infusion and methanol extract of *S. hydrangea* were shown to have luteolin glycoside and rosmarinic acid as major phenolic components, respectively. The remarkable biological effects of *S. hydrangea* might be attributed to the presence of these compounds and also their synergistic effects with other secondary metabolites. Moreover, the infusion of *S. hydrangea* showed promising anticholinesterase action for addressing neurological issues and seems worthy of more research. A connection between the potential for biological activity and the phytochemical composition of the samples is crucial to help to identify the molecules that are responsible for the effects. Our findings revealed the need for more detailed future studies to identify different bioactivity of the samples as well as to prove their bioavailability and mechanisms.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

References

Afonso, A.F., Pereira, O.R., Cardoso, S.M., 2021. *Salvia* species as nutraceuticals: focus on antioxidant, antidiabetic and anti-obesity properties. Appl. Sci. 11 (20), 9365.

Apak, R., Güçlü, K., Özyürek, M., Karademir, S.E., 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method". J. Agric. Food Chem. 52 (26), 7970–7981.

Al-Qudah, M.A., Al-Jaber, H.I., Abu Zarga, M.H., Abu Orabi, S.T., 2014. Flavonoid and phenolic compounds from *Salvia palaestina* L. growing wild in Jordan and their antioxidant activities. Phytochemistry 99 (1), 115–120.

Al-Bakri, A.G., Othman, G., Afifi, F.U., 2010. Determination of the antibiofilm, antiadhesive, and anti-MRSA activities of seven *Salvia* species. Pharmacogn Mag. 6 (24), 264.

Akkol, E.K., Orhan, I.E., Yeşilada, E., 2012. Anticholinesterase and antioxidant effects of the ethanol extract, ethanol fractions and isolated flavonoids from Cistus laurifolius L. leaves. Food Chem. 131 (2), 626–631.

Asadi, S., Ahmadiani, A., Esmaeili, M.A., Sonboli, A., Ansari, N., Khodagholi, F., 2010. *In vitro* antioxidant activities and an investigation of neuroprotection by six *Salvia* species from Iran: a comparative study. Food Chem. Toxicol. 48 (5), 1341–1349.

Askari, S.F., Avan, R., Tayarani-Najaran, Z., Sahebkar, A., Eghbali, S., 2021. Iranian *Salvia* species: a phytochemical and pharmacological update. Phytochemistry 183, 112619.

Bahadori, M.B., Mirzaei, M., 2015. Cytotoxicity, antioxidant activity, total flavonoid and phenolic contents of *Salvia urmiensis* Bunge and *Salvia hydrangea* DC. ex Benth. Res. J. Pharmacogn. 2 (2), 27–32.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils−a review. Food Chem. Toxicol. 46 (2), 446–475.

Barazandeh, M.M., 2004. Volatile constituents of the oil of *Salvia hydrangea* DC. ex Benth. from Iran. J. Essent. Oil Res. 16 (1), 20–21.

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239 (1), 70–76.

Baser, K.H.C., 2002. Aromatic biodiversity among the flowering plant taxa of Turkey. Pure Appl. Chem. 74 (4), 527–545.

Baytop, T., 1999. Therapy With Medicinal Plants in Turkey, Past and Present, 2nd ed. Nobel Medical Publishing, Istanbul, pp. 142–144.

Bibi Sadeer, N., Montesano, D., Albrizio, S., Zengin, G., Mahomoodally, M.F., 2020. The versatility of antioxidant assays in food science and safety−Chemistry, applications, strengths, and limitations. Antioxidants 9, 709.

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

Büyüktuncel, E., 2013. Toplam fenolik içerik ve antioksidan kapasite tayininde kullanılan başlıca spektrofotometrik yöntemler. Marmara Pharm. J. 17 (2), 93–103.

Chen, H., Zhang, Q., Wang, X.M., Yang, J., Wang, Q., 2011. Qualitative analysis and simultaneous quantification of phenolic compounds in the Aerial Parts of *Salvia miltiorrhiza* by HPLC-DAD and ESI/MSn. Phytochem. Anal. 22 (3), 247–257.

Cvetkovikj, I., Stefkov, G., Acevska, J., Stanoeva, J.P., Karapandzova, M., Stefova, M., Dimitrovska, A., Kulevanova, S., 2013. Polyphenolic characterization and chromatographic methods for fast assessment of culinary *Salvia* species from South East Europe. J. Chromatogr. A 1282 (0), 38–45.

