

Phenolic Contents, *in vitro* Antioxidant and Cytotoxicity Activities of *Salvia aethiopsis* L. and *S. ceratophylla* L. (Lamiaceae)

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Abstract: Along with present study it is designed to examine phenolic compositions, *in vitro* antioxidant and cytotoxicity activities of methanol and ethyl acetate extracts of *Salvia aethiopsis* L. and *S. ceratophylla* L. (Lamiaceae) from Turkey. Total phenolic contents of *S. aethiopsis* methanol (SA-ME) and ethyl acetate (SA-EA) extracts were between 94.36±1.36-290.62±1.51 mg GAE/g extract while *S. ceratophylla* methanol (SC-ME) and ethyl acetate (SC-EA) extracts were between 168.35±1.97-330.14±2.28 mg GAE/g extract. The main phenolic acid of the methanol and ethyl acetate extracts was rosmarinic acid (40.25 and 140.6 µg/100 g plant for *S. aethiopsis*; 74 and 234.5 µg/100 g plant for *S. ceratophylla*). Phenolic acids of *S. aethiopsis* and *S. ceratophylla* extracts has much more cinnamic acid derivatives then benzoic acid derivatives. Studied *Salvia* extracts showed dose-dependent radical scavenging activities. HPLC results allow to make a correlation between antioxidant capacity and quantity of these phenolic acids showed as strongly antioxidant components. It is suggested that to utilize the potential antioxidant properties of these plant extracts, they can be used in safe under 266 µg mL⁻¹, 230 µg mL⁻¹, 150 µg mL⁻¹ and 133.3 µg mL⁻¹ for SC-ME, SA-ME, SA-EA and SC-EA extracts, respectively. The proliferation of the cells was assessed by the MTT assay. Viability percentage of the extracts was determined relative to controls and measured on 15.6 - 1000 µg mL⁻¹ extract concentrations. IC₅₀ value of SA-ME extract at 24 hours was 230.0 ± 17.3 and at 48 hours was 93.3 ± 5.8 while 266.7 ± 41.6 (24 h) and 180.0 ± 20.0 (48 h) for SC-ME extract. Toxicities of extracts were decreased in turn, SA-EA>SC-EA>SA-ME>SC-ME. The most toxic extract was SA-EA also with high phenolic contents while SC-ME was the lowest.

Keywords: *Salvia aethiopsis* L.; *S. ceratophylla* L.; antioxidant activity; phenolic contents; cytotoxicity. © 2017 ACG Publications. All rights reserved.

1. Introduction

Plants are potential sources of natural bioactive compounds known as secondary metabolites. There is a relationship between the chemical structures of the most abundant compounds in the plants and their functional properties [1, 2]. Many disorders may be the result of increased concentrations of

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free radicals in an organism [3, 4]. Harmful effects of free radicals can be largely prevented by intake of antioxidant substances [5]. Antioxidants have an importance on the prevention of oxidative stress and inhibit the oxidation of oxidizable substrates as reactive oxygen species (ROS) [6]. Medicinal and aromatic plants contain a wide variety of free radical scavenging molecules, such as polyphenolic compounds (flavonoids, phenolic acids, anthocyanidins and procyanidins, etc.). Epidemiological studies have shown that many of antioxidant compounds from plants possess to anti-inflammatory, antibacterial, antiproliferative, anticarcinogenic, antimutagenic etc. activities substantially [5]. Many plants used as food or in traditional medicine can have mutagenic, cytotoxic or genotoxic hazards resulting from the long-term usage of such plants [7]. Usage of *in vitro* cytotoxicity tests has a necessity defining the basal cytotoxicity. Cytotoxicity tests are also useful to define the concentration range for further and more detailed *in vitro* testing to provide meaningful information on parameters such as genotoxicity, induction of mutations or programmed cell death. By establishing the dose at which 50% of the cells are affected, it is possible to compare quantitatively responses of a single compound in different systems or of several compounds in individual systems. [8]. Especially to screening the internal usage of plant extracts' can be accepted a combination of compounds acts together synergistic has a noteworthy dignity. Several studies have been carried out on natural sources to unravel the components which possess antioxidant properties and with low cytotoxicities. Natural antioxidants are generally more desirable for consumption than the synthetic one such as butylated hydroxytoluene (BHT) which reported to being carcinogenic to humans [9]. *Salvia* is one of the largest genus of Lamiaceae, represented by nearly 1.000 species all around the World [10] and nearly 100 species in Turkey [11]. In Turkey *Salvia* species have ethnobotanically usages for the treatment of stomachache, asthma, analgesic, sore throat, cold, bronchitis as an infusion of leaves and inflamed wounds as a decoction, analgesic as boiled leaves in water [12-15]. The objective of this study is to evaluate the phenolic contents and antioxidant activities of *Salvia aethiopsis* L. and *S. ceratophylla* L. from Turkey and make a suggestion about a possible link between these results and reliable doses to get these plants potential healing properties.

