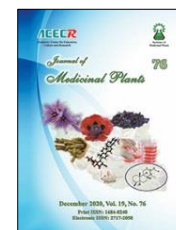




Institute of
Medicinal Plants

Journal of Medicinal Plants

Journal homepage: www.jmp.ir



Research Article

Comparison of cytotoxic and antioxidant activities and phenol content of four *Salvia* L. species from Iran

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ARTICLE INFO

Keywords:

Antioxidant activity

Cytotoxicity

Salvia

Phenol content

Flavonoid content

ABSTRACT

Background: The use of medicinal plants has been one of the most common treatments since ancient times. Various plants are used in traditional and modern medicine, due to their antioxidant, antimicrobial and anticancer properties, and other biological potentials. **Objective:** In this study, the aerial parts of four species of *Salvia* including two populations of *S. reuteriana* Boiss., two populations of *S. limbata* C.A.Mey. and one population of each *S. syriaca* L. and *S. ceratophylla* L. species from Kashan region have been investigated. **Methods:** The antioxidative activity of the methanol extracts of plant samples were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The total phenol and flavonoid contents were determined via the Folin-Ciocalteu and aluminum chloride methods, respectively. The cytotoxic effects of samples on HeLa cells were determined through MTT assay. **Results:** Based on the results of DPPH assay, IC₅₀ levels of methanolic extracts were in the range of 39.08 ± 0.15 to 163.77 ± 0.63 µg/ml, and total phenol contents were in the range of 42.7 ± 3.09 to 105.8 ± 1.15 µg/mg. In addition, the flavonoid contents ranged from 30.07 ± 2.52 to 82.46 ± 2.2 µg/mg. There was a direct relationship between antioxidant activity and phenol compound contents. According to our study, the methanolic extracts of *Salvia* species showed toxicity effects on HeLa cells. **Conclusion:** The extracts of two populations of *S. reuteriana* were associated with the highest cytotoxicity, compared to other species of *Salvia*. Furthermore, all examined extracts exhibited weak to moderate antioxidant activities. The *S. ceratophylla* extract was associated with the highest antioxidant activity.

1. Introduction

Currently, the high level of free radical production in the body plays a critical role in the progression of a variety of pathological

disturbances like heart diseases, cancer, atherosclerosis, diabetes mellitus and inflammation by detrimental cellular components of DNA, proteins, and lipids [1-3].

Abbreviations: DPPH, 2,2-Diphenyl-1-picrylhydrazyl; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; IC₅₀, Medium Inhibitory Concentration; BHT, Butylated Hydroxytoluene; HDF, Human Dermal Fibroblast.

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doi: 10.29252/jmp.19.76.59

Received 4 February 2020; Received in revised form 3 August 2020; Accepted 21 August 2020

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Antioxidants are chemical compounds that delays or prevents free radicals formation and can help in preventing some diseases such as cancer. They can prevent cancer progression by inhibiting cell proliferation, maintaining normal cell cycle regulation and inducing apoptosis [4]. Therefore, a daily intake of antioxidants in the human diet is an important way for disease prevention and reducing the risk of cancer. Many researchers have recently focused on natural compounds, medicinal plants, and phytochemical components with antioxidant properties, since according to toxicologists and nutritionists; synthetic antioxidants can neutralize noxious effects in the human body [5-7].

Cancer is among the most dangerous diseases, being regarded as one of the primary causes of death, worldwide. According to the results of WHO assessments, the worldwide deaths due to cancer until 2030 would probably exceed 11 million cases [8, 9]. The most common type of cancer that causes death in women worldwide is breast cancer. Gastric, liver, and then cervical cancers are considered as the second to fourth types of cancer that cause death among women [10, 11]. Currently, multiple chemo-preventive agents are available for treating cancer. However, they cause serious side effects and toxicities, making many patients seek alternative therapies for cancer [12, 13].

Medicinal plants have been long investigated to find new compounds that might have therapeutic properties and prevent cancer formation [12, 14]. Different species of *Salvia* have been used in traditional and modern medicine, due to their active and effective constituents [15, 16]. *Salvia* species, commonly known as sage and genus *Salvia*, is one of the most important genera in the Lamiaceae family. This plant comprises over 900 species all over the world [5, 17]. In Iran, 58 species are growing, with 17 endemic cases [18]. *Salvia*

plants are great sources of new bioactive constituents and different phytochemical components including phenolic acid, flavonoids, terpenoids, and polysaccharides. Such compounds are known to be responsible for the intrinsic biological activities of *Salvia* plants such as their antioxidant, antibacterial, anti-diabetic, antitumor, anti-tuberculosis, and anti-inflammatory activities [10, 16, 18, 19].

