ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/306330675

Salvia nemorosa L.: A novel source of bioactive agents with functional connections

Article *in* LWT- Food Science and Technology · January 2017

DOI: 10.1016/j.lwt.2016.08.048

CITATION
1

READS
298

7 authors, including:

Mir Babak Bahadori
Tabriz University of Medical Sciences
43 PUBLICATIONS **180** CITATIONS

SEE PROFILE

Behvar Asghari
Imam Khomeini International University
33 PUBLICATIONS **229** CITATIONS

SEE PROFILE

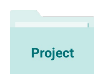
Gokhan Zengin
Selcuk University
195 PUBLICATIONS **749** CITATIONS

SEE PROFILE

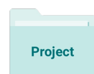
Cengiz Sarikurkcu
T.C. Süleyman Demirel Üniversitesi
67 PUBLICATIONS **1,113** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Innovative extraction techniques from biological and complex samples View project



Anticancer properties of natural compounds and their mechanism of action. View project

All content following this page was uploaded by Mir Babak Bahadori on 24 August 2016.

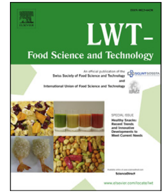
The user has requested enhancement of the downloaded file.

LWT - Food Science and Technology 75 (2017) 42–50

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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



Salvia nemorosa L.: A novel source of bioactive agents with functional connections

Mir Babak Bahadori [a], Behvar Asghari [b], Leila Dinparast [c], Gokhan Zengin [d, *], Cengiz Sarikurkcu [e], Mahdi Abbas-Mohammadi [f], Shahram Bahadori [g]

[a] Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Department of Plant Production and Breeding Engineering, Faculty of Engineering and Technology, Imam Khomeini International University, Qazvin, Iran
[c] Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[d] Department of Biology, Science Faculty, Selcuk University, Konya, Turkey
[e] Suleyman Demirel University, Faculty of Pharmacy, Department of Analytical Chemistry, Isparta, Turkey
[f] Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran
[g] Department of Pharmacognosy, Faculty of Pharmacy, Urmia University of Medical Sciences, Urmia, Iran

ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form
2 July 2016
Accepted 20 August 2016
Available online 21 August 2016

Keywords:
Salvia
Acetylcholinesterase
Diabetes mellitus
Alzheimer's disease
Phenolics

Chemical compounds studied in this article:
Rosmarinic acid (CID: 5281792)
Gallic acid (PubChem CID: 370)
DPPH (PubChem CID: 2735032)
BHT (PubChem CID: 31404)
Acarbose (PubChem CID: 41774)
Quercetin (PubChem CID: 5280343)
Xanthine (PubChem CID: 1188)
Podophyllotoxin (PubChem CID: 10607)
Gentamicin (PubChem CID: 3467)
Nystatin (PubChem CID: 6433272)

ABSTRACT

Salvia species are used as food and medicinal plants all around the world. In this study, chemical composition and antimicrobial, toxicity, antioxidant, and enzyme inhibitory activity of *Salvia nemorosa* were evaluated. Phenolic components were characterized by the HPLC-DAD and 11 components were identified, in which rosmarinic acid was detected as the major compound (7584 μg/g extract). Sixteen volatile components were determined in both leaves and flowers essential oils. Oxygenated sesquiterpenes were the major components. Methanolic extract showed the highest total phenolic and flavonoid contents (294 mg GAE/g and 117 mg QE/g extract, respectively) and the strongest DPPH radical scavenging activity (IC_{50}: 82 μg/mL). Plant samples showed moderate to high aldehyde oxidase, xanthine oxidase, acetylcholinesterase, and α-glucosidase inhibitory activity and moderate cytotoxicity. Also, EOs exhibited great antimicrobial potential (IZ: 8.8–33.7 mm). Our findings indicated that *S. nemorosa* may be useful for novel applications in functional food and pharmaceutical industries.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

In recent years, the essential oils and extracts of plants have gained remarkable interest in food industries (Albayrak & Aksoy, 2013; Ertas et al., 2015; Nikolic et al., 2014). There is a growing interest for medicinal plants as therapeutic agents against many illnesses such as Alzheimer's disease, diabetes mellitus, and oxidative damages (Zengin, Sarikurkcu, Aktumsek, & Ceylan, 2014). The genus *Salvia* is the most important and the largest genus of Lamiaceae family with over 1000 species (Farimani et al., 2015). *Salvia* species are traditionally used all around the world, from China to Europe and from Mexico to South Africa. Several *Salvia* species have economic importance because of their utilization as food, spices, and flavors (Bahadori, Valizadeh, & Farimani, 2016a). The name *Salvia* comes from Latin word “Salvare” which means “health” because of its wide range of medicinal effects (Ulubelen

* Corresponding author.
E-mail address: gokhanzengin@selcuk.edu.tr (G. Zengin).

http://dx.doi.org/10.1016/j.lwt.2016.08.048
0023-6438/© 2016 Elsevier Ltd. All rights reserved.

et al., 2002). *S. officinalis* is a common medicinal plant in Europe with antioxidant, anti-Alzheimer, and anti-inflammatory activity. It is important for its uses as flavor and preservative in food industries (Topcu, 2006). *S. sclarea* is used in wine production, cosmetic, and aromatherapy (Tkachuk & Shapoval, 1987; Topcu, 2006). Another valuable *Salvia* is *S. fruticosa* (Turkish sage). Its decoction is utilized to lower blood glucose level and blood pressure. Also, it is used for treatment of colds and digestive disorders (Perfumi, Arnold, & Tacconi, 1991). *S. hydrangea* is used in Iranian folk medicine for several purposes such as antispasmodic, sedative, carminative, anti-inflammatory, antileishmanial, and anthelmintic (Farimani et al., 2011, 2012). *S. miltiorrhiza* is well known in eastern Asia. It has many biological effects and is widely used for treatment of heart and liver disease (Nan et al., 2001). Other important *Salvia* species are *S. hispanica*, *S. divinorum*, and *S. yunnanensis* which are cultivated for their utilization in food, perfume, and pharmaceutical industries (Bahadori & Mirzaei, 2015; Wu et al., 2012).

Salvia nemorosa L. (syn. *S. sylvestris*), commonly known as wood sage, is growing in central Europe and Western Asia (Skala & Wysokinska, 2004). Leaves of *S. nemorosa* are traditionally used in Turkish folkloric medicine for stop bleeding when applied externally (Takeda et al., 1997). In Russia, *S. nemorosa* is utilized for much the same ailments as *S. officinalis*. In the Bulgarian traditional medicine, *S. nemorosa* is used mainly for treatment of stomach ache, diarrhea, hemorrhages, and furuncles (Daskalova, 2004).

There are many studies on isolation and characterization of bioactive constituents from *Salvia* species. This genus is rich in terpenoids and phenolic compounds (Bautista et al., 2013; Lu & Foo, 2002). In this sense, many reports focused on biological activities of extracts, purified compounds, and essential oils of these plants such as antimicrobial, cytotoxicity, anti-malarial, anti-tumor, cardiovascular, and anti-inflammatory (Farimani et al., 2013; Ulubelen, 2003). But unfortunately there are limited studies on nutritional value and the importance of the genus as a functional food. Due to the lack of information on this field, the present study was performed to explore the potential of *S. nemorosa* as a famous medicinal plant for its possible pharmaceutical and nutritional uses.

Herein, we report the chemical composition of the essential oils obtained from leaves and flowers of *S. nemorosa*. The phenolic composition of the methanolic extract was also analyzed by the HPLC-DAD. Several biological activities such as antimicrobial, antioxidant, cytotoxic, α-glucosidase, xanthine oxidase, aldehyde oxidase, and acetylcholinesterase inhibitory activities of the plant are presented.