2. Materials and Methods

2.1. Plant Materials

S. aethiopsis L. (Lamiaceae) was collected from Turkey, Eskisehir, Anadolu University, Yunus Emre Campus, 750 m, 17.06.2011 and *S. ceratophylla* L. (Lamiaceae) was collected from Turkey, Eskisehir, Gürleyik-Gürleyik stream spring road, 688 m, 06.06.2010. Collected plants were deposited in Anadolu University Faculty of Pharmacy Herbarium (ESSE 14584, 14579). Species were identified according to Flora of Turkey and the East Aegean Islands [16].

2.2. Preparation of the extracts

Dried herba of *S. aethiopsis* and *S. ceratophylla* were ground and extracted with petroleum ether in a Soxhlet apparatus. Fat-free air-dried material was extracted with methanol:water (70:30, v/v) at 40°C, 30 min four times. Extracts were concentrated to dryness in vacuum and the aqueous solution was lyophilized (*S. aethiopsis* methanol extract=SA-ME and *S. ceratophylla* methanol extract=SC-ME). Second extracts were prepared as follows: fat-free air-dried material was extracted with methanol:water (70:30 v/v) at 40°C, 30 min four times, and it was concentrated in a vacuum, and the aqueous phase was extracted with ethyl acetate at room temperature (*S. aethiopsis* ethyl acetate extract=SA-EA and *S. ceratophylla* ethyl acetate extract=SC-EA). Then it was concentrated to dryness in vacuum. Thus, recovery of the phenolic acids with some solvents of different polarity was attempted. All extracts obtained were weighed to determine the yields of soluble constituents [17].

2.3. Determination of total phenolic content (Folin-Ciocalteu Method)

Determination of total phenolic content in ethyl acetate and methanol extracts from studied *Salvia* species was carried out according to Singleton and Rossi [18] using a Folin-Ciocalteu colorimetric method, calibrating against gallic acid as the reference standard and expressing the results as gallic acid equivalents (GAE) and the calibration formula obtained from gallic acid graph were given in supporting information (S1).

2.4. Separation and analysis of phenolic acids by HPLC

An HPLC system consisting of a model of 600 E HPLC pump, 717 plus autosampler, 996 photodiode array detector (PAD), and a data processor (Millennium 32) was used (Waters Corp., Massachusetts, USA). Ultrapure water ($18.2 \mu\text{S cm}^{-1}$) from a Millipore (Molsheim, France) water purification system and an octadecylsilane (ODS, C18). Ultrasphere column from Phenomenex (100x4.6 mm inner diameter, the particle size of $3 \mu\text{m}$) were utilized in the HPLC analysis. The mobile phase consisted of (A) methanol:water:formic acid [10:88:2 by volume] and (B) methanol:water:formic acid [90:8:2 by volume] using a gradient elution of 0% B at 0–15 min, 15% B at 15–20 min, 50% at 20–30 min, 100% B at 30–35 min, then back to 100% A at 36–42 min. The flow rate was always 1 mL/min and the injection volume was 10 μL . The signals were detected at 280 nm. Standard phenolic acids were prepared in a solvent consisting of water:methanol (50:50; v/v). The IS technique was applied to the analysis; propylparaben was the suitable IS. The relevant extracts were dissolved in a mixture of methanol and water (1:1 v/v), and the mixture was injected into the HPLC apparatus [19, 20]. The supportive information of the HPLC method are given in supporting information part (the original chromatograms of standard, *S. aethiopsis* and *S. ceratophylla* ethyl acetate extracts are shown in S2-S5).

2.5. DPPH Free Radical Scavenging Activity

The DPPH assay was used as a rapid spectroscopic method to provide an evaluation of antioxidant activity due to scavenging free radicals. Free radical scavenging potentials tested by the DPPH method [21, 22] of different extracts from *Salvia* species at different concentrations are determined. The reaction mixture was left at ambient temperature for 30 min in dark; absorbance of the resulting solution was then measured spectrophotometrically at 517 nm. The results were compared to those of BHT as a synthetic antioxidant. The extracts were tested for their antioxidant activity at 9.4×10^{-4} , 1.8×10^{-3} , $3.6 \times 10^{-3} \mu\text{g mL}^{-1}$ concentrations.