CLSI (NCCLS) M27-A2, 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard−second edition.

CLSI (NCCLS) M7-A7, 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, Seventh Edition.

Delamare, A.P.L., Moschen-Pistorello, I.T., Artico, L., Atti-Serafini, L., Echeverrigaray, S., 2007. Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chem 100 (2), 603–608.

Demirci, B., Başer, K.H.C., Yıldız, B., Bahçecioğlu, Z., 2003. Composition of the essential oils of six endemic *Salvia* spp. from Turkey. Flavour Fragr. J. 18 (2), 116–121.

Dong, J., Zhu, Y., Gao, X., Chang, Y., Wang, M., Zhang, P., 2013. Qualitative and quantitative analysis of the major constituents in Chinese medicinal preparation Dan-Lou tablet by ultra high performance liquid chromatography/diode-array detector/quadrupole time-of-flight tandem mass spectrometry. J Pharm Biomed Anal 80, 50–62.

Ellman, G.L., Courtney, K.D., Andress, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7 (2), 88–95.

Esposito, E., Rotilio, D., Di Matteo, V., Di Giulio, C., Cacchio, M., Algeri, S., 2002. A review of specific dietary antioxidants and the effects on biochemical mechanisms related to neurodegenerative processes. Neurobiol. Aging 23 (5), 719–735.

Farimani, M.M., Taheri, S., Ebrahimi, S.N., Bahadori, M.B., Khavasi, H.R., Zimmermann, S., Brun, R., Hamburger, M., 2012. Hydrangenone, a new isoprenoid with an unprecedented skeleton from *Salvia hydrangea*. Org. Lett. 14 (1), 166–169.

Firuzi, O., Javidnia, K., Gholami, M., Soltani, M., Miri, R., 2010. Antioxidant activity and total phenolic content of 24 Lamiaceae species growing in Iran. Nat. Prod. Commun. 5 (2) 1934578X1000500219.

Firuzi, O., Miri, R., Asadollahi, M., Eslami, S., Jassbi, A.R., 2013. Cytotoxic, antioxidant and antimicrobial activities and phenolic contents of eleven *Salvia* species from Iran. Iran. J. Pharm. Res. IJPR 12 (4), 801.

Gibbons, S., 2005. Plants as a source of bacterial resistance modulators and anti-infective agents. Phytochem. Rev. 4 (1), 63–78.

Guner, A., Aslan, S., Ekim, T., Vural, M., Babaç, M., 2012. Türkiye Bitkileri Listesi (Damarlı Bitkiler). Flora Araştırmaları Derneği ve Nezahat Gökyigit Botanik Bahcesi ve Flora Arastırmaları Dernegi Yayını (in Turkish), Istanbul.

Gürdal, B., Kültür, Ş., 2013. An ethnobotanical study of medicinal plants in Marmaris (Muğla, Turkey). J. Ethnopharmacol. 146 (1), 113–126.

Ghavam, M., Manca, M.L., Manconi, M., Bacchetta, G., 2020. Chemical composition and antimicrobial activity of essential oils obtained from leaves and flowers of *Salvia hydrangea DC. ex Benth*. Sci. Rep. 10 (1), 1–10.

Hossain, M.B., Rai, D.K., Brunton, N.P., Martin-Diana, A.B., Barry-Ryan, C., 2010. Characterization of phenolic composition in Lamiaceae spices by LC-ESI-MS/MS. J. Agric. Food Chem. 58 (19), 10576–10581.

Kahnamoei, M.B., Tabefam, M., Ebrahimi, S.N., Danton, O., Hamburger, M., Farimani, M.M., 2019. Chemical constituents from the ethyl acetate extract of *Salvia hydrangea*. Nat. Prod. Commun. 14 (6) 1934578X19848852.

Kontogianni, V.G., Tomic, G., Nikolic, I., Nerantzaki, A.A., Sayyad, N., Stosic-Grujicic, S., Stojanovic, I., Gerothanassis, I.P., Tzakos, A.G., 2013. Phytochemical profile of *Rosmarinus officinalis* and *Salvia officinalis* extracts and correlation to their antioxidant and anti-proliferative activity. Food Chem. 136 (1), 120–129.

Koşar, M., Göger, F., Başer, K.H.C., 2008. *In vitro* antioxidant properties and phenolic composition of *Salvia virgata* Jacq. from Turkey. J. Agric. Food Chem. 56 (7), 2369–2374.