2. Materials and methods

2.1. Plant material

The aerial parts of *Salvia nemorosa* L. were collected during the flowering stage in April 2014 from Urmia, West Azerbaijan province of Iran. The taxonomic identification of the plant was confirmed by Mr. Shahram Bahadori in Urmia School of Pharmacy Herbarium and a voucher specimen was deposited for the plant (USPH-103).

2.2. Preparation of essential oils

The essential oils of the flowers and leaves of the plant were extracted by hydrodistillation method using a Clevenger apparatus during three hours. The obtained oils were dried using anhydrous sodium sulphate. The extracted oils were kept in sealed glass vials at 4 °C in dark until analysis.

2.3. GC and GC/MS analysis

The GC-FID analysis was performed on an Agilent instrument employing the following conditions: flame ionization detector (FID), DB-5 column (30 m × 0.32 mm i.d., film thickness 0.25 μm), and Helium as carrier gas (1.1 mL/min). Temperatures of the detector and the injector were 250 and 240 °C, respectively. The column temperature programming was 60–250 °C at the rate of 5 °C/min, and at 250 °C for 10 min. The injection volumes were 1 μL.

The GC-MS analysis was carried out using a Thermoquest Finnigan instrument. The GC conditions were the same as above. The obtained oils were diluted in dichloromethane and 0.5 μL of the solution was injected into the GC-MS apparatus with a split ratio of 1:100. EI-MS measurements were carried out with ionization voltage of 70 eV. Mass range was scanned from 35 to 456 amu.

2.4. Identification and quantification of volatile compounds

Identification of compounds was performed based on the GC retention indices of the compounds relative to *n*-alkanes (C_6–C_{24}). Components of the oils were identified by matching of their mass spectra with the Wiley, NIST and Adams Mass Spectral libraries as well as by comparison of retention indices with those published in the literature (Adams, 2007). Relative area percentages obtained from GC were used for quantification of the components.

2.5. Extraction

The powdered aerial parts of *Salvia nemorosa* (50 g) were extracted successively by *n*-hexane, dichloromethane, and methanol using maceration method at room temperature (3 × 200 mL). The solvent of the extracts was removed *in vacuo* at 40 °C to obtain solventless *n*-hexane (2.8 g), DCM (2.5 g), and MeOH (6.2 g) extracts.

2.6. Determination of total phenolic (TPC) and flavonoid contents (TFC)

The total phenolic contents of the extracts were measured using Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999). The TPCs were expressed as mg of gallic acid equivalents per gram of dry weight of extracts (mg GAE/g) using the calibration curve of gallic acid. Measurement of the TFCs was carried out through the aluminium chloride colorimetric method (Lesjak et al., 2014). The TFCs were expressed as mg of quercetin equivalents per gram of dry weight of extracts (mg QE/g) using the calibration curve of quercetin. All analysis were carried out in triplicates and the results were expressed as mean ± standard error of the mean (SEM).

2.7. Antioxidant activity

We employed the DPPH and FRAP methods to evaluate the antioxidant activity of *S. nemorosa*. The DPPH radical scavenging potential of the extracts and EOs of the plant was determined using a previously published method (Ercetin, Senol, Orhan, & Toker, 2012). This is performed from the discoloration of DPPH solution. Butylated hydroxy toluene (BHT) was used as a standard antioxidant. The results were expressed as mean value ± SEM of three experiments. The ferric reducing capacity of the samples was also determined (Zhao et al., 2008). Increased reducing power of the tested samples was indicated with increased absorbance of the reaction mixture. Ascorbic acid was used as a standard reference and the results were expressed as observed absorbance values.

2.8. Brine shrimp larva assay

The Brine shrimp lethality assay (BSLA) was performed for the evaluation of cytotoxicity of the plant samples (Meyer et al., 1982). DMSO was used as untreated control and Berberine as a standard drug. The LC_{50} values were expressed as mean of triplicates ± SEM.

2.9. Xanthine oxidase inhibition activity

The xanthine oxidase inhibitory (XOI) activity of the EOs and extracts were determined using a Shimadzu spectrophotometer (2550 UV/VIS) (Pirouzpanah, Hanaee, Razavieh, & Rashidi, 2009). In this method, XO activity is monitored by measurement of uric acid production at 295 nm. At first step, all solutions were equilibrated at 37 °C (the enzyme was kept in ice). 50 mM xanthine as the substrate of XO was mixed with Sorenson's phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The reaction was started by the addition of the enzyme and the initial oxidation rate was measured up to 3 min. The reaction was also monitored in the presence of the essential oils and the extracts (15.6–500 μg/mL). The results were compared with the inhibitory effect of quercetin (0.31–2.5 μg/mL) as a positive control. Obtained IC_{50} values were expressed as mean ± SEM.

2.10. Aldehyde oxidase inhibition activity

We employed a spectroscopic method using a Shimadzu spectrophotometer (2550 UV/VIS) for evaluation of aldehyde oxidase inhibitory (AOI) activity of the plant samples (Hamzeh-Mivehroud, Rahmani, Rashidi, Feizi, & Dastmalchi, 2013). All solutions were incubated at 37 °C except the enzyme, which was stored in ice before addition to the reaction mixture. Phenanthridine as the substrate was mixed with Sorenson's phosphate buffer (pH 7.0) containing 0.1 mM of Ethylenediaminetetraacetic (EDTA) acid at 37 °C (at final concentration of 50 mM). The reaction was started by addition of the enzyme solution and monitored up to 3 min. The reaction was also monitored in the presence of plant samples (15.6–500 μg/mL) and the results were compared with the inhibitory effect of quercetin (0.31–2.5 μg/mL) as positive control.

2.11. Acetylcholinesterase inhibition activity

In vitro acetylcholinesterase inhibitory activities of the extracts and EOs were measured using a spectrophotometric method described by Ellman, Courtney, Andres, and Featherstone (1961) using a Shimadzu spectrophotometer (2550 UV/Vis). Briefly, 500 μL of sodium phosphate buffer (100 mM, pH 8.0), 150 μL of DTNB (3.5 mM), 150 μL of substrate (acetylthiocholine Iodide), and 150 μL inhibitor solution were added into the reaction mixture in a 1 mL cell and incubated for 15 min at 37 °C. Thereafter, the reaction was started by addition of 50 μL of enzyme. After immediate mixing of the reaction mixture, the changing of the absorbance was measured at 412 nm for 10 min. The inhibition rate of the samples on acetylcholinesterase was calculated by the following formula:

$$\text{Inhibition percentage} = \left[\left(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}\right) \times \Big/ \text{Absorbance}_{\text{control}}\right] \times 100$$

The IC_{50} values were calculated from inhibition curves (inhibitor concentration vs. percent of inhibition). Galantamine was used as a standard drug and the results were expressed as mean ± SEM of three independent experiments.