2.6. Antioxidant Activity using β -Carotene/Linoleic acid system

Antioxidant activity of extracts from relevant *Salvia* species was determined according to β -Carotene bleaching methods. In β -Carotene/Linoleic acid system, β -Carotene undergoes a rapid discoloration in the absence of an antioxidant. The presence of an antioxidant such as phenolics can hinder the extent of β -Carotene destruction by “neutralizing” the linoleate free radical and any other free radicals formed within the system [23]. Obtained results were compared to those of BHT as a synthetic antioxidant.

2.7. Cell culture and extracts treatment and MTT assay for cytotoxicity of extracts

NIH/3T3 cells were obtained from the American Type Culture Collection (ATCC, USA). The cells were incubated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, Paisley, Scotland), 100 IU/mL penicillin (Gibco) and 100 mg/mL streptomycin (Gibco) at 37°C in a humidified atmosphere of 95% air and 5% CO_2 . Exponentially growing cells were plated at 2×10^4 cells/mL into 96-well microtiter tissue culture plates (Nunc, Denmark) and incubated for 24 and 48 hours. Before the addition of the extracts (the optimal cell number for

cytotoxicity assays was determined in preliminary experiments) stock solutions of extracts were prepared in dimethyl sulphoxide (DMSO; Sigma & Aldrich, Poole, UK) and further dilutions were prepared with fresh culture medium (the concentration of DMSO in the final culture medium was <0.1% which had no effect on the cell viability).

MTT is widely used as a measure of cytotoxicity. After 24 or 48 hours of preincubation, the tested extracts were added to give a final concentration in the range 15.6-1000 µg/mL and the cells were incubated for 24 and 48 hours. At the end of this period, MTT was added to a final concentration of 0.5 mg/mL and the cells were incubated for 4 h at 37°C. After the medium was removed, the formazan crystals formed by MTT metabolism were solubilized by addition of 200 µL DMSO to each well and absorbance was read at 540 nm with a microtitre plate spectrophotometer (Bio-Tek Plate Reader). Each concentration was repeated in three wells and IC₅₀ values were defined as the extract concentrations that reduced absorbance to 50% of control values [24-26] and rosmarinic acid were used as positive control.

2.8. Statistical Analysis

All data were the average of triplicate analyses. Mean values, standard deviations (SD), medians, and both minimum and maximum contents of all achieved results were also determined. Correlation analysis of phenolic contents was carried out using the correlation and regression program in the Microsoft EXCEL program.

3. Results and Discussion

3.1. Total phenolic contents and phenolic acids

Table 1 shows the results of comprising the extraction yields, total phenolic contents of the studied *Salvia* species extracts recovered with solvents of different polarities. The extraction yield as a percentage of plant material ranges from 2.21 to 22.4. It was obtained methanol extracts of all species showed a higher extraction yield when compared with ethyl acetate extracts. The total phenolic contents of *S. aethiopsis* extracts were in the range between 94.36 ± 1.53 – 290.62 ± 3.51 mg GAE/g extract while *S. ceratophylla* extracts were between 168.35 ± 1.97 - 330.14 ± 2.28 mg GAE/g extract. SC-EA was found to the richest as a source of phenolics. SA-EA contained a higher amount of total phenolic contents then SC-ME and SA-ME. The highest result of total phenolic contents was found in SC-EA extract, in turn, increasingly SA-ME>SC-ME>SA-EA>SC-EA (Table 1).

Table 1. Extraction yields and total phenolic contents of extracts of studied *Salvia* species.

Extracts*	Yield (%)	Total Phenolic Contents**
SA-ME	21	94.36±1.53
SA-EA	2.69	290.62±3.51
SC-ME	22.4	168.35 ± 1.97
SC-EA	2.21	330.14 ± 2.28

**S. aethiopsis* and *S. ceratophylla* methanol (SA-ME and SC-ME) and ethyl acetate (SA-EA and SC-EA) extracts.

The phenolic acids amounts of extracts were detected using RP-HPLC-DAD method and were shown in Table 2. SC-EA is the richest one containing the *tr*-CIN (122.02 µg/100 g plant) and SA-EA is richest one having the *p*-COU (63.44 µg/100 g plant). SC-ME and SA-ME are the richest extracts containing the ChA (25.80-26.07 µg/100 g plant). SA-ME, SA-EA and SC-EA were contained in the different amount of all studied phenolic acids, except GA. Also seen in Table 2, the main phenolic acid of the extracts was rosmarinic acid (RA) (40.25 - 234.54 µg/100 g plant). RA has antioxidant and anti-inflammatory properties [27]. The polyphenolic structure of RA has related with antioxidant effect. Two diphenolic rings of the RA can be oxidized to their respective *o*-quinones by

autooxidation, to generate ROS. Superoxide anion and hydrogen peroxide are formed by an auto-catalytic mechanism and RA is a substrate of peroxidase which leads to the formation of free radicals [28]. RA is the most abundant caffeic acid dimer in *Salvia* species. It was reported to be the major phenolic compound responsible for the high antioxidant activity of *Salvia* samples [29].