The main goal of this study was to evaluate the antioxidant and cytotoxic activities of the methanolic extracts of four species of *salvia* against the human HeLa cervical cancer cell line and a normal cell line. In addition, the studied species have been collected from different areas of Kashan for the first time.

2. Materials and Methods

2.1. Reagents

Methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, butylated hydroxytoluene (BHT), quercetin, sodium carbonate, sodium sulfate, dimethyl sulfoxide (DMSO), aluminum chloride ($AlCl_3$), sodium acetate and standard Folin-Ciocalteu's phenol reagent were all purchased from Merck (Germany). Gallic acid, fetal bovine serum (FBS), 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), RPMI 1640, trypsin and phosphate buffered saline (BPS) were provided by Sigma-Aldrich (USA).

2.2. Plant material

In this study, four species of *Salvia* were collected in April and May 2014 from different areas of Kashan, Iran (Table 1). These plants were identified [20, 21] in the University of Kashan, Iran. The voucher specimens were deposited in the University of Kashan Herbarium (UKH).

Table 1. Locations and voucher specimens of the plant species

Number	Plant	Location	Altitude	Voucher specimens	Date
1	<i>Salvia syriaca</i>	Eznaveh-Kashan N=34° 00' 54" E= 51° 00' 46"	2690 m	UKH ^a 247	April 2014
2	<i>Salvia ceratophylla</i>	Eznaveh-Kashan N=34° 06' 30" E=51°00'41"	2690 m	UKH246	April 2014
3	<i>Salvia limbata</i>	Ghazaan-Kashan N= 33° 42' 38" E=51° 24' 13"	2316 m	UKH215	April 2014
4	<i>Salvia limbata</i>	Dorreh-Kashan N= 33° 52' 37" E= 51° 20' 34"	1630 m	UKH454	April 2014
5	<i>Salvia reuteriana</i>	Dorreh-Kashan N=33° 52' 40" E=51° 20' 29"	1570 m	UKH453	April 2014
6	<i>Salvia reuteriana</i>	Maragh-Kashan N= 33° 54' 42" E= 51° 06' 30"	2056 m	UKH700	May 2014

^a. University of Kashan Herbarium

2.3. Preparation of the extracts

After washing and drying the plants at room temperature in the shade, aerial parts were powdered. Then, 20 g of each dried sample was extracted with 300 ml of methanol in a soxhlet apparatus during 8 h. The extracts were concentrated through rotary evaporation under pressure, at controlled temperature (45 to 50 °C). Then, the residual solvent was removed using a vacuum oven at 50 °C. The obtained extracts were kept in the dark at 4 °C until subsequent analyses within a few days.

2.4. Cell lines and culture

The HeLa cell line and normal human dermal fibroblast (HDF) cell line were provided by the National cell bank of Iran, Pasteur Institute of Iran, Tehran, Iran. The cell lines were incubated with RPMI 1640 medium supplemented with FBS (10 %) and, Penicillin (1 %) and Streptomycin (1 %) at 37 °C and 5 % CO₂. The

viability of cells was assessed using the trypan blue dye exclusion test.

2.5. Cytotoxicity assay

The cytotoxicity of samples was determined based on MTT assay [22]. Briefly, cells were transferred to 96-well plates (at 15000 cells/ml density) and incubated at 37 °C and 5 % CO₂. After 24 h, cells in each well were treated with 100 µl of extract, at a concentration in the range of 0.03125 to 1 mg/ml. After 72 h of incubation, each well was added with 100 µl of MTT solution (0.5 mg/ml). Following incubation for 4 h, 100 µl of isopropanol was added to each well. After 20 min of incubation, the absorbance levels of different wells were measured using an ELISA Readers device at 570 nm. The percentage of cell growth inhibition was calculated according to the following formula:

$$\text{Growth inhibition \%} = 100 - \left[\frac{\text{OD extract treated cells}}{\text{control cells}} \right] \times 100$$

In all experiments, the cells incubated with solvent (without any extract) were used as the control group. Then we determined IC₅₀ values (the concentrations of plant extracts that resulted in 50 % cell viability).