2.12. α-Glucosidase inhibition activity

The α-glucosidase inhibitory (AGI) potential of *S. nemorosa* was evaluated spectroscopically (Dinparast et al., 2016). Briefly, 20 μL of 0.5 unit/mL of α-glucosidase solution was mixed with 120 μL of 100 mM potassium phosphate buffer (pH 6.9) and 10 μL of the plant samples (25, 50, 100, and 200 μg/mL for *n*-hexane and dichloromethane extracts and 5, 10, 25, and 50 μg/mL for the EOs, methanol extract, and acarbose). The mixture was incubated at 37 °C for 15 min and the reaction was initiated by addition of 20 μL 5 mM 4-nitrophenyl-α-D-glucopyranoside (*p*NPG) in buffer. The reaction was incubated at 37 °C for another 15 min and was stopped by addition of 80 μL sodium carbonate solution (0.2 M). Finally, the absorbance of 4-nitrophenol released from *p*NPG was measured at 405 nm. The mixture without enzyme was used as a blank for correcting the background absorbance. The increasing of absorbance was compared with that of the control (buffer instead of sample solution) to calculate the inhibitory activity. Acarbose was used as a standard inhibitor. Inhibition percentage and IC_{50} values were calculated. Analyses were carried out in triplicates and the results were expressed as average value with SEM.

2.13. Antimicrobial activity

The antimicrobial activity of the extracts and essential oils of *S. nemorosa* was assessed against Two Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*), two Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*), and two fungi (*Aspergillus niger*, *Candida albicans*). Disk diffusion (NCCLS, 2006) and minimal inhibitory concentration (Ebrahimabadi, Mazoochi, Kashi, Djafari-Bidgoli, & Batooli, 2010) methods were employed for this purpose. Microbial strains were obtained from the Pasteur Institute of Iran. The diameter of inhibition zone (IZ) was measured in mm (including 6 mm diameter of paper disc). Gentamicin (10 μg/disc) and nystatin (50 IU) were used as standard drugs in the antibacterial and antifungal assays, respectively. The MIC values were determined according to micro-well dilution assay. The MICs were considered as the minimum concentration of the sample which could inhibit the growth of microorganisms.

2.14. Phenolic compounds characterization by reversed-phase high-performance liquid chromatography

Phenolic compounds were evaluated by the reversed-phase high performance liquid chromatography (RP-HPLC) (Shimadzu Scientific Instruments, Kyoto, Japan). Detection and quantification were carried out with a LC-10ADvp pump, a Diode Array Detector, a CTO-10Avp column heater, SCL-10Avp system controller, DGU-14A degasser, and SIL-10ADvp auto sampler (Shimadzu Scientific Instruments, Columbia, MD). Separations were conducted at 30 °C on Agilent® Eclipse XDB C-18 reversed-phase column (250 mm × 4.6 mm length, 5 μm particle size). Phenolic compositions of the extracts were determined by a modified method of Sarikurkcu, Uren, Tepe, Cengiz, and Kocak (2014). Gallic acid, protocatechuic acid, (+)-catechin, *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, (−)-epicatechin, syringic acid, vanillin, *p*-coumaric acid, ferulic acid, sinapic acid, benzoic acid, *o*-coumaric acid, rutin, naringin, hesperidin, rosmarinic acid, eriodictyol, *trans*-cinnamic acid, quercetin, luteolin, kaempferol, and apigenin were used as standards. Identification and quantitative analysis were done by comparison with standards. The amount of each phenolic compound was expressed as microgram per gram of extract using external calibration curves, which were obtained for each phenolic standard.

2.15. Statistical analysis

The experiments were carried out in triplicates. Obtained results were expressed as average value ± standard error mean. Statistical comparisons were evaluated using one-way ANOVA followed by Duncan's post-hoc test for multiple comparisons with control. Statistical analyses were carried out by SPSS 16.0 software. A value of $p < 0.05$ was considered to show statistical significance.

3. Results and discussion

3.1. Chemical composition of the essential oils

Essential oils are complex mixture of volatile compounds. These metabolites have wide usage in the fragrance, flavor, food, and pharmaceutical industries. The present work is the first comparative screening of the EO compositions of leaves (EO-L) and flowers (EO-F) of *S. nemorosa*. Chemical composition, content percentages, and retention indices of the volatile compounds of the EOs obtained from leaves and flowers of the plant are given in Table 1. EO-L yield was 0.1% v/w and EO-F yield was 0.2% v/w. The isolated oils had light yellow color and strong perfume. Sixteen components were identified in the oil extracted from flowers (91.3% of the total oil) and 8 components were identified in the oil extracted from leaves (94.0% of the total oil). Obtained oils from leaves and flowers contain similar major compounds which are spathulenol (57.8 and 23.0%) and caryophyllene oxide (28.2 and 45.0%, respectively). EO-L and EO-F are also dominated by oxygenated sesquiterpenes (86 and 68%, respectively). However, leden oxide, the third major compound in EO-F with 9.2% was found with 1.0% in EO-L. Furthermore, E-caryophyllene and humulene epoxide were relatively abundant in EO-F but were not detected in EO-L. There are some difficulties in classification of *Salvia nemorosa* sub species. Chemical composition of essential oils is a powerful tool for chemotaxonomic studies (Sonboli et al., 2013). Accordingly, our findings could be useful for exact classification of this species.

3.2. Total bioactive compounds

Flavonoids and phenolics have attracted considerable interest due to their wide biological activities such as antioxidant, antibacterial, cardioactive, and anti-cancer effects (Lazarova et al., 2015). Also, these phenolic compounds have toxicity potential against pathogens (Tuominen, 2013). The total phenolic contents (TPC) of the extracts of *S. nemorosa* are presented in Table 2. The methanolic extract exhibited the highest TPC (294.9 ± 10.9 mg GAE/g). This amount of phenolic compounds is comparable with the values reported in the previous studies (Loizzo et al., 2014) but is higher compared to 8 *Salvia* species reported by Tosun et al. (2009). Regarding flavonoids, in a similar trend with TPC, the methanolic extract had the highest TFC between the extracts. The previous works on *Salvia* species using similar procedures, reported flavonoid content from 7 to 36 mg QE/g dried extract for 9 *Salvia* species (Loizzo et al., 2014). Thus, *S. nemorosa* appears to have higher flavonoid contents than the previously studied *Salvia* plants. Our findings showed that the yield of the extraction of phenolic and flavonoid compounds depend on the polarity of used solvents. Accordingly, polar fractions of leaves and flowers of *S. nemorosa* could be rich sources of valuable phenolic constituents with high nutritional and health benefits for consumers. This is in agreement with the fact that phenolic compounds are better extracted by polar solvents like MeOH and water (Zengin et al., 2015). Also, the results indicated that flavonoids are a major portion of phenolic compounds in all of the tested extracts.

3.3. Antioxidant activity

Antioxidant compounds and radical scavengers are valuable

Table 1
Chemical composition of the essential oils from leaves and flowers of *Salvia nemorosa*.

No	Compounds	RT[a]	RI	Concentration (%)		Identification method
				Flowers	Leaves	
1	2*E*-Hexenal	3.25	863	0.1	–	RI, MS
2	α-Thujene	4.39	924	0.9	–	RI, MS
3	Sabinene	5.27	971	0.9	–	RI, MS
4	α-Terpinene	6.16	1016	0.1	–	RI, MS
5	*o*-Cymene	6.33	1023	0.8	–	RI, MS
6	γ-Terpinene	7.12	1057	0.3	–	RI, MS
7	Terpinene-4-ol	10.12	1180	1.0	–	RI, MS
8	Carvacrol	13.29	1309	–	1.2	RI, MS
9	E-Caryophyllene	16.45	1424	7.0	–	RI, MS
10	α-Humulene	17.28	1457	0.1	–	RI, MS
11	β-Ionene	18.28	1496	–	1.0	RI, MS
12	Bicyclogermacrene	18.34	1498	0.3	–	RI, MS
13	Spathulenol	20.37	1585	23.0	57.8	RI, MS
14	Caryophyllene oxide	20.48	1590	45.0	28.2	RI, MS
15	α-Cadinol	20.93	1603	–	0.5	RI, MS
16	Humulene epoxide	21.02	1614	2.4	–	RI, MS
17	Leden oxide	22.4	1676	9.2	1.0	MS
18	14-hydroxy α-Humulene	23.19	1712	0.1	–	RI, MS
19	2*E*,6*E*-Farnesol	23.86	1743	0.1	–	RI, MS
20	Hexahydrofarnesyl acetone	27.09	1756	–	3.8	RI, MS
21	Phytol	30.84	2085	–	0.5	RI, MS
	Monoterpene hydrocarbons			**3.0**	**-**	
	Oxygenated monoterpenes			**1.0**	**1.2**	
	Sesquiterpene hydrocarbons			**7.4**	**-**	
	Oxygenated sesquiterpenes			**79.8**	**87.5**	
	Others			**0.1**	**5.3**	
	Total identified			**91.3**	**94.0**	

Bold items indicate the Grouped compounds.
[a] RT: Retention time (min).

