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Original article

Cytotoxicity, antioxidant activity, total flavonoid and phenolic contents of *Salvia urmiensis* Bunge and *Salvia hydrangea* DC. ex Benth.

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Abstract

Background and objectives: *Salvia* species are important because of their medicinal, traditional and economical uses. They are used traditionally for treatment of several diseases. The genus *Salvia* is represented in the Iranian flora by 61 species of which, 17 are endemic. In the present study, the phytochemical and biological effects of two Iranian *Salvia* species have been evaluated. **Methods**: 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals scavenging activities of extracts of *Salvia urmiensis* Bunge and *Salvia hydrangea* were evaluated. Total flavonoid and phenolic contents and brine shrimp lethality potential of the extracts were also determined. **Results**: Compared to podophyllotoxin (LC_{50} =42 µg/mL), the ethyl acetate extract of *S. hydrangea* demonstrated a significant cytotoxicity (LC_{50}=36 µg/mL). The ethyl acetate extract of *S. urmiensis* was found to have significant antioxidant properties with IC_{50} value of 10.0±0.2 µg/mL. All tested extracts showed moderate to high flavonoid and phenolic contents. **Conclusion**: Findings showed that these plants contain important metabolites and could be suggested for discovery of biologically active natural compounds.

Keywords: antioxidant, brine shrimp, flavonoid content, *Salvia hydrangea*, *Salvia urmiensis*

Introduction

Plants have been used for treatment of human illnesses during history [1,2]. Many medicinal plants have been screened extensively for their medicinal potential worldwide [3,4]. *Salvia* is the largest genus of the Lamiaceae family, with over 1000 species [5]. *Salvia* species are used traditionally for medicinal purposes all around the world [6]. Several biological properties have been reported for these species including antidiabetic, antibacterial, antifungal, antitumor, antioxidant, cytotoxic, antiplasmodial, antiprotozoal and HIV inhibitory activities [7,8]. Some *Salvia* species have economic importance because of their use as medicinal plants, spices, and sources of essential oils for the perfume industries [9]. *Salvia* species produce biologically active natural compounds that could be classified as terpenoids, steroids, flavonoids and polyphenols. Some of them have interesting and novel structures [10]. These plants are rich sources of terpenoids and polyphenols [11]. Iranian flora comprises 61 *Salvia* species, of

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which 17 are endemic [12]. *Salvia urmiensis* Bunge is an endemic species growing wildly in the West Azerbaijan province, northwestern of Iran. A new antimalarial triterpenoid has been recently isolated from this species [13]. *Salvia hydrangea* DC. ex Benth. is an aromatic plant that grows widely in Anatolia, Iran and Transcaucasia. The aerial parts of S. *hydrangea* have been used in Iranian Traditional Medicine as carminative, anti-inflammatory, sedative and antispasmodic remedies [14]. Infusions from the flowers of this plant have shown anthelmintic and antileishmanial activities [15]. Some flavonoids, terpenoids and biologically active new isoprenoids have been isolated from *S. hydrangea* in Iran [14,15]. In the present study, we have evaluated the cytotoxicity, antioxidant activity, total flavonoid and phenolic contents of several extracts of two important Iranian *Salvia* species, *S. urmiensis* and *S. hydrangea*. To the best of our knowledge, this is the first report on the cytotoxic properties and bioactive constituents of these *Salvia* species in Iran.

Experimental

Plant materials

The aerial parts of *S. urmiensis* were collected at full flowering stage in May 2013 from Takab, West Azarbaijan province. The aerial parts of *S. hydrangea* DC. ex Benth. were collected from Koohin region in Qazvin province, Iran, in May 2012. A voucher specimen has been deposited for *S. urmiensis* (MPH-1220) at the Herbarium of Medicinal Plants and Drug Research Institute (MPH) of Shahid Beheshti University, Tehran, Iran and a voucher specimen has been deposited for *S. hydrangea* (6719-TEH) at the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Preparation of plant extracts

The air-dried and powdered plant samples (10 g) were extracted for 48 h using 200 mL *n*-hexane, ethyl acetate and methanol, successively by maceration on a shaker at room temperature. The extracts were filtered and concentrated using a rotary evaporator at 40 °C. The filtered extracts were stored at -20 °C until the experiment.