Table 2. The contents of phenolic acids ($\mu\text{g}/100$ g extract) in extracts of studied *Salvia* species.

Extracts*	Phenolic acids** ($\mu\text{g}/100$ g extract)											
	GA	protoCA	<i>p</i> -OHBA	VA	CA	ChA	SA	<i>p</i> -COU	FA	RA	<i>o</i> -COU	<i>tr</i> -CIN
SA-ME	---	1.41	7.39	0.56	1.06	25.80	2.93	1.47	3.00	40.26	1.07	21.30
SA-EA	---	8.03	10.12	4.01	4.46	3.08	8.40	63.44	15.97	140.65	1.61	11.02
SC-ME	1.16	1.46	13.30	2.43	12.23	26.07	2.61	2.38	25.10	74.00	13.65	22.76
SC-EA	---	5.98	7.53	5.63	8.91	63.84	7.10	6.75	7.42	234.54	7.27	122.02

**S. aethiopsis* and *S. ceratophylla* methanol (SA-ME and SC-ME) and ethyl acetate (SA-EA and SC-EA) extracts.

**GA: Gallic acid, protoCA: Protocatechuic acid, *p*-OHBA: *p*-Hydroxybenzoic acid, VA: Vanillic acid, CA: Caffeic acid, ChA: Chlorogenic acid, SA: Syringic acid, *p*-COU: *p*-Coumaric acid, FA: Ferulic acid, RA: Rosmarinic acid, *o*-COU: *o*-Coumaric acid, *tr*-CIN: *tr*-Cinnamic acid.

Lamiaceae has phenolics rich plants [30, 31]. A member of this family the genus *Salvia* L. has a lot of species used in traditional medicine in the world and also in Turkey [32, 33]. The total phenolic contents of different extracts of some *Salvia* species were studied before. Loizzo *et al.* [34] were found total phenolic contents of *S. ceratophylla* methanol extract as 32.7 mg GAE / g dried extract. It was found for the EtOAc extract from the aerial parts of *S. glutinosa* had the highest total phenol amount (126.75 ± 4.68 mg g⁻¹ extract), while the EtOH extract of the aerial parts of *S. cryptantha* was found to contain the highest total phenol amount (392.18 ± 4.70 mg g⁻¹ extract) as gallic acid equivalent by Erdoğan Orhan *et al.* [35].

With another study, the total phenolics of infusions of *S. tomentosa* was found 87.87 mg g⁻¹ and *S. fruticosa* was found as 129.94 mg g⁻¹ while gallic acid was 92.57 mg g⁻¹ [36]. Belmekki and Bendimerad [37] were reported the total phenolic content of *S. verbenaca* methanol-water (7:3) extract as 0.845 ± 9.33 while mg GAE/g dried extract. It has an awareness and interest in the antioxidant behaviour and potential health benefits associated with plant based simple phenolic acids and their role as dietary antioxidants recently.

Because of their ubiquitous presence in plant-based foods, humans consume phenolic acids on a daily basis. The estimated range of consumption is 25 mg-1 g a day depending on diet (fruit, vegetables, grains, teas, coffees, spices) [38]. It was reported 10 phenolic acids for ethyl acetate and methanol extracts of 16 Turkish *Salvia* species for their inhibitory activity against some enzymes linked to neurodegeneration as acetylcholinesterase and butyrylcholinesterase (BuChE) Along with this study, *S. cassia* methanolic extract was the most effective the species towards BuChE and its high inhibitory activity on mentioned enzyme might be a result of some other components as well as rosmarinic acid. Also reported that *S. ekimiana* methanol extract was the highest amounts of phenolic acids as represent with present like protocatechuic, *p*-hydroxybenzoic, vanillic, chlorogenic, *p*-coumaric, ferulic, *o*-coumaric, rosmarinic and *tr*-cinnamic [39]. Kan *et al.* [40] found the values of the phenolic acids ranges from *S. ceratophylla* flowers, leaves, branches and roots were reported as 1.28 - 380 ng g⁻¹ for rosmarinic acid, 5.08 - 8.11 ng g⁻¹ for gallic acid, 21 - 173 ng g⁻¹ for caffeic acid and 94 - 450 ng g⁻¹ for chlorogenic acid which was detected in trace amount from roots. Koşar *et al.* [41] were studied five different extracts of *S. virgata* for their contents of phenolic acids and it was found that methanol extract was the richest for caffeic, *o*-coumaric and rosmarinic acids while 50% methanolic extract was the richest for gallic and *p*-hydroxybenzoic acids. Koşar *et al.* [42] were investigated phenolic acids of *S. halophila* extracts from Turkey and they found that methanol extract was the richest for gallic acid content while 50% methanol extract was the richest for *p*-hydroxybenzoic,