Table 2
Total phenolic and flavonoid contents and biological activities of the EOs and extracts of *Salvia nemorosa*.

Samples	EO-F	EO-L	*n*-Hexane	DCM	MeOH	Standard
Phenolic content (mg GAE/g)	–	–	17.3 ± 3.2[a]	31.2 ± 4.2	294.9 ± 10.9	–
Flavonoid content (mg QE/g)	–	–	14.3 ± 2.6	24.0 ± 4.8	117.9 14.5	–
DPPH (IC_{50} μg/mL)	>500	>500	>500	>500	82.1 ± 4.2	64.0 ± 4.5
FRAP (SRP)	0.67 ± 0.09	0.77 ± 0.01	0.31 ± 0.03	0.68 ± 0.07	0.99 ± 0.08	1.02 ± 0.15
BSLA (LC_{50} μg/mL)	>1000	>1000	194.8 ± 14.6	404.5 ± 13.9	850.1 ± 14.3	40.0 ± 2.5
XOI (IC_{50} μg/mL)	222.3 ± 12.3	207.2 ± 6.9	226.8 ± 7.4	>500	358.1 ± 16.6	1.18 ± 0.04
AOI (IC_{50} μg/mL)	19.4 ± 1.4	15.4 ± 1.0	12.6 ± 0.9	16.4 ± 1.9	20.6 ± 1.5	1.22 ± 0.03
AChEI (IC_{50} μg/mL)	488.9 ± 12.4	434.1 ± 11.6	338.9 ± 14.7	223.4 ± 8.4	469.9 ± 11.8	12.4 ± 1.1
AGI (IC_{50} μg/mL)	61.1 ± 2.9	47.2 ± 1.6	121.8 ± 3.1	113.6 ± 2.5	19.0 ± 2.1	17.0 ± 0.9

[a] Values expressed are mean ± standard error of the mean (SEM) of three independent experiments ($p < 0.05$).

Table 3
Antimicrobial activity of the essential oils and extracts of *Salvia nemorosa* against human pathogenic microorganisms.

Samples		Gram positive		Gram negative		Fungi	
		B. cereus	*S. aureus*	*P. aeruginosa*	*E. coli*	*A. niger*	*C. albicans*
EO-F	IZ	31.0 ± 0.7	32.0 ± 2.2	9.7 ± 0.7	9.1 ± 0.6	23.5 ± 2.9	19.7 ± 2.5
	MIC	25	10	1000	>1000	100	200
EO-L	IZ	29.7 ± 1.6	33.7 ± 1.2	8.8 ± 0.8	8.8 ± 0.5	24.9 ± 9.0	15.8 ± 2.3
	MIC	25	10	>1000	>1000	50	400
n-Hexane	IZ	22.9 ± 1.7	25.0 ± 1.6	7.0 ± 0.1	–	11.3 ± 3.7	7.9 ± 0.3
	MIC	100	100	>1000	>1000	1000	1000
DCM	IZ	21.2 ± 1.6	31.5 ± 0.8	14.9 ± 2.0	12.1 ± 1.4	21.5 ± 2.5	11.8 ± 1.3
	MIC	100	10	800	800	200	1000
MeOH	IZ	27.5 ± 1.2	33.0 ± 1.9	18.2 ± 9.0	21.3 ± 1.3	23.1 ± 0.3	20.1 ± 1.7
	MIC	50	10	400	100	50	200
Standard	IZ	34.7 ± 2.1	31.8 ± 1.1	28.6 ± 1.9	23.8 ± 1.3	24.2 ± 1.2	27.0 ± 1.4
	MIC	10	10	50	25	50	50

dietary supplements. They protect the human health against many diseases (Bahadori, Dinparast, Valizadeh, Farimani, & Ebrahimi, 2016b; Sarikurkcu et al., 2014; Torbati et al., 2014). We used DPPH radicals to determine free radical scavenging activity of *S. nemorosa* EOs and extracts. The radical scavenging test is performed for investigation of the free radical scavenging activity of natural products based on the reduction of free radicals. In the DPPH assay, the methanol extract (IC_{50} = 82.1 μg/mL) showed superior scavenging activity (Table 2). The IC_{50} values for the EOs and other extracts were higher than 500 μg/mL and were not determined. This could be explained with higher level of TPC and TFC in the methanolic extract.

Table 4
Quantitative results for determination of phenolic compounds of *Salvia nemorosa*.

No	Phenolic compounds	Concentration (μg/g extract)	Analytical characteristics			
			Linear range (mg/L)	r^2	LOD (mg/L)	LOQ (mg/L)
1	Gallic acid	nd[a]	0.20–25.0	0.9993	0.075	0.227
2	Protocatechuic acid	44 ± 4	0.20–25.0	0.9991	0.086	0.260
3	(+)-Catechin	nd	0.90–113	0.9988	0.172	0.522
4	*p*-Hydroxybenzoic acid	23 ± 1	0.20–25.0	0.9994	0.007	0.020
5	Chlorogenic acid	87 ± 1	0.35–45.0	0.9988	0.080	0.241
6	Caffeic acid	109 ± 2	0.16–21.0	0.9993	0.054	0.162
7	(−)-Epicatechin	nd	0.50–66.0	0.9990	0.170	0.514
8	Syringic acid	28 ± 1	0.05–12.0	0.9995	0.030	0.090
9	Vanillin	nd	0.08–10.0	0.9995	0.020	0.060
10	*p*-Coumaric acid	19 ± 1	0.04–6.0	0.9996	0.066	0.199
11	Ferulic acid	83 ± 3	0.12–17.0	0.9993	0.004	0.011
12	Sinapic acid	nd	0.12–17.0	0.9993	0.017	0.053
13	Benzoic acid	nd	0.85–55.0	0.9998	0.111	0.335
14	*o*-Coumaric acid	nd	0.24–32.0	0.9988	0.023	0.069
15	Rutin	nd	0.40–56.0	0.9989	1.113	3.373
16	Naringin	nd	0.24–0.32	0.9988	0.023	0.069
17	Hesperidin	nd	0.43–55.0	0.9992	1.080	3.280
18	Rosmarinic acid	7584 ± 142	0.02–7.0	0.9998	0.148	0.447
19	Eriodictyol	nd	0.33–21.0	0.9998	0.140	0.410
20	*trans*-Cinnamic acid	nd	0.02–7.0	0.9998	0.148	0.447
21	Quercetin	151 ± 2	0.40–55.0	0.9999	0.013	0.040
22	Luteolin	nd	0.13–17.0	0.9999	0.020	0.060
23	Kaempferol	nq[b]	0.05–15.0	0.9996	0.021	0.062
24	Apigenin	nq	0.17–11.0	0.9997	0.034	0.104

[a] nd: not detected.
[b] nq: not quantified (The exact quantification was not possible due to overlapping of the related peaks).