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenothiazoline-6-sulphonic acid) diammonium salt (ABTS), Gallic acid, potassium persulphate, ethanol, Folin-Ciocalteu, sodium carbonate, quercetin, butylated hydroxy toluene (BHT), *n*-hexane, ethyl acetate and methanol were obtained from Merck (Germany). Podophyllotoxin and sea salt were obtained from Sigma-Aldrich (Germany).

DPPH free radical-scavenging assay

DPPH radical scavenging activity of the six extracts were measured according to the method described by Xu *et al.* [16]. 50 µL of various concentrations (5, 10, 20, 40, 80 µg/mL) of the extract solutions in methanol were added to 200 µL of 100 µM DPPH solution in methanol. BHT was used as the standard antioxidant. The reaction mixture was incubated for 30 min at room temperature in darkness, and then absorbance was determined at 517 nm with a microplate reader spectrophotometer (BioTek XS2 model). The control contained 50 µL of methanol in place of the test sample, and the blank contained pure methanol instead of DPPH solution. Experiments were carried out in triplicates. The percentage of inhibition for each concentration was calculated according to the following equation:

$$\%\text{inhibition} = [1 - (As - Ab)/Ac] \times 100$$

Where As is the absorbance of the mixture in the presence of the samples, Ab is the absorbance of the blank and Ac is the absorbance of control. A lower absorbance of the mixture indicated a higher DPPH radical scavenging potential. IC_{50} value (µg extract/mL) is the concentration at which 50% of DPPH radicals are inhibited and is obtained by interpolation from linear regression calculation.

Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC assay is a general and simple method

used for evaluation of antioxidant capability of extracts to scavenge ABTS radicals. It could measure antioxidant activity of hydrophilic and lipophilic components. The ABTS radical cation was prepared by mixing a solution of 7 mM ABTS in ethanol and 2.45 mM potassium persulphate in a volume ratio of 1:1. The mixture was incubated in dark for 12 h at room temperature until the reaction was complete and used during 1 day [17]. For measurements, the ABTS radical cation solution was diluted to an absorbance of 0.70±0.04 at 734 nm. ABTS solution (3.8 mL) and 100 µL of the plant samples were mixed for 45 s and the absorbance at 734 nm were recorded after 1 min of incubation. The percentage of inhibition of absorbance at 734 nm was calculated. Trolox was used as the standard reagent, and the results were expressed as µmol of Trolox equivalents per gram dry weight of plants.

Brine shrimp lethality assay

The experiments were carried out according to the method described by Meyer with some modifications [18]. Brine shrimp (*Artemia salina*) eggs were allowed to hatch in flask containing artificial seawater (300 mL, 3.8% w/w salt in distilled water) for 24 h at room temperature under constant aeration. The extracts were dissolved in dimethyl sulfoxide (DMSO). Ten larvae were collected with a pipette and added to the two-fold serially diluted solutions (1000 –15.625 µg/mL extract) in the test tubes. After 24 h, a magnifying glass was used to count the number of dead larvae and the mortality percentage was calculated. The mean value of three replications of mortality percentage was plotted against the concentrations logarithm using Microsoft Excel 2013 software. LC_{50} values were determined from the linear equation. Podophyllotoxin was used as the positive and DMSO as the negative controls. Final DMSO concentration was 0.1%.

Total phenolic contents evaluation

The total phenolic contents of the plant extracts were estimated using Folin-Ciocalteu assay according to the method of Zhou *et al.* with some modification [19]. 2.5 mL of sample was mixed with 2.5 mL of Folin-Ciocalteu reagent. Then 50 µL of sodium carbonate (7%) was added to the mixture and the volume was adjusted to 250 mL by adding distilled water. The mixture was mixed thoroughly for 30 min at room temperature in the dark. Absorbance of the sample solutions against a blank was determined at 765 nm using a micro plate reader. Total phenolic contents were expressed as mg of gallic acid equivalents per gram of dry extract (mg GAE/g of extract). Different concentrations of gallic acid as standard (12.5, 25, 50, 100, 200 µg/mL) were used to construct a calibration curve. All measurements were carried out in triplicate.