Koşar, M., Göger, F., Başer, K.H.C., 2011. *In vitro* antioxidant properties and phenolic composition of *Salvia halophila* Hedge from Turkey. Food Chem. 129 (2), 374–379.

Kwapong, A.A., Stapleton, P., Gibbons, S., 2019. Inhibiting plasmid mobility: the effect of isothiocyanates on bacterial conjugation. Int. J. Antimicrob. Agents 53 (5), 629–636.

Kotan, R., Kordali, S., Cakir, A., Kesdek, M., Kaya, Y., Kilic, H., 2008. Antimicrobial and insecticidal activities of essential oil isolated from Turkish *Salvia hydrangea* DC. ex Benth. Biochem. Syst. Ecol. 36 (5), 360–368.

Li, B., Zhang, C., Peng, L., Liang, Z., Yan, X., Zhu, Y., Liu, Y., 2015. Comparison of essential oil composition and phenolic acid content of selected *Salvia* species measured by GC−MS and HPLC methods. Ind. Crops Prod. 69, 329–334.

Lin, L.Z., Harnly, J.M., 2010. Identification of the phenolic components of chrysanthemum flower (*Chrysanthemum morifolium* Ramat). Food Chem. 120 (1), 319–326.

Lopresti, A.L., 2017. *Salvia* (Sage): a review of its potential cognitive-enhancing and protective effects. Drugs R D 17, 53–64.

Maltaş, E., Uysal, A., Yildiz, S., Durak, Y., 2010. Evaluation of antioxidant and antimicrobial activity of *Vitex agnus-castus* L. Fresenius Environ. Bull. 19, 3094–3099.