The reducing potential of natural compounds is considered as their antioxidant capacity. In this regard, the FRAP test was used in this work for evaluation of reducing power of the samples. The slopes of the trend lines were calculated and summarized in Table 2. Methanolic extract indicated the strongest reducing activity (SRP = 0.99) comparing with ascorbic acid as a standard (SRP = 1.02) followed by the essential oil of leaves (SRP = 0.77). Hydrogen donating components like phenolics and flavonoids in the extracts and spathulenol in the essential oils could be responsible for observed reducing power of *S. nemorosa*.

3.4. Brine shrimp larva toxicity

The Brine shrimp lethality test is a reliable bench-top assay for preliminary evaluation of toxicity of natural and synthetic compounds (Valizadeh, Sonboli, Kordi, Dehghan, & Bahadori, 2015). Freeze-dried eggs of *Artemia salina* are available. Obtained results from Brine shrimp lethality assay could be correlated well with cytotoxicity assays against cancer cell lines (Anderson, Goetz, McLaughlin, & Suffness, 1991). In the present work, several extracts and EOs of *S. nemorosa* were evaluated for their toxicity and the LC_{50} results are summarized in Table 2. The *n*-hexane extract were especially active (LC_{50} = 194 μg/mL). Other extracts showed moderate toxicity and EOs had no significant activity. According to Meyer et al. (1982) plant extracts with LC_{50} values less than 1000 μg/mL are considered as active substances. At this point, the *n*-hexane extract could be regarded as a candidate for discovery of novel anti-tumor natural products.

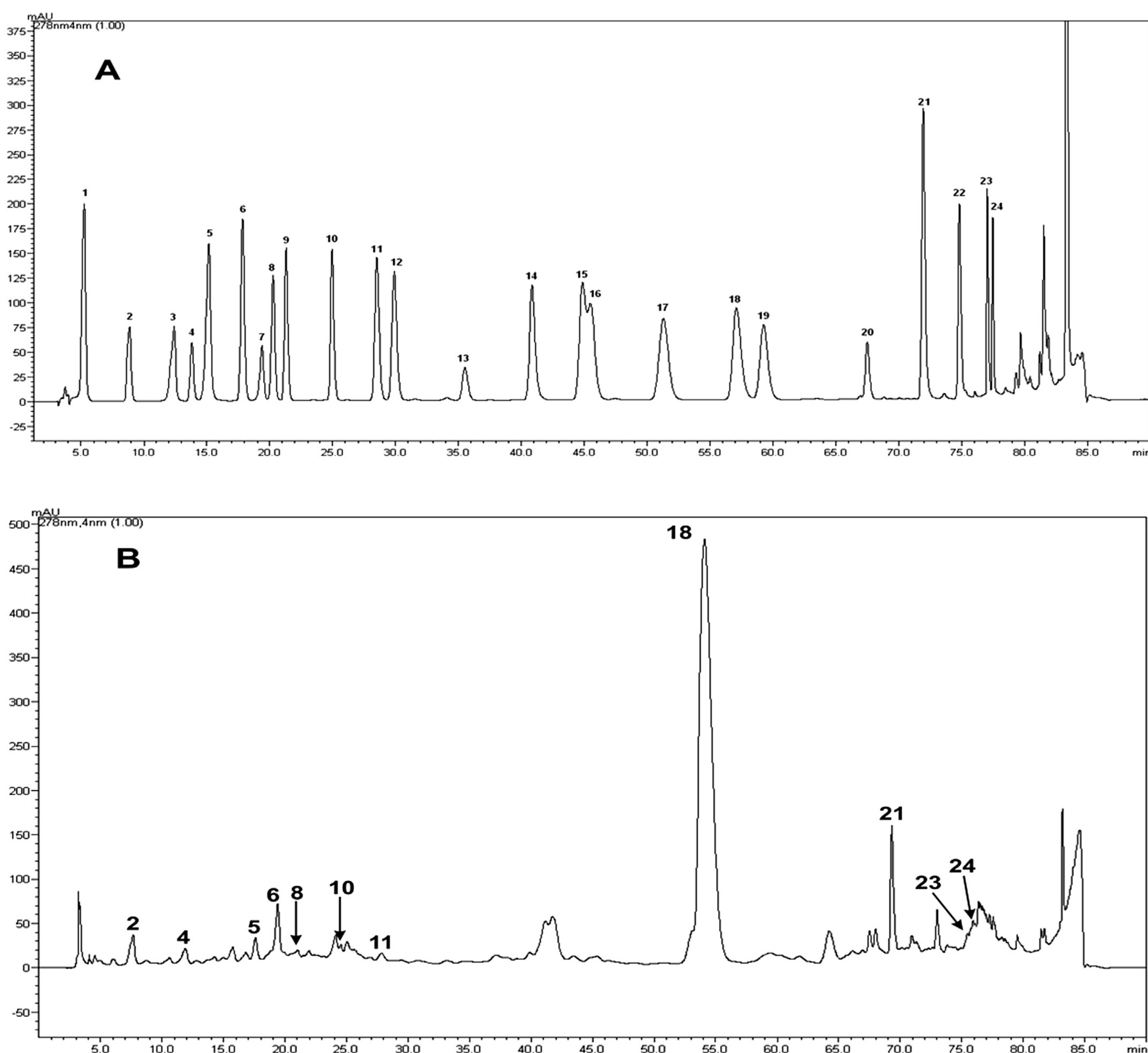


Fig. 1. HPLC chromatograms of standard phenolic compounds (A) and the methanolic extract (B) of *Salvia nemorosa*.

3.5. Enzyme inhibitory activity

3.5.1. Xanthine oxidase inhibition

Xanthine oxidase catalyzes the conversion of xanthine to uric acid and is involved in goat, hyperuricemia, and oxidative damages. All of the analyzed plant materials indicated moderate inhibitory activities towards xanthine oxidase. The poorest result was obtained for the DCM extract (Table 2).

3.5.2. Aldehyde oxidase inhibition

Aldehyde oxidase (AO) has an important role in metabolization of many drugs. AO catalyzes the conversion of aldehydes to related carboxylic acids. This enzyme is involved in disorders relative to oxidative stress. Studied samples showed high aldehyde oxidase inhibitory potential (IC_{50} of 16–20 μg/mL) (Table 2). The findings indicated that the extracts and EOs of *S. nemorosa* could be considered for several uses in food supplementary and cosmetics as oxidative stress reducing agents.

3.5.3. Acetylcholinesterase inhibition

Alzheimer's disease (AD) causes neurodegeneration resulting in cognitive disorders and finally death. Recently, inhibition of cholinesterases is considered as the best strategy for treatment of AD (Sarikurkcu et al., 2015). *Salvia* species are known in European traditional medicine as memory enhancer plants (Bahadori et al., 2016b; Çulhaoğlu, Yapar, Dirmenci, & Topçu, 2013). In addition, *Salvia* species are effective on central nervous system disorders (Karami, Gohari, & Naghshvar, 2012). Accordingly, we investigated the anti-Alzheimer's activity of *S. nemorosa* in the present study. DCM extract of the plant exhibited the strongest acetylcholinesterase inhibitory potential (Table 2). Other extracts and essential oils were generally less potent than DCM. AChE inhibitory activity of *S. nemorosa* is moderate in comparison with similar data published in the literature (Kolak et al., 2009; Loizzo et al., 2010).