Total flavonoid contents evaluation

The total flavonoid content of the extracts was estimated according to a previously described method [20]. The absorbance was measured against a blank at 510 nm. Results were expressed as mg of quercetine equivalents per gram of dried extract. Different concentrations of quercetine as standard (12.5, 25, 50, 100, 200 µg/mL) were used to construct a calibration curve. All measurements were carried out in triplicate.

Statistical analysis

The samples were evaluated in triplicates and the results were shown as mean±standard deviation (S.D.). Analysis of correlation was determined by bivariate correlations test using IBM SPSS Statistics V21.

Results and Discussion

The DPPH radical scavenging activity of different extracts has been illustrated in table 1. With regard to IC_{50} values among the examined samples, the ethyl acetate extract of *S. urmiensis* with the lowest IC_{50} (10.0±0.2 µg/mL), exhibited considerable DPPH radical scavenging activity compared with BHT (15.6±0.8 µg/mL), followed by the methanol extract of this plant (IC_{50} =

13.6±1.9 µg/mL) and the *n*-hexane extract of *S. hydrangea* (IC_{50} value of 14.8±1.2 µg/mL). Table 1 shows the ABTS radical scavenging activity of the extracts via TEAC values. Generally, these *Salvia* species have shown moderate ABTS radical scavenging ability. The TEAC values varied from 0.3±0.02 to 7.8±0.3 µmol Trolox/g dry weight of plants. Among the tested samples, the methanol extract of *S. hydrangea* was the strongest ABTS radical cation scavenger with TEAC value about 7.8±0.3 µmol Trolox/g dry weight of plants.

Table 1. DPPH and ABTS radical scavenging activity of *Salvia* extracts

Samples	Extract	DPPH IC_{50} (µg/mL)	ABTS (µmoL Trolox/g)
S. urmiensis	*n*-hexane	45.0±2.3	0.3±0.02
	Ethyl acetate	10.0±0.2	6.4±0.25
	Methanol	13.6±1.9	3.65±0.15
S. hydrangea	*n*-hexane	14.8±1.2	4.9±0.21
	Ethyl acetate	32.2±1.6	5.2±0.18
	Methanol	42.8±2.1	7.8±0.3
BHT	-	15.6±0.8	-

The LC_{50} values of *Salvia* extracts against brine shrimp larvae have been shown in table 2. The LC_{50} values for all tested extracts were below 1000 µg/mL. The ethyl acetate extract of *S. hydrangea* demonstrated high toxicity against brine shrimp larvae (LC_{50} = 36.0 µg/mL) compared to the positive control, podophyllotoxin (LC_{50} = 42.0 µg/mL). Other extracts were found to have moderate toxicity (table 2). The lethality rate of brine shrimp larvae were found to get more with increasing the extract concentration. Values have been expressed as an average of triplicates.

Table 2. Brine shrimp lethality assay of *Salvia* extracts

	Extract	LC_{50} (µg/mL)
S. urmiensis	*n*-hexane	211.2
	Ethyl acetate	324.4
	Methanol	850.1
S. hydrangea	*n*-hexane	180.8
	Ethyl acetate	36.0
	Methanol	452.5
Standard Drug	Podophyllotoxin	42.0

As shown in figure 1, the total phenolic contents varied from 30.5±3.2 to 109.25±10.5 mg GAE/g.

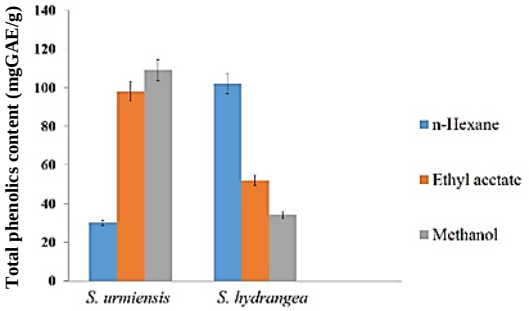

Figure 1. Total phenolics content of *Salvia* extracts

The methanol extract of *Salvia urmiensis* showed the highest total phenolic content (109.25±10.5 GAE/g), followed by *n*-hexane extract of *S. hydrangea* (102.54 ± 8.2 mg GAE/g).
All tested *Salvia* extracts exhibited moderate to high phenolic content.
Using the standard curve of quercetine, the flavonoid contents of the extracts were found ranging from 16.5 to 170.5 mg quercetine equivalents/g of dry extract as shown in Figure 2. The results showed the greatest total flavonoid content in ethyl acetate extract of *Salvia urmiensis* (170.5 mg quercetine equivalents/g).