caffeic, and *o*-coumaric acids. The highest rosmarinic acid content was obtained from ethyl acetate extract ($48.9 \pm 2.12 \text{ mg g}^{-1}$). With another study, it was reported rosmarinic acid contents of *S. virgata*, *S. staminea* and *S. verbenaceae* methanolic extracts also from Turkey. There were contained rosmarinic acid in the ranges between $4.82 - 26.12 \pm 0.27 - 0.73 \text{ } \mu\text{g mg}^{-1}$ [43]. It has not been reached any study evaluated on phenolic acid contents of *S. aethiopsis* and *S. ceratophylla* methanol and ethyl acetate extracts before. Depend on our results, phenolic acids of *S. aethiopsis* and *S. ceratophylla* extracts has much more cinnamic acid derivatives.

3.2. Antioxidant activity

To evaluate the antioxidant activities of plants two different methods were used; radical scavenging capacities on DPPH and β -Carotene/Linoleic acid test systems. In DPPH test, the extracts were tested for their antioxidant activity at 9.4×10^{-4} , 1.8×10^{-3} , $3.6 \times 10^{-3} \text{ } \mu\text{g mL}^{-1}$ concentrations. The highest antiradical activity was observed in SA-EA, while the lowest activity was observed in SC-ME (S6 in supporting information part). The results were compared to those of BHT as a synthetic antioxidant and RA as natural antioxidant. The studied *Salvia* extracts showed dose-dependent radical scavenging activities.

Usually screening antioxidative activity in various model systems is important prior to the application of antioxidants in food. Free radical scavenging is one of the generally accepted mechanisms against lipid oxidation. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability [44]. There were some of the studies about antioxidant potentials of different *Salvia* species. It was found by Loizzo et al. [34], IC_{50} value for DPPH with $5.5 \pm 0.1 \text{ } \mu\text{g mL}^{-1}$ while ascorbic acid with 5.0 ± 0.8 for *S. ceratophylla* methanol extract. Antioxidant activities of eleven *Salvia* species from Iran reported by Firuzi et al. [45], IC_{50} values of 80% methanol extracts of the plants were 1.38 ± 4.6 and 557.40 ± 12.73 and as a reference quercetin was 1.79 ± 0.046 (mg plant extracted) while for *S. aethiopsis* it was 237.37 ± 8.05 . It was reported that antioxidant activities of *S. tomentosa* and *S. fruticosa* infusions were determined using DPPH radical scavenging test at a concentration of 0.25, 0.50 and 1.0 mg mL^{-1} by Erdogan-Orhan et al. [35]. The infusion of *S. fruticosa* (65.04%) had the highest scavenging effect towards DPPH while *S. tomentosa* had 34.01% and BHA had 81.60% at the concentration of 1.0 mg mL^{-1} . With another study, it was reported that *S. sericeo-tomentosa* var. *sericeo-tomentosa* methanol extract was showed the highest DPPH radical scavenging activity (90.29 ± 1.94) at $1000 \text{ } \mu\text{g mL}^{-1}$ while gallic acid was showed 93.42 ± 1.02 [39]. In another study it was analysed the antioxidant activity assayed by DPPH, aerial parts and roots of CH_2Cl_2 , EtOAc and EtOH extracts of different *Salvia* species collected from Turkey. The CH_2Cl_2 extract of aerial parts of *S. cryptantha* showed a better scavenging activity ($37.50 \pm 5.77\%$) against DPPH ($1000 \text{ } \mu\text{g mL}^{-1}$) then other mentioned extracts of *Salvia* sp. The EtOAc extract of aerial parts of *S. glutinosa* showed a better scavenging activity ($71.56 \pm 1.60\%$) against DPPH ($1000 \text{ } \mu\text{g mL}^{-1}$). The EtOH extract of aerial parts of *S. multicaulis* showed a better scavenging activity ($86.16 \pm 0.01\%$) against DPPH ($1000 \text{ } \mu\text{g mL}^{-1}$). For DPPH scavenging activity the gallic acid was used as reference (92.57 ± 0.10) [35]. Along with another study the IC_{50} value of methanolic extract of the *S. verbenaca* was found $9.79 \pm 0.47 \text{ } \mu\text{g mL}^{-1}$ and ascorbic acid was $0.27 \pm 0.12 \text{ } \mu\text{g mL}^{-1}$ which used as reference [37]. Along with this present study results, antioxidant activity of SA-EA was similar to the activity of BHT, but lower than RA. The studied *Salvia* extracts showed dose-dependent radical scavenging activities. HPLC results allow us to make a correlation between antioxidant capacity and quantity of these phenolic acids showed as strongly antioxidant components.

