

# Phytochemical and Antioxidant Evaluation of *Salvia mirzayanii* Extracts Prepared with Different Solvents

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## ABSTRACT

*Salvia mirzayanii* Rech.f. & Esfand. is a member of the Lamiaceae family and is known to possess a wide range of pharmacological properties including