3.5.4. α-Glucosidase inhibition

α-Glucosidase inhibitor drugs reduce the glucose level of blood as a strategy for treatment of type 2 diabetes (Eskandani, Babak Bahadori, Zengin, Dinparast, & Bahadori, 2016). In this study, methanolic extract showed the strongest α-glucosidase inhibitory activity (IC_{50} 19 μg/mL). Comparing with acarbose as a standard antidiabetic drug (IC_{50} 16 μg/mL), the methanolic extract could be regarded highly active. So, further phytochemical analyses for isolation and structure elucidation of active constituents is warranted. The methanolic extract of *S. nemorosa* has high amount of phenolic and flavonoid compounds which may be the responsible agents for observed activity. This hypothesis is in agreement with some previous studies that reported phenolic compounds as potent α-glucosidase inhibitors (Asghari, Salehi, Sonboli, & Ebrahimi, 2015; Moradi-Afrapoli et al., 2012).

3.6. Antimicrobial activity

In the recent years, natural antimicrobial compounds have gained a great attention because of microorganisms resistance to current antibiotics (Shahbazi, Shavisi, Karami, & Kakaei, 2015; Zare et al., 2014). In the present study, the oils and extracts of *S. nemorosa* were investigated against a microorganism panel of 4 bacteria and 2 fungi. Diameter of inhibition zone and minimum inhibitory concentration values (MICs) were determined (Table 3). Gram-positive bacteria were more sensitive. Leaves EO showed high inhibitory activity against *S. aureus* (IZ = 33.7 mm, MIC = 10 μg/mL) followed by methanolic extract (IZ = 33.0 mm, MIC = 10 μg/mL) and flowers EO (IZ = 32.0 mm, MIC = 10 μg/mL). These activities are higher than the standard antibiotic Gentamicin. Gram-negative bacteria were resistant and just methanolic extract inhibited their growth moderately. Between the fungi, *C. albicans* was the most resistant but flowers EO and methanolic extract could strongly inhibit its growth (19.7 and 20.1 mm, respectively). A number of studies concerning with the antimicrobial potential of *Salvia* species could be found in the literature (Bahadori et al., 2016a; Ulukanli et al., 2013; Yousefzadi et al., 2007). Our findings demonstrated that *S. nemorosa* has high antimicrobial activity against Gram-positive bacteria and fungi strains in comparison

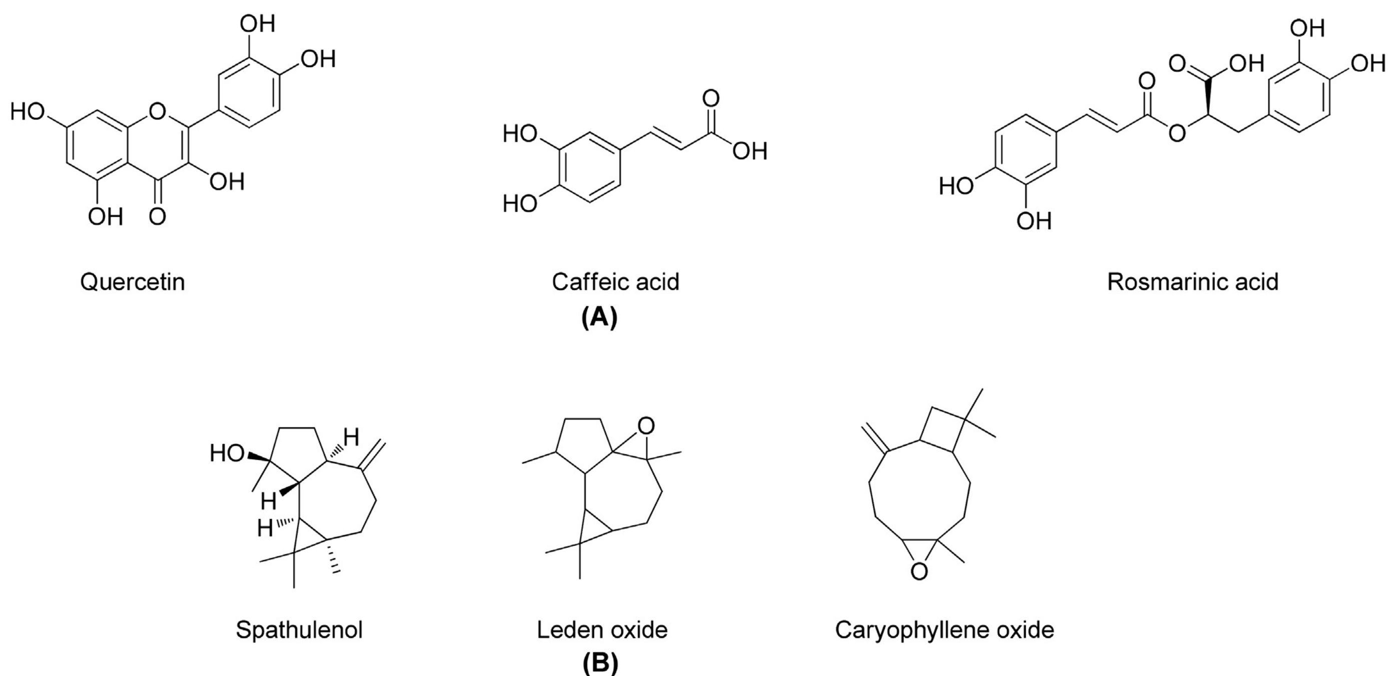


Fig. 2. Structures of the major compounds in the methanolic extract (A) and essential oils (B) of *Salvia nemorosa*.

with other *Salvia* species. Bioactivity-guided isolation of active components may be resulted in purification of new natural antibiotics from this plant.

3.7. Phenolic composition of methanolic extract

The methanolic extract of *S. nemorosa* showed high phenolic and flavonoid contents. There is a good correlation between the total phenolic content (TPC) and total flavonoid content (TFC) of methanolic extract with several bioactivities of this extract (DPPH, FRAP, AOI, and AGI). In this context, we aimed to analyze the phenolic profile of this extract to have an insight on its bioactive compounds. Identification and quantification of phenolic compounds in *S. nemorosa* were determined using the RP-HPLC–DAD. Analytical characteristics such as linearity range, limits of detection (LOD), and quantification (LOQ) were performed to validate the HPLC method for the quantification of phenolic components (Table 4).

Phenolic components of *S. nemorosa* were investigated and quantified according to the mentioned method. Twenty four standard phenolic compounds were analyzed and 11 of them were detected in the extract (Fig. 1). Caffeic acid, rosmarinic acid, and quercetin were found as the major compounds. Main phenolic components detected in *S. nemorosa* are presented in Fig. 2. These metabolites are well known for several biological properties such as antioxidant, antimicrobial, aldehyde oxidase inhibitory, and anticancer activities. The most abundant compound was characterized as rosmarinic acid (7584 μg/g extract). Main biological properties reported for rosmarinic acid are adstringent, antiinflammatory, antioxidative, antibacterial, antimutagen, and antiviral (Petersen & Simmonds, 2003). Accordingly, the above-mentioned phenolics could be responsible for observed high DPPH radical scavenging capacity, antibacterial, and enzyme inhibitory activity of the methanolic extract. These results suggest that methanolic extract of *S. nemorosa* could be regarded for some applications as health-care and food-supplements. To the best of our knowledge, this is the first study on the phenolic profile of *S. nemorosa*.