β -Carotene/Linoleic acid test system is commonly used to evaluate the antioxidant properties of various natural and synthetic antioxidants. In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [46]. It was reported that the inhibition ratio of linoleic acid oxidation by former system as 29.0 ± 2.05 for *S. aethiopsis* from Turkey [47]. According to our results, the highest antioxidant activity on the system of β -Carotene/Linoleic acid in four extracts of two *Salvia* species, in turn, increasingly BHT>RA>SA-ME>SC-ME>SA-EA>SC-EA(S7 in supporting information part).

Contrary to DPPH, in this test system, ethyl acetate extracts exhibited the lowest antioxidant activity. For radical scavenging activities (Inhibition %) of studied extracts, the highest inhibition of DPPH were found, in turn, increasingly RA>BHT>SA-EA>SC-EA>SC-ME>SA-ME while RA and BHT was reference. Ethyl acetate extracts of these two *Salvia* species was a distinctive antioxidant activity compared with the methanol extracts evaluated antioxidant activity by DPPH, depending on the highest phenolics and rosmarinic acid contents.

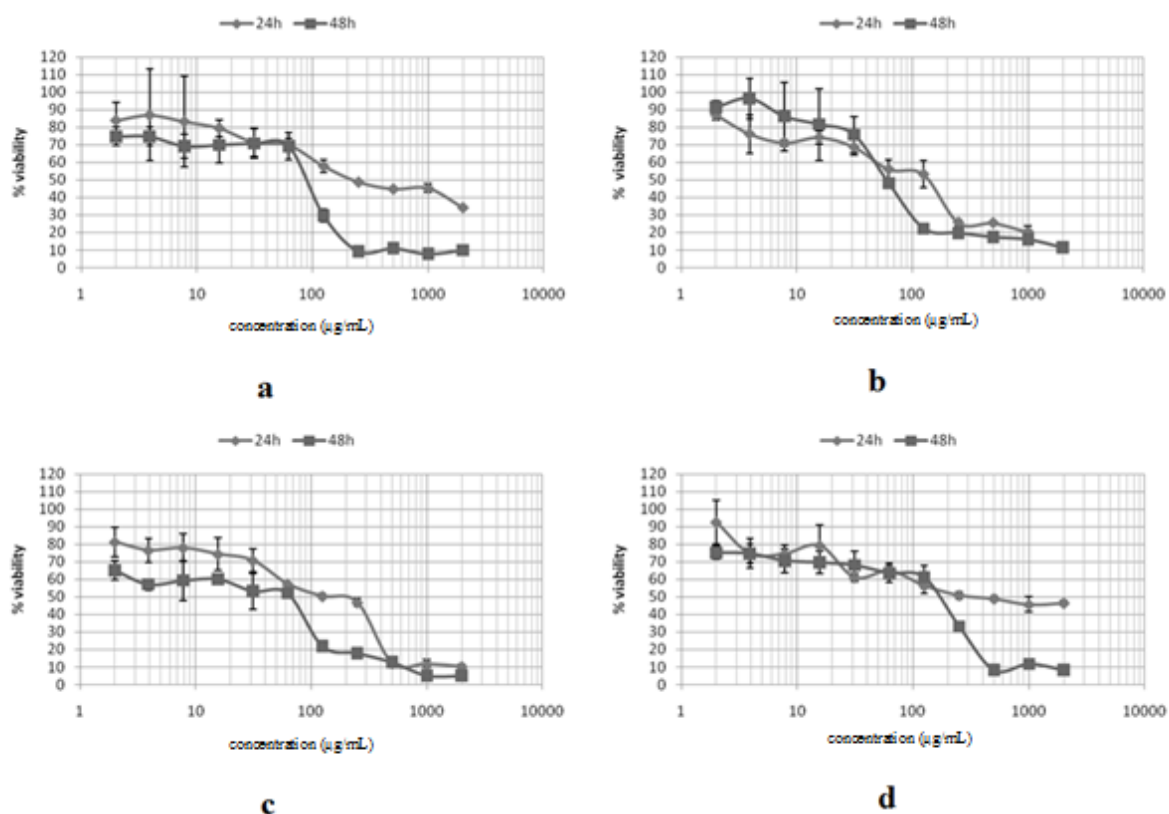


Figure 1. a. SA-ME, b. SA-EA, c. SC-ME and d. SC-EA extract against NIH/3T3 cell line (viability % to concentration ($\mu\text{g/mL}$) graphics in 24 and 48 h.

3.3. Cytotoxicity

The proliferation of the NIH/3T3 cells was assessed by the MTT assay, which based on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product [20]. Viability percentage of the extracts was determined compared to controls and measured on 15.6 - 1000 $\mu\text{g mL}^{-1}$ extract concentrations and rosmarinic acid were used as positive control. IC_{50} value of SA-ME at 24 hours was 230.0 ± 17.3 and at 48 hours was 93.3 ± 5.8 while 266.7 ± 41.6 (24 h) and 180.0 ± 20.0 (48 h) for SC-ME (Table 3). Toxicities of extracts were decreased in turn, SA-EA>SC-EA>SA-ME>SC-ME. The most toxic extract was SA-EA also with high phenolic contents while SC-ME was the lowest. Also, viability per cent to concentration ($\mu\text{g