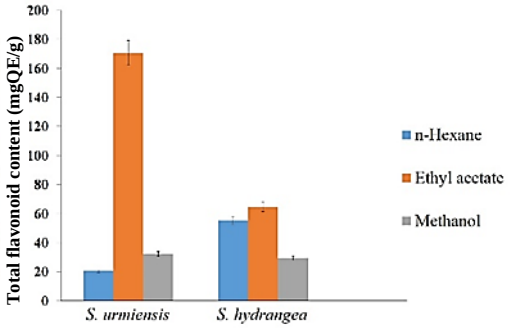

Figure 2. Total flavonoid content of *Salvia* extracts

The present investigation has provided useful information about cytotoxicity, antioxidant properties, and flavonoid and phenolic contents of two Iranian *Salvia* species. The six extracts that have been evaluated in the current study exhibited moderate to high cytotoxicity to brine shrimp larvae with LC_{50} values ranging between 35 and 850 µg/mL. All of them exhibited considerable DPPH and ABTS scavenging activities. A weak correlation between the TEAC

value and total phenolic content proved that phenolic compounds could not be the main components responsible for free radicals scavenging ability of these *Salvia* species. Weak correlations were observed between ABTS values and total flavonoid contents, ABTS and total phenolic contents as well as DPPH values and total flavonoid contents; However, a good correlation between DPPH values and total phenolic contents of the examined plants (figure 3) indicated that phenolic compounds could be responsible for ability of reducing oxidants in these *Salvia* species.

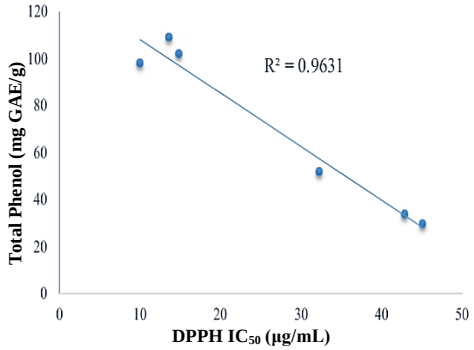

Figure 3. Correlation between the total phenolic content and DPPH values

The findings of our study are in agreement with previous phytochemical studies on these species. Firuzi *et al.* have reported remarkable cytotoxic activity of eleven Iranian *Salvia* species using MTT assay on three human cancer cell lines [21]. In the same study total phenolic contents of *Salvia* species were determined. Our findings showed that *S. urmiensis* and *S. hydrangea* have higher phenolic contents than those 11 *Salvia* species studied by Firuzi *et al.* There are several reports on cytotoxic activity of terpenoids from *Salvia* genus [6,22,23]. 2α-hydroxy ursolic acid, luteolin and urmiensolide are the major compounds isolated from *S. urmiensis* [13]. Salvadione C, perovskone B, 5-hydroxy-4′,7-dimethoxyflavone, oleanolic acid, salvigenin and hydrangenone are the natural products isolated from *S. hydrangea* in previous works [14,15]. All these compounds belong to terpenoids and flavonoids. So these two groups of natural compounds could be responsible for biological activities of studied *Salvia* species in this work. Further phytochemical investigations and bioactivity evaluations could clarify the biological role of phytoconstituents found in these two species.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

References

[1] Valizadeh H, Mahmoodi FK, Kouhkan R, Bahadori MB. Isolation and structure elucidation of coumarin and cinamate derivatives from *Lycium ruthenicum*. *Iran Chem Commun.* 2014; 2: 277-282.

[2] Valizadeh H, Mahmoodi FK, Alizadeh Z, Bahadori MB. Isolation and structure elucidation of secondary metabolites from *Echinophora platyloba* DC from Iran. *J Med Plant.* 2014; 13: 15-21.