4. Conclusion

This work was carried out to evaluate the biological properties and chemical composition of *Salvia nemorosa*. Our findings showed that *S. nemorosa* is a rich source of bioactive metabolites. The methanolic extract was rich in phenolics and the major compounds of the EOs were oxygenated sesquiterpenes. Also, *S. nemorosa* exhibited a considerable antibacterial, antioxidant, and enzyme inhibitory activities. In conclusion, *S. nemorosa* is a valuable source of natural products and could be used for preparing novel functional foods, cosmetics, and pharmaceutical ingredients.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

Adams, R. P. (2007). *Identification of essential oil components by gas chromatography/mass spectrometry*. Carol Stream, IL, USA: Allured Publishing Corporation.

Albayrak, S., & Aksoy, A. (2013). Essential oil composition and in vitro antioxidant and antimicrobial activities of *Thymus cappadocicus* Boiss. *Journal of Food Processing and Preservation, 37*(5), 605–614.

Anderson, J. E., Goetz, C. M., McLaughlin, J. L., & Suffness, M. (1991). A blind comparison of simple bench-top bioassays and human tumour cell cytotoxicities as antitumor prescreens. *Phytochemical Analysis, 2*(3), 107–111.

Asghari, B., Salehi, P., Sonboli, A., & Ebrahimi, S. N. (2015). Flavonoids from *Salvia chloroleuca* with a-amylsae and a-glucosidase inhibitory effect. *Iranian Journal of Pharmaceutical Research, 14*(2), 609–615.

Bahadori, M. B., Valizadeh, H., & Farimani, M. M. (2016a). Chemical composition and antimicrobial activity of the volatile oil of *Salvia santolinifolia* Boiss. from Southeast of Iran. *Pharmaceutical Sciences, 22*(1), 42–48.

Bahadori, M. B., Dinparast, L., Valizadeh, H., Farimani, M. M., & Ebrahimi, S. N. (2016b). Bioactive constituents from roots of *Salvia syriaca* L.: Acetylcholinesterase inhibitory activity and molecular docking studies. *South African Journal of Botany, 106*, 1–4.

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

Bautista, E., Toscano, A., Calzada, F., Diaz, E., Yepez-Mulia, L., & Ortega, A. (2013). Hydroxyclerodanes from *Salvia shannoni*. *Journal of Natural Products, 76*(10), 1970–1975.

Çulhaoğlu, B., Yapar, G., Dirmenci, T., & Topçu, G. (2013). Bioactive constituents of *Salvia chrysophylla* Stapf. *Natural Product Research, 27*(4–5), 438–447.

Daskalova, T. (2004). On some specificities of seed formation in *Salvia nemorosa* (Lamiaceae). *Phytologia Balcanica, 10*, 79–84.

Dinparast, L., Valizadeh, H., Bahadori, M. B., Soltani, S., Asghari, B., & Rashidi, M.-R. (2016). Design, synthesis, α-glucosidase inhibitory activity, molecular docking and QSAR studies of benzimidazole derivatives. *Journal of Molecular Structure, 1114*, 84–94.

Ebrahimabadi, A. H., Mazoochi, A., Kashi, F. J., Djafari-Bidgoli, Z., & Batooli, H. (2010). Essential oil composition and antioxidant and antimicrobial properties of the aerial parts of *Salvia eremophila* Boiss. from Iran. *Food and Chemical Toxicology, 48*(5), 1371–1376.

Ellman, G. L., Courtney, K. D., Andres, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology, 7*(2), 88–95.

Ercetin, T., Senol, F. S., Orhan, I. E., & Toker, G. (2012). Comparative assessment of antioxidant and cholinesterase inhibitory properties of the marigold extracts from *Calendula arvensis* L. and *Calendula officinalis* L. *Industrial Crops and Products, 36*(1), 203–208.

Ertas, A., Boga, M., Yilmaz, M. A., Yesil, Y., Tel, G., Temel, H., … Ugurlu, P. (2015). A detailed study on the chemical and biological profiles of essential oil and methanol extract of *Thymus nummularius* (Anzer tea): Rosmarinic acid. *Industrial Crops and Products, 67*, 336–345.

Eskandani, M., Babak Bahadori, M., Zengin, G., Dinparast, L., & Bahadori, S. (2016). Novel natural agents from Lamiaceae family: An evaluation on toxicity and enzyme inhibitory potential linked to diabetes mellitus. *Current Bioactive Compounds, 12*(1), 34–38.

Farimani, M. M., Bahadori, M. B., Koulaei, S. A., Salehi, P., Ebrahimi, S. N., Khavasi, H. R., et al. (2015). New ursane triterpenoids from *Salvia urmiensis* Bunge: Absolute configuration and anti-proliferative activity. *Fitoterapia, 106*, 1–6.

Farimani, M. M., Bahadori, M. B., Taheri, S., Ebrahimi, S. N., Zimmermann, S., Brun, R., … Hamburger, M. (2011). Triterpenoids with rare carbon skeletons from *Salvia hydrangea*: Antiprotozoal activity and absolute configurations. *Journal of Natural Products, 74*(10), 2200–2205.

Farimani, M. M., Ebrahimi, S. N., Salehi, P., Bahadori, M. B., Sonboli, A., Khayasi, H. R., … Hamburger, M. (2013). Antitrypanosomal triterpenoid with an epsilon-lactone E-ring from *Salvia urmiensis*. *Journal of Natural Products, 76*(9), 1806–1809.

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

Hamzeh-Mivehroud, M., Rahmani, S., Rashidi, M. R., Feizi, M. A. H., & Dastmalchi, S. (2013). Structure-based investigation of rat aldehyde oxidase inhibition by flavonoids. *Xenobiotica, 43*(8), 661–670.

Karami, M., Gohari, A. R., & Naghshvar, F. (2012). Comparison effects of methanolic extracts of *Salvia macrosiphon* and withania coagulans on withdrawal syndrome in mice. *Pharmaceutical Sciences, 18*(3).

Kolak, U., Hacibekiroglu, I., Ozturk, M., Ozgokce, F., Topcu, G., & Ulubelen, A. (2009). Antioxidant and anticholinesterase constituents of *Salvia poculata*. *Turkish Journal of Chemistry, 33*(6), 813–823.

Lazarova, I., Zengin, G., Bender, O., Zheleva-Dimitrova, D., Uysal, S., Ceylan, R., … Gunduz, M. (2015). A comparative study of Bulgarian and Turkish *Asphodeline lutea* root extracts: HPLC-UV profiles, enzyme inhibitory potentials and anti-proliferative activities against MCF-7 and MCF-10A cell lines. *Journal of Functional Foods, 15*, 254–263.

Lesjak, M. M., Beara, I. N., Orcic, D. Z., Petar, K. N., Simin, N. D., Emilija, S. D., et al. (2014). Phytochemical composition and antioxidant, anti-inflammatory and antimicrobial activities of *Juniperus macrocarpa* Sibth. et Sm. *Journal of Functional Foods, 7*, 257–268.

Loizzo, M. R., Abouali, M., Salehi, P., Sonboli, A., Kanani, M., Menichini, F., et al. (2014). In vitro antioxidant and antiproliferative activities of nine *Salvia* species. *Natural Product Research, 28*(24), 2278–2285.

Loizzo, M. R., Tundis, R., Conforti, F., Menichini, F., Bonesi, M., Nadjafi, F., … Menichini, F. (2010). *Salvia leriifolia* Benth (Lamiaceae) extract demonstrates in vitro antioxidant properties and cholinesterase inhibitory activity. *Nutrition Research, 30*(12), 823–830.

Lu, Y. R., & Foo, L. Y. (2002). Polyphenolics of *Salvia* – A review. *Phytochemistry, 59*(2), 117–140.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., & Mclaughlin, J. L. (1982). Brine shrimp – A convenient general bioassay for active-plant constituents. *Planta Medica, 45*(1), 31–34.