SI value expresses the selectivity grades of the compounds under investigation. Therefore, a compound with a high SI value (> 2) is suggested to cause selective toxicity on cancer cells, while lower SI values (< 2) result in general toxicity [23, 24].

Here, SI value was calculated as the following:

SI value = IC₅₀ of normal cell/IC₅₀ of cancer cell

2.6. Determination of the free radical scavenging activity using DPPH assay

The ability of the extracts to scavenge DPPH was determined using a published method for DPPH radical scavenging activity assay [25], with some modifications. Briefly, the stock solutions (1 mg/ml) of each plant extract and also BHT (synthetic standard antioxidant) were separately prepared in methanol. Then, serial dilutions were prepared to obtain different concentrations of the extract (0.0005 - 1 mg/ml). One ml of each dilution was added to 1 ml of 94 µg/ml DPPH solutions in methanol, which were then incubated for 30 min in the dark, at room temperature. The absorbance levels of solutions were recorded using a spectrophotometer (UV1800, Shimadzu, Japan) at 517 nm. The inhibition percentage of free radical in DPPH assay was calculated as the following:

$$I \% = \left[\frac{(\text{blank} - \text{sample})}{\text{blank}} \right] \times 100$$

In this method, the concentration of the blank sample was similar to those of extracts or BHT samples in the absence of DPPH. The IC₅₀ value for each sample was calculated through plotting the inhibition percentages against concentrations of the methanolic extracts.

2.7. Total phenol content

The total phenol contents of methanol extracts of *Salvia* were determined using a previously described Folin-Ciocalteu method [26] with minor modifications. Briefly, 0.02 ml of the extract solution was pipetted into a 5ml volumetric flask, which was then added with 3 ml of distilled water and 0.1 ml of Folin-Ciocalteu. Then, the mixture was thoroughly shaken. After 3 minutes, 0.3 ml of 2 % Na₂CO₃ solution was added, followed by incubation at room temperature for 2 h with periodic shaking. The absorbance values were then measured at 760 nm against the blank sample.

The above-mentioned procedure was repeated for the standard gallic acid solution and the following equation was obtained according to the standard curve:

$$\text{Absorbance} = 0.001 X + 0.003$$

X: Concentration of gallic acid (µg/ml)

The total phenol content of each extract was expressed as µg of gallic acid equivalent in 1 mg of dried extract.

2.8. Total flavonoid content

The calorimetric method with aluminum chloride was used to determine the total flavonoid content [27]. Briefly, 0.5 ml of the solution of each plant extract was mixed with 1.5 ml of methanol. Then 0.1 ml of aluminum chloride (10 %), 0.1 ml of sodium acetate (1M), and 2.8 ml of distilled water were added. The solution was kept at room temperature for 30 min. Then, the absorbance levels of mixtures were measured at 415 nm, using a spectrophotometer (UV1800, Shimadzu, Japan).

The same procedure was repeated for the standard quercetin solution and the following equation was obtained according to the standard curve:

$$\text{Absorbance} = 0.0078 X + 0.02$$

X: Concentraion of quercetin ($\mu\text{g/ml}$)

The total flavonoid content was expressed as μg of quercetin equivalent per mg of dried extract.

2.9. Statistical analysis

In this study, all experiments were performed in triplicate. The data shown in Tables 2 and 3 have been expressed as mean \pm SD. The IC_{50} levels were acquired using a curve expert statistical program. One-way analysis of variance (ANOVA) followed by Post hoc tests were used to compare the results for the examined extracts, using SPSS 22.0 software (SPSS Ins, USA). P value < 0.05 was considered to be statistically significant.

3. Results

3.1. Cytotoxic activity

In this study, the cytotoxicity effects of all methanol extracts of *S. limbata*, *S. syriaca*, *S. reuteriana* and *S. ceratophylla* on HeLa cervical cancer and human dermal fibroblast (HDF) cell line were examined. The results have been shown in Table 2. The cytotoxic activity was observed in three of the six tested extracts on the HDF and five of the six tested extracts on the HeLa cells. The extracts of *S. reuteriana* showed the highest cytotoxicity levels among the four tested species of *Salvia*, with an IC_{50} value of $279 \pm 30.0 \mu\text{g/ml}$ (Fig. 1).

However, none of the tested methanolic extracts exhibited an SI value greater than 2, indicating the high selectivity of these samples.