[3] Valizadeh H, Mahmoodi FK, Hosseinzadeh Z, Bahadori MB. Furanocoumarins from *Heracleum rawianum* in Iran. *Iran Chem Commun.* 2015; 3: 1-6.

[4] Sonboli A, Bahadori MB, Dehghan H, Aarabi L, Savehdoroudi P, Nekuei M, Pournaghi N, Mirzania F. Chemotaxonomic importance of the essential oil composition in two subspecies of *Teucrium stocksianum* Boiss. from Iran. *Chem Biodivers.* 2013; 10: 687-694.

[5] Wu YB, Ni ZY, Shi QW, Dong M, Kiyota H, Gu YC, Cong B. Constituents from *Salvia* species and their biological activities. *Chem Rev.* 2012; 112: 5967-6026.

[6] Topcu G. Bioactive triterpenoids from *Salvia* Species. *J Nat Prod.* 2006; 69: 482-487.

[7] Ulubelen A. Cardioactive and antibacterial terpenoids from some *Salvia* species. *Phytochemistry*. 2003; 64: 395-399.
[8] Imanshahidi M, Hosseinzadeh H. The pharmacological effects of *Salvia* species on the central nervous system. *Phytother Res.* 2006; 20: 427-437.
[9] Kamatou GPP, Makunga NP, Ramogola WPN, Viljoen AM. South African *Salvia* species: a review of biological activities and phytochemistry. *J Ethnopharmacol*. 2008; 119: 664-672.
[10] Shirota O, Nagamatsu K, Sekita S. Neo-clerodane diterpenes from the hallucinogenic Sage *Salvia divinorum*. *J Nat Prod.* 2006; 69: 1782-1786.
[11] Lu Y, Foo LY. Polyphenolics of *Salvia*-a review. *Phytochemistry.* 2002; 59: 117-140.
[12] Jamzad Z. *Flora of Iran: Lamiaceae*. 1st ed. Tehran: Research Institute of Forests and Rangelands press, 2012.
[13] Farimani MM, Ebrahimi SN, Salehi P, Bahadori MB, Sonboli A, Khavasi HR, Zimmermann S, Kaiser M, Hamburger M. Antitrypanosomal triterpenoid with an ε-Lactone E-Ring from *Salvia urmiensis*. *J Nat Prod.* 2013; 76: 1806-1809.
[14] Farimani MM, Bahadori MB, Taheri S, Ebrahimi SN, Zimmermann S, Brun R, Amin G, Hamburger M. Triterpenoids with rare carbon skeletons from *Salvia hydrangea*: antiprotozoal activity and absolute configurations. *J Nat Prod.* 2011; 74: 2200-2205.
[15] Farimani MM, Taheri S, Ebrahimi SN, Bahadori MB, Khavasi HR, Zimmermann S, Brun R, Hamburger M. Hydrangenone, a new isoprenoid with an unprecedented skeleton from *Salvia hydrangea*. *Org Lett.* 2012; 14: 166-169.
[16] Xu X, Xie H, Wang Y, Wei X. A-Type proanthocyanidins from lychee seeds and their antioxidant and antiviral activities. *J Agric Food Chem.* 2010; 58: 11667-11672.
[17] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice EC. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med.* 1999; 26: 1231-1237.
[18] Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols De, McLaughlin Jl. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med.* 1982; 45: 31-34.
[19] Zhou K, Su L, Yu L. Phytochemicals and antioxidant properties in wheat barn. *J Agric Food Chem.* 2004; 52: 6108-6114.
[20] Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 1999; 64: 555-559.
[21] Firuzi O, Miri R, Asadollahi M, Eslami S, Jassbi AR. Cytotoxic, antioxidant and antimicrobial activities and phenolic contents of eleven *Salvia* species from Iran. *Iran J Pharm Res.* 2013; 12: 801-810.
[22] Pan ZH, Wang YY, Li MM, Xu G, Peng LY, He J, Zhao Y, Li Y, Zhao QS. Triterpenoids from *Salvia trijuga. J Nat Prod.* 2010; 73: 1146-1150.
[23] Hussein AA, Meyer JJM, Jimeno JM, Rodriguez B. Bioactive diterpenoids from *Orthosiphon labiatus* and *Salvia Africana-lutea. J Nat Prod.* 2007; 70: 293-295.