mL}^{-1}$) graphics for 24 and 48 hours were given in Figure 1. IC_{50} values of the RA were 201.7 ± 7.6 for 24 hours while it was 173.3 ± 32.1 for 48 hours. Viability percent of the cells was increasing for 24 hours depend on the concentration doses also same for 48 hours and the specific toxic potential of RA can interpret by these IC_{50} values. SA-EA extract had the highest radical scavenging activity at $3.6 \times 10^{-3} \text{ mg mL}^{-1}$ with IC_{50} of 45.68 while the highest antioxidant activity exerted by SA-ME extract at the same concentration with IC_{50} of 27.26.

Table 3. Viability of *S. aethiopsis* and *S. ceratophylla* methanol (SA-ME and SC-ME) and ethyl acetate (SA-EA and SC-EA) extracts and rosmarinic acid against NIH/3T3 cell line (viability % was determined compare to controls)

Concentrations ($\mu\text{g/mL}$)	% Viability									
	SA-ME		SA-EA		SC-ME		SC-EA		RA ($\mu\text{g/mL}$)	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
15.6	79.6 \pm 8.0	70.8 \pm 8.6	72.6 \pm 3.0	71.1 \pm 2.0	74.7 \pm 11.7	70.6 \pm 1.9	78.0 \pm 6.6	59.2 \pm 1.8	16.9 \pm 6.7	15.7 \pm 3.0
31.25	71.5 \pm 4.9	69.3 \pm 6.7	68.7 \pm 4.6	53.8 \pm 2.3	64.9 \pm 2.9	63.6 \pm 7.9	76.7 \pm 9.4	53.2 \pm 10.1	26.3 \pm 4.7	20.9 \pm 1.5
62.5	69.9 \pm 3.8	64.3 \pm 7.6	55.9 \pm 5.4	37.9 \pm 0.4	61.2 \pm 4.9	58.5 \pm 5.4	57.4 \pm 0.8	51.9 \pm 0.8	37.9 \pm 3.2	35.9 \pm 12.1
125	57.9 \pm 3.6	19.7 \pm 3.7	53.1 \pm 7.7	22.2 \pm 1.7	56.5 \pm 4.6	52.6 \pm 6.7	50.5 \pm 1.7	21.8 \pm 2.7	79.5 \pm 4.2	56.3 \pm 6.6
250	48.9 \pm 0.5	10.9 \pm 1.2	25.7 \pm 1.9	19.6 \pm 1.3	50.9 \pm 2.1	33.3 \pm 1.4	49.9 \pm 2.2	17.8 \pm 1.7	80.7 \pm 11.3	79.2 \pm 7.7
500	45.3 \pm 2.4	9.2 \pm 2.9	25.4 \pm 3.5	15.9 \pm 0.7	48.9 \pm 1.6	11.9 \pm 0.8	12.2 \pm 2.1	12.0 \pm 0.4	85.5 \pm 4.1	82.8 \pm 6.6
1000	34.3 \pm 0.6	7.7 \pm 2.1	14.6 \pm 1.2	11.3 \pm 0.1	46.5 \pm 1.6	8.4 \pm 2.9	11.8 \pm 2.3	5.1 \pm 0.2	93.6 \pm 12.1	91.1 \pm 9.0
IC₅₀ values	230.0\pm17.3	93.3\pm5.8	133.3\pm36.1	61.0\pm3.6	266.7\pm41.6	180.0\pm20.0	150.0\pm36.1	73.3\pm5.8	201 \pm 7.6	173.3 \pm 32.1