3.2. Antioxidant properties and total phenol content

In this study, the antioxidant activities of extracts were evaluated based on free radical scavenging using DPPH method. The total phenol and flavonoid contents were also determined. The results have been shown in Table 3. The IC_{50} levels of methanolic extracts in DPPH assay were in the range of 39.8 ± 0.15 to $163.77 \pm 0.63 \mu\text{g/ml}$. The highest DPPH radical-scavenging activity was observed in the case of *S. ceratophylla* extracts with an IC_{50} value of $39.08 \pm 0.15 \mu\text{g/ml}$ and then, *S. limbata* and *S. reuteriana* (both from Dorreh) with IC_{50} values of $80.83 \pm 0.22 \mu\text{g/ml}$ and $86.76 \pm 0.3 \mu\text{g/ml}$, respectively. The total phenol contents ranged from 42.7 ± 3.09 to $105.8 \pm 1.15 \mu\text{g/mg}$, in which the highest values were corresponding to *S. ceratophylla*. In this research, the total flavonoid content ranged from 30.07 ± 2.52 to $82.46 \pm 2.2 \mu\text{g/mg}$, such that *S. reuteriana* from Dorreh and *S. ceratophylla* displayed the highest contents of flavonoid (82.46 ± 2.2 and $78.36 \pm 2.68 \mu\text{g/mg}$, respectively). Based on the results, there was a direct relationship between the antioxidant activity and phenol content of the compounds.

Table 2. Cytotoxic activity of different methanolic extracts of *salvia* species on HeLa cell line and normal human fibroblast (HDF) cell line

Plant	IC_{50} ($\mu\text{g/ml}$)		
	HeLa cell line	HDF	SI
<i>Salvia syriaca</i> 247	520 ± 30.0	605 ± 20.0	1.16
<i>Salvia ceratophylla</i> 246	907 ± 40.0	> 1000	1.24
<i>Salvia limbata</i> 215	885 ± 60.0	> 1000	1.19
<i>Salvia limbata</i> 454	> 1000	> 1000	> 1
<i>Salvia reuteriana</i> 453	279 ± 30.0	373 ± 40.0	1.33
<i>Salvia reuteriana</i> 700	500 ± 10.0	760 ± 30.0	1.52