Orhan, I.E., Aslan, S., Kartal, M., Şener, B., Başer, K.H.C., 2008. Inhibitory effect of Turkish *Rosmarinus officinalis* L. on acetylcholinesterase and butyrylcholinesterase enzymes. Food Chem. 108 (2), 663–668.
Lu, Y., Foo, L.Y., 2002. Polyphenolics of Salvia-a review. Phytochemistry 59 (2), 117–140.
Orhan, I.E., Senol, F.S., Ozturk, N., Akaydin, G., Sener, B., 2012. Profiling of *in vitro* neurobiological effects and phenolic acids of selected endemic *Salvia* species. Food Chem. 132 (3), 1360–1367.
Ozsoy, N., Can, A., Yanardağ, R., Akev, N., 2008. Antioxidant activity of *Smilax excelsa* L. leaf extracts. Food Chem. 110 (3), 571–583.
Perry, N., Court, G., Bidet, N., Court, J., Perry, E., 1996. European herbs with cholinergic activities: potential in dementia therapy. Int. J. Geriatr. Psychiatry 11 (12), 1063–1069.
Petersen, M., Simmonds, M.S., 2003. Rosmarinic acid. Phytochemistry 62 (2), 121–125.
Poulios, E., Giaginis, C., Vasios, G.K., 2020. Current state of the art on the antioxidant activity of sage (*Salvia* spp.) and its bioactive components. Planta Med. 86 (4), 224–238.
Righi, N., Boumerfeg, S., Deghima, A., Fernandes, P.A., Coelho, E., Baali, F., Cardosa, S., Coimbra, R., Baghiani, A., 2021. Phenolic profile, safety assessment, and anti-inflammatory activity of *Salvia verbenaca* L. J. Ethnopharmacol. 272, 113940.
Roowi, S., Crozier, A., 2011. Flavonoids in tropical Citrus species. J. Agric. Food Chem. 59 (22), 12217–12225.
Sharifi-Rad, M., Ozcelik, B., Altın, G., Daşkaya-Dikmen, C., Martorell, M., Ramírez-Alarcón, K., Leal, A.L., 2018. *Salvia* spp. plants-from farm to food applications and phytopharmacotherapy. Trends Food Sci. Technol. 80, 242–263.
Sharma, Y., Fagan, J., Schaefer, J., 2019. Ethnobotany, phytochemistry, cultivation and medicinal properties of Garden sage (*Salvia officinalis* L.). J. Pharmacogn. Phytochem. 8, 3139–3148.
Sairafianpour, M., Bahreininejad, B., Witt, M., Ziegler, H.L., Jaroszewski, J.W., Staerk, D., 2003. Terpenoids of *Salvia hydrangea*: two new, rearranged 20-norabietanes and the effect of oleanolic acid on erythrocyte membranes. Planta Med. 69 (9), 846–850.
Shahidi, F., 2000. Antioxidants in food and food antioxidants. Food Nahrung 44, 158–163.
Shahidi, F., Zhong, Y., 2010. Novel antioxidants in food quality preservation and health promotion. Eur. J. Lipid Sci. Technol. 112, 930–940.
Shojaeifard, Z., Hemmatinejad, B., Jassbi, A.R., 2021. Chemometrics-based LC-UV-ESIMS analyses of 50 Salvia species for detecting their antioxidant constituents. J. Pharm. Biomed. Anal. 193, 113745.
Stešević, D., Ristić, M., Nikolić, V., Nedović, M., Caković, D., Šatović, Z., 2014. Chemotype diversity of indigenous Dalmatian sage (*Salvia officinalis* L.) populations in Montenegro. Chem. Biodivers. 11 (1), 101–114.
Toplan, G., Kurkcuoglu, M., Goger, F., İşcan, G., Ağalar, H.G., Mat, A., Başer, K.H.C., Koyuncu, M., Sarıyar, G., 2017. Composition and biological activities of *Salvia veneris* Hedge growing in Cyprus. Ind. Crops Prod. 97, 41–48.
Şenol, F.S., Orhan, I., Celep, F., Kahraman, A., Doğan, M., Yilmaz, G., Şener, B., 2010. Survey of 55 Turkish Salvia taxa for their acetylcholinesterase inhibitory and antioxidant activities. Food Chem. 120 (1), 34–43.
Temel, H.E., Demirci, B., Demirci, F., Celep, F., Kahraman, A., Doğan, M., Başer, K.H.C., 2016. Chemical characterization and anticholinesterase effects of essential oils derived from Salvia species. J. Essent. Oil Res. 28 (4), 322–331.
Tundis, R., Bonesi, M., Pugliese, A., Nadjafi, F., Menichini, F., Loizzo, M.R., 2015. Tyrosinase, acetyl-and butyryl-cholinesterase inhibitory activity of *Stachys lavandulifolia* Vahl (Lamiaceae) and its major constituents. Rec. Nat. Prod. 9 (1), 81.
Wei, F., Jinglou, C., Yaling, C., Yongfang, L., Liming, C., 2010. Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from Parathelypteris nipponica (Franch.etSav.) Ching. J. Ethnopharmacol. 130 (3), 521–528.
Wu, Y.B., Ni, Z.Y., Shi, Q.W., Dong, M., Kiyota, H., Gu, Y.C., Cong, B., 2012. Constituents from Salvia species and their biological activities. Chem. Rev. 112 (11), 5967–6026.
Xie, Y., Xiao, X., Luo, J., Fu, C., Wang, Q., Wang, Y., Liang, Q., Luo, G., 2014. Integrating qualitative and quantitative characterization of traditional Chinese medicine injection by high-performance liquid chromatography with diode array detection and tandem mass spectrometry. J. Sep. Sci. 37 (12), 1438–1447.
Xu, J., Wei, K., Zhang, G., Lei, L., Yang, D., Wang, W., Han, Q., Xia, Y., Bi, Y., Yang, M., Li, M., 2018. Ethnopharmacology, phytochemistry, and pharmacology of Chinese Salvia species: a review. J. Ethnopharmacol. 225, 18–30.
Zare, S., Hatam, G., Firuzi, O., Bagheri, A., Chandran, J.N., Schneider, B., Jassbi, A.R., 2021. Antileishmanial and pharmacophore modeling of abietane-type diterpenoids extracted from the roots of Salvia hydrangea. J. Mol. Struct. 1228, 129447.
Zeb, A., Sadiq, A., Ullah, F., Ahmad, S., Ayaz, M., 2014. Investigations of anticholinestrase and antioxidant potentials of methanolic extract, subsequent fractions, crude saponins and flavonoids isolated from *Isodon rugosus*. Biol. Res. 47 (1), 1–10.
Zengin, G., Llorent-Martínez, E.J., Fernández-de Córdova, M.L., Bahadori, M.B., Mocan, A., Locatelli, M., Aktumsek, A., 2018. Chemical composition and biological activities of extracts from three Salvia species: *S. blepharochlaena*, *S. euphratica* var. *leiocalycina*, and *S. verticillata* subsp. *amasiaca*. Ind. Crops Prod. 111, 11–21.
Zhang, Q.W., Lin, L.G., Ye, W.C., 2018. Techniques for extraction and isolation of natural products: a comprehensive review. Chin. Med. 13 (1), 1–26.