RA: Rosmarinic acid

There were some studies made on cytotoxic activity on the different cancer cells of various extracts of *S. aethiopsis* and *S. ceratophylla* while none was made on the healthy cells. It was recorded that methanol and dichloromethane extracts of *S. aethiopsis* showed considerable cytotoxic activity against HL60 (human acute promyelocytic leukemia cells), K562 (human chronic myelogenous leukemia cells) and MCF-7 (human breast adenocarcinoma cells). IC₅₀ values of dichloromethane extract of the species were 44.6 ± 8.2 µg mL⁻¹, 41.3 ± 4.5 µg mL⁻¹ and 44.4 ± 5.0 µg mL⁻¹ on HL60, K562 and MCF 7 cells, respectively while they were 50.1 ± 4.2 µg mL⁻¹ and 79.4 ± 12.3 µg mL⁻¹ on K562 and MCF 7 cells respectively for methanol extract [45]. Another study reported *in vitro* cytotoxic activity of *S. aethiopsis* collected from Uzbekistan, MeOH and CHCl₃ extracts, using 5637 cells, a human bladder carcinoma cell line (ATCC HTB-9). The MeOH extracts of *S. aethiopsis* exhibited strong cytotoxicity with IC₅₀ values 64.6 µg mL⁻¹ [48]. With another study, *in vitro* antiproliferative activities reported using sulforhodamine B (SRB) assay against cancer cell lines namely C32, ACHN, COR-L23, A375, Caco-2, Huh-7D12, A549, MCF-7 and LNCaP of *S. ceratophylla* methanol extract. This extract exhibited the strongest inhibitory activity against the cell lines of C32 (amelanotic melanoma) (IC₅₀=20.8 µg mL⁻¹) and ACHN (human renal cell adenocarcinoma) (IC₅₀=27.2 µg mL⁻¹) [34]. Along with present study it may mention that this is the first cytotoxicity investigation of methanol and ethyl acetate extracts of *S. aethiopsis* and *S. ceratophylla* made on healthy cells. The toxicity of the relevant *Salvia* species extracts was showed an increase depending on time and concentration. The highest dose for SC-ME was 266.7 µg mL⁻¹, for SA-ME was 230 µg mL⁻¹, for SA-EA 150 µg mL⁻¹ and for SC-EA 133.3 µg mL⁻¹.

The genus *Salvia* L. has a lot of species used in traditional medicine in the world and also in Turkey. Sage is known as “adaçayı” and different species of the genus have a common use as teas or folk medicine in Turkey [15,49,50]. Two members of this genus, *S. aethiopsis* and *S. ceratophylla* methanol and ethyl acetate extracts were investigated for their antioxidant activities using DPPH and β-Carotene/Linoleic acid systems. As it is known that phenolics are the most characteristic phytochemical compounds of the genus demonstrated by plenty of research to build up the understandings on the potential healing properties of species. For this base, mentioned species extracts total phenolic contents and phenolic acids were evaluated by the present study. RA is the most abundant phenolic acid in mentioned extracts as usual in *Salvia* species and also to be the major phenolic compound responsible for the high antioxidant activity with other phenolic acids. Also to show the beneficial antioxidant and toxicity potentials of the mentioned *Salvia* species, they were applied on NIH/3T3 cells. Plants with antioxidant properties have the cytoprotective effect on normal cells while exerting antitumor activity on cancer cells. However strong antioxidants may cause toxic effects on normal cells due to their pro-oxidant activities. It is suggested by these results, to utilize the potential antioxidant properties of these plants extracts, they can be use in safe under 266 µg mL⁻¹, 230 µg mL⁻¹, 150 µg mL⁻¹ and 133.3 µg mL⁻¹ for SC-ME, SA-ME, SA-EA and SC-EA, respectively.

Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/RNP>

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