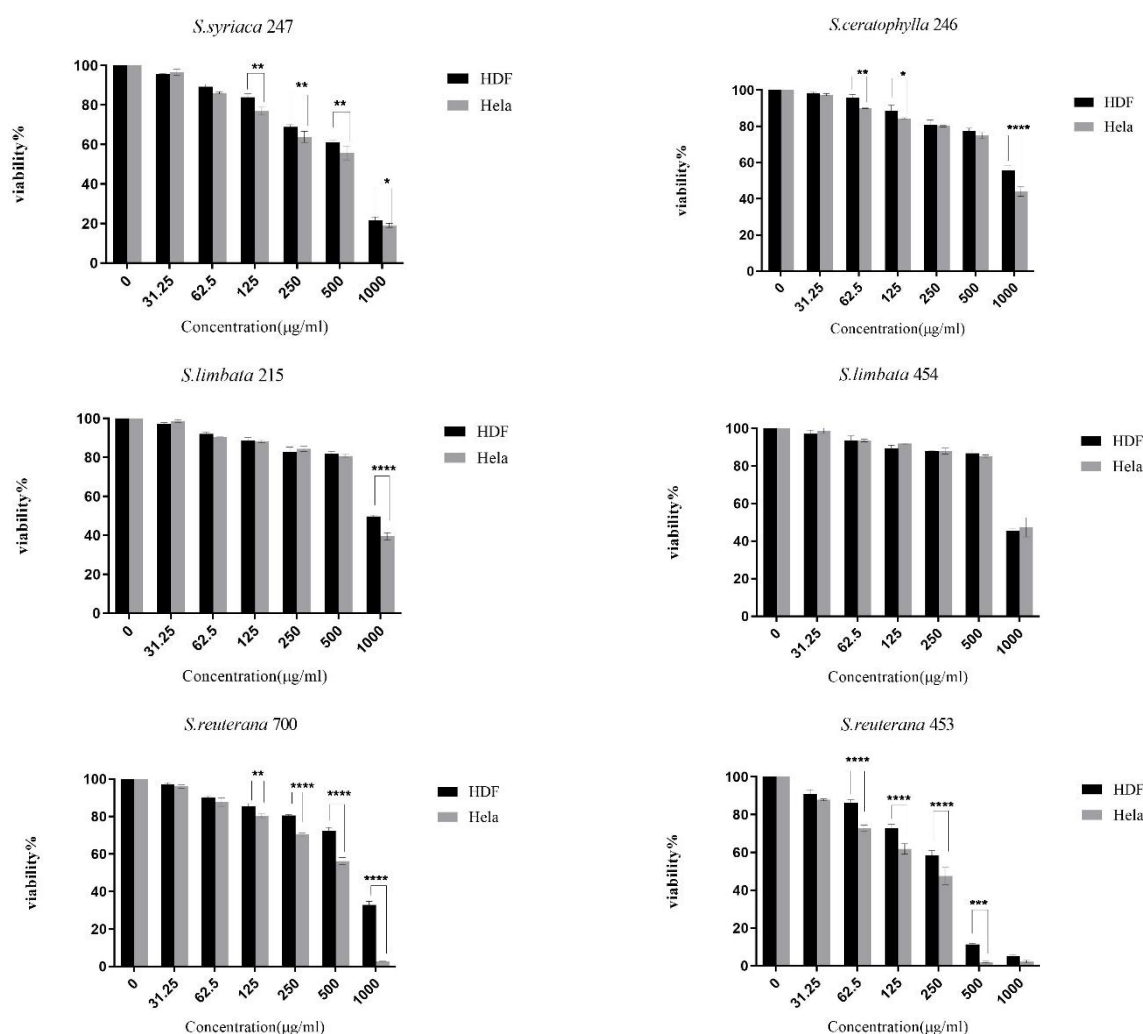


Fig. 1. Dose-dependent growth inhibition of HeLa and normal fibroblast (HDF) cell line by different extracts of *salvia* species (namely, *S. reuteriana*, *S. limbata*, *S. syriaca* and *S. ceratophylla*). HeLa and HDF cell lines were incubated in the presence of different concentrations of methanol extracts for 72 h. Cytotoxicity was determined through the MTT assay. Data have been presented as the mean \pm SD, from three independent experiments. (****) $P < 0.0001$; (***) $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$. (One-way ANOVA followed by Tuckey's post Hoc test).

4. Discussion

Many studies have been recently conducted on the cytotoxicity of various *Salvia* species. In a previous study in 2011, the cytotoxic activities of the methanolic extracts from leaves of 23 species of sage plant have been investigated against human cervix adenocarcinoma (HeLa), skin carcinoma (A431) and breast

adenocarcinoma (MCF7) cells using MTT assay. According to the obtained results, none of the tested extracts had a significant cytotoxic effect (above 50 %) on HeLa and MCF7 cells. *S. ringens* exhibited the strongest activity among the studied *salvia* species with a 61.8 % cell growth inhibitory activity on A431 [28].

Table 3. Phenol contents and DPPH radical scavenging potentials of the methanol extracts of plants

Plant name	IC ₅₀ DPPH ^a	Total phenol ^b	Total flavonoid ^c
<i>Salvia syriaca</i> 247	155.71 ± 0.005	51.2 ± 3.1	40.51 ± 0.77
<i>Salvia ceratophylla</i> 246	39.08 ± 0.15	105.8 ± 1.15	78.36 ± 2.68
<i>Salvia limbata</i> 215	163.77 ± 0.15	42.7 ± 3.09	30.07 ± 2.52
<i>Salvia limbata</i> 454	80.83 ± 0.22	79.4 ± 0.5	58.01 ± 3.57
<i>Salvia reuteriana</i> 453	86.76 ± 0.3	71.9 ± 0.65	82.46 ± 2.2
<i>Salvia reuteriana</i> 700	112.5 ± 0.74	59.73 ± 0.3	69.13 ± 3.8
BHT	19.72 ± 0.82	-	-

Notes: Data are expressed as means ± SD

^a IC₅₀ µg/ml ± SD

^b Microgram of gallic acid equivalents/mg of dried extracts ± SD

^c Microgram of quercetin equivalents/mg of dried extracts ± SD

Positive controls: BHT

DPPH test: one-way ANOVA analysis: P < 0.0001. Duncan post test: significant differences were observed between all extracts (P < 0.05). Total phenol content: one-way ANOVA analysis: P < 0.0001. Duncan post test: significant differences were observed between all extracts (P < 0.05). Total flavonoid content: one-way ANOVA analysis: P < 0.0001. Duncan post test: significant differences were observed between all extracts (P < 0.05) except for *S. ceratophylla* 246 and *S. reuteriana* 453 (P > 0.05)

In a research, *S. ceratophylla* exhibited the highest inhibitory activity against amelanotic melanoma (C32) in comparison with other tested *salvia* species [29]. In another study, *S. reuteriana* methanolic extracts have been reported to exhibit a strong cytotoxic activity on the Raji lymphoma cell line. These extracts with an IC₅₀ value of 156 ± 5 µg/ml, caused inhibition of HeLa cell growth [30].