Moradi-Afrapoli, F., Asghari, B., Saeidnia, S., Ajani, Y., Mirjani, M.,

Malmir, M., … Yassa, N. (2012). In vitro a-glucosidase inhibitory activity of phenolic constituents from aerial parts of Polygonum hyrcanicum. *DARU-Journal of Pharmaceutical Sciences, 20*.

Nan, J. X., Park, E. J., Kang, H. C., Park, P. H., Kim, J. Y., & Sohn, D. H. (2001). Anti-fibrotic effects of a hot-water extract from Salvia miltiorrhiza roots on liver fibrosis induced by biliary obstruction in rats. *Journal of Pharmacy and Pharmacology, 53*(2), 197–204.

NCCLS. (2006). *Performance standards for antimicrobial disk susceptibility tests: Approved standards*. National Committee for Clinical Laboratory Standards.

Nikolic, M., Glamoclija, J., Ferreira, I. C. F. R., Calhelha, R. C., Fernandes, A., Markovic, T., … Sokovic, M. (2014). Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. and Reut and *Thymus vulgaris* L. essential oils. *Industrial Crops and Products, 52*, 183–190.

Perfumi, M., Arnold, N., & Tacconi, R. (1991). Hypoglycemic activity of *Salvia fruticosa* Mill from Cyprus. *Journal of Ethnopharmacology, 34*(2–3), 135–140.

Petersen, M., & Simmonds, M. S. J. (2003). Molecules of interest – Rosmarinic acid. *Phytochemistry, 62*(2), 121–125.

Pirouzpanah, S., Hanaee, J., Razavieh, S. V., & Rashidi, M. R. (2009). Inhibitory effects of flavonoids on aldehyde oxidase activity. *Journal of Enzyme Inhibition and Medicinal Chemistry, 24*(1), 14–21.

Sarikurkcu, C., Uren, M. C., Tepe, B., Cengiz, M., & Kocak, M. S. (2014). Phenolic content, enzyme inhibitory and antioxidative activity potentials of *Phlomis nissolii* and *P. pungens* var. *pungens*. *Industrial Crops and Products, 62*, 333–340.

Sarikurkcu, C., Zengin, G., Oskay, M., Uysal, S., Ceylan, R., & Aktumsek, A. (2015). Composition, antioxidant, antimicrobial and enzyme inhibition activities of two *Origanum vulgare* subspecies (subsp *vulgare* and subsp *hirtum*) essential oils. *Industrial Crops and Products, 70*, 178–184.

Shahbazi, Y., Shavisi, N., Karami, N., & Kakaei, S. (2015). Chemical composition and in vitro antibacterial activity of *Ferulago angulata* (Schlecht.) Boiss essential oil. *Pharmaceutical Sciences, 21*(1), 6–11.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.

Skala, E., & Wysokinska, H. (2004). In vitro regeneration of *Salvia nemorosa* L. from shoot tips and leaf explants. *In Vitro Cellular & Developmental Biology-Plant, 40*(6), 596–602.

Sonboli, A., Bahadori, M. B., Dehghan, H., Aarabi, L., Savehdroudi, P., Nekuei, M., … Mirzania, F. (2013). Chemotaxonomic importance of the essential-oil composition in two subspecies of *Teucrium stocksianum* Boiss. from Iran. *Chemistry & Biodiversity, 10*(4), 687–694.

Takeda, Y., Zhang, H. J., Matsumoto, T., Otsuka, H., Oosio, Y., Honda, G., … Yesilada, E. (1997). Megastigmane glycosides from *Salvia nemorosa*. *Phytochemistry, 44*(1), 117–120.

Tkachuk, V. G., & Shapoval, V. V. (1987). Effect of *Salvia sclarea* essential oil on indices of the hematopoietic, immune and enzyme systems. *Vrachebnoe Delo*, (5), 83.

Topcu, G. (2006). Bioactive triterpenoids from *Salvia* species. *Journal of Natural Products, 69*(3), 482–487.

Torbati, M., Nazemiyeh, H., Lotfipour, F., Nemati, M., Asnaashari, S., & Fathiazad, F. (2014). Chemical composition and in vitro antioxidant and antibacterial activity of *Heracleum transcaucasicum* and *Heracleum anisactis* roots essential oil. *BioImpacts, 4*(2), 69.

Tosun, M., Ercisli, S., Sengul, M., Ozer, H., Polat, T., & Ozturk, E. (2009). Antioxidant properties and total phenolic content of eight *Salvia* species from Turkey. *Biological Research, 42*(2), 175–181.

Tuominen, A. (2013). Defensive strategies in *Geranium sylvaticum*, Part 2: Roles of water-soluble tannins, flavonoids and phenolic acids against natural enemies. *Phytochemistry, 95*, 408–420.

Ulubelen, A. (2003). Cardioactive and antibacterial terpenoids from some *Salvia* species. *Phytochemistry, 64*(2), 395–399.

Ulubelen, A., Birman, H., Oksuz, S., Topcu, G., Kolak, U., Barla, A., et al. (2002). Cardioactive diterpenes from the roots of *Salvia eriophora*. *Planta Medica, 68*(9), 818–821.

Ulukanli, Z., Karaborklu, S., Cenet, M., Sagdic, O., Ozturk, I., & Balcilar, M. (2013). Essential oil composition, insecticidal and antibacterial activities of *Salvia tomentosa* Miller. *Medicinal Chemistry Research, 22*(2), 832–840.

Valizadeh, H., Sonboli, A., Kordi, F. M., Dehghan, H., & Bahadori, M. B. (2015). Cytotoxicity, antioxidant activity and phenolic content of eight fern species from North of Iran. *Pharmaceutical Sciences, 21*(1), 18.

Wu, Y. B., Ni, Z. Y., Shi, Q. W., Dong, M., Kiyota, H., Gu, Y. C., et al. (2012). Constituents from *Salvia* species and their biological activities. *Chemical Reviews, 112*(11), 5967–6026.

Yousefzadi, M., Sonboli, A., Karimi, F., Ebrahimi, S. N., Asghari, B., & Zeinali, A. (2007). Antimicrobial activity of some *Salvia* species essential oils from Iran. *Zeitschrift Fur Naturforschung Section C – A Journal of Biosciences, 62*(7–8), 514–518.

Zare, K., Nazemyeh, H., Lotfipour, F., Farabi, S., Ghiamirad, M., & Barzegari, A. (2014). Antibacterial activity and total phenolic content of the *Onopordon acanthium* L. seeds. *Pharmaceutical Sciences, 20*(1), 6.

Zengin, G., Sarikurkcu, C., Aktumsek, A., & Ceylan, R. (2014). *Sideritis galatica* Bornm.: A source of multifunctional agents for the management of oxidative damage, Alzheimer's's and diabetes mellitus. *Journal of Functional Foods, 11*, 538–547.

Zengin, G., Sarikurkcu, C., Gunes, E., Uysal, A., Ceylan, R., Uysal, S., … Aktumsek, A. (2015). Two *Ganoderma* species: Profiling of phenolic compounds by HPLC-DAD, antioxidant, antimicrobial and inhibitory activities on key enzymes linked to diabetes mellitus, Alzheimer's disease and skin disorders. *Food & Function, 6*(8), 2794–2802.

Zhao, H. F., Fan, W., Dong, J. J., Lu, J., Chen, J., Shan, L. J., … Kong, W. B. (2008). Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. *Food Chemistry, 107*(1), 296–304.

View publication stats