In 2012, researchers investigated the antiproliferative activities of *S. ceratophylla*, *S. hormium*, *S. syriaca*, *S. dominica*, *S. fruticosa* and *S. spinosa* on models of breast cancer; MCF-7, T47D, ZR-75-1 and, BT474 cells. The authors suggested that this *Salvia* species is a natural source for novel anticancer therapy [31].

In an investigation in 2013, the cytotoxic activities of *S. limbata*, *S. aegyptiaca*, *S. xanthocheila*, *S. aethiopsis*, *S. syriaca*, *S. sclarea*, *S. eremophila*, *S. santolinifolia*, *S. hypoleuca*, *S. atropatana* and *S. nemorosa* from different regions of Iran have investigated. Among the tested plants, the methanol and dichloromethane extracts of *S. limbata*, *S. hypoleuca* and *S. aethiopsis* exhibited significant

cytotoxic effects on HL60, K562, and MCF-7 cells [32]. In a study published in the 2016 year, the methanolic extract of *S. hypargeia* exhibited no cytotoxic activity against the HeLa cells and Vero normal cell line [33].

It has been reported in 2013, that ethanol extract of *S. officinalis* showed cytotoxic activity to HeLa cell line with an IC₅₀ value of 122.22 ± 3.30 µg/ml [34]. According to work published in 2019 *S. libanotica* extracts is not able to suppress cancer cell viability in HeLa cervical cancer cell line, but this extracts in combination with balsam oil exhibited moderate anti-cancer activity [35].

Researchers have investigated the antioxidant activities of two populations of *S. limbata* from Takab (Azerbaijan province) and Mashhad Ardehal (Kashan province) using DPPH assay and reported IC₅₀ values in the range 40.5 ± 0.3 to 32.3 ± 0.42 µg/ml [36].

In another study on the antioxidant activity of 11 *Salvia* species from Iran, *S. limbata* exhibited the weakest antioxidant activity with an IC₅₀ value of 557 ± 12.73 µg/ml and total phenol content of 12.95 ± 0.70 mg/g [32].

In a study on the antioxidant properties of five *Salvia* species, *S. reuteriana* showed an IC₅₀ value of 125.1 µg/ml and total flavonoid content of 46.97 µg/mg [37]. According to the findings of researchers using DPPH assay (published in 2014), *S. ceratophylla* showed an IC₅₀ value of 5.5 ± 0.1 µg/ml and total phenol and flavonoid contents of 32.7 mg gallic acid/g dried extract and 27 mg catechin / g dried extract, respectively [29].

The outcomes of the present research and previous studies indicate that *Salvia* species exhibit weak to moderate antioxidant activities.

5. Conclusion

The plants of the genus *Salvia* family include various metabolites such as phenolic acids, flavonoids, terpenoids and polysaccharides. These metabolites are responsible for different pharmacological effects. In this study, four Iranian *Salvia* species were investigated in terms of their cytotoxic and antioxidant activities. According to the obtained results, the methanolic extracts of this species showed moderate to low cytotoxic effects on the HeLa cells. Among the studied extracts, *S. reuteriana* from Dorreh showed the highest cytotoxicity

level. All examined extracts also exhibited good to low antioxidant activities. The *S. ceratophylla* extract exhibited the highest antioxidant activity. In this study the concentration of phenol and antioxidant activity is higher in *S. ceratophylla* extract than in *S. reuteriana*, but cytotoxic activity of *S. reuteriana* extract against HeLa cells is better than that of *S. ceratophylla*. Further detailed studies on these extracts and various cancer cell lines, are still required to find active components that can be used in cancer treatment.

Author contributions

All authors contributed toward data analysis, drafting, and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgements

This research was financially supported by the University of Kashan and National Cell Bank of Iran, Pasteur Institute of Iran.

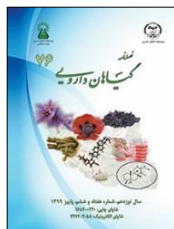
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How to cite this article: Iravani M, Mahinpour R, Zahraei Z, Toluei Z, Asgari F and Haghighipour N. Comparison of cytotoxic and antioxidant activities and phenolic content of four *Salvia* L. species from Iran. *Journal of Medicinal Plants* 2020; 19(76): 59-68. doi: 10.29252/jmp.19.76.59



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مقاله تحقیقاتی

مقایسه فعالیت آنتی‌اکسیدانی و سمیت سلولی و محتوای فنلی چهار گونه مریم‌گلی از ایران

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چکیده

اطلاعات مقاله

گل‌واژگان:

فعالیت آنتی‌اکسیدانی

سمیت سلولی

مریم‌گلی

محتوای فنلی

محتوای فلاونوئیدی

مقدمه: استفاده از گیاهان دارویی از زمان‌های قدیم یکی از بهترین روش‌های درمانی محسوب می‌شدند. امروزه نیز گیاهان به علت خواص آنتی‌اکسیدانی، ضد میکروبی، ضد سرطانی و دیگر پتانسیل‌های زیستی در طب سنتی و مدرن مورد استفاده قرار می‌گیرند. هدف: اندام‌های هوایی چهار گونه مریم‌گلی (*Salvia*) شامل دو جمعیت از مریم‌گلی اصفهانی (*S. reuteriana* Boiss.)، دو جمعیت از مریم‌گلی لبه‌دار (*S. limbata* C.A.Mey.) و یک جمعیت از هر یک از گونه‌های مریم‌گلی سوری (*S. syriaca* L.) و مریم‌گلی شاخ‌گوزنی (*S. ceratophylla* L.) از منطقه کاشان مورد بررسی قرار گرفت. روش بررسی: فعالیت آنتی‌اکسیدانی عصاره‌های متانلی نمونه‌های گیاهی به روش مهار رادیکال آزاد DPPH ارزیابی شد. محتوای ترکیب‌های فنلی و فلاونوئیدی تام با روش فولین سیوکالتیو و آلومینیوم کلرید سنجش شد. همچنین اثر سمیت سلولی عصاره متانلی نمونه‌ها بر رده سلولی HeLa با آزمون MTT بررسی شد. نتایج: در آزمایش DPPH میزان IC₅₀ عصاره‌های متانلی بین ۰/۱۵ ± ۳۹/۰۸ تا ۰/۶۳ ± ۱۶۳/۷۷ میکروگرم بر میلی‌لیتر متغیر بودند. میزان فنل بین ۳/۰۹ ± ۴۲/۷ تا ۱/۱۵ ± ۱۰۵/۸ میکروگرم بر میلی‌گرم و میزان فلاونوئید بین ۲/۵۲ ± ۳۰/۰۷ تا ۲/۲ ± ۸۲/۴۶ میکروگرم بر میلی‌گرم متغیر بودند. میزان فعالیت آنتی‌اکسیدانی گونه‌ها، با پلی‌فنل‌های موجود در آنها رابطه مستقیم داشت. عصاره‌های متانلی گونه‌های مریم‌گلی در این مطالعه بر رده سلولی HeLa اثر سمیت سلولی نشان دادند. نتیجه‌گیری: دو جمعیت مریم‌گلی اصفهانی بیشترین اثر سمیت سلولی را در مقایسه با دیگر گونه‌های مریم‌گلی نشان دادند. تمام عصاره‌های مورد بررسی دارای فعالیت‌های آنتی‌اکسیدانی ضعیف تا متوسط بودند. عصاره مریم‌گلی شاخ‌گوزنی با بالاترین فعالیت آنتی‌اکسیدانی همراه بود.

مخفف‌ها: DPPH، ۲،۲-دی فنیل-۱-پیکریل هیدرازیل؛ MTT، ۳-(۴،۵-دی متیل تیازول-۲-ایل)-۵،۲-دی فنیل تترازیولیم بروماید؛ IC₅₀ متوسط غلظت مهارکنندگی؛ BHT، بوتیل هیدروکسی تولوئن؛ HDF، فیبروبلاست پوست انسانی

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تاریخ دریافت: ۱۵ بهمن ۱۳۹۸؛ تاریخ دریافت اصلاحات: ۱۳ مرداد ۱۳۹۹؛ تاریخ پذیرش: ۳۱ مرداد ۱۳۹۹

doi: 10.29252/jmp.19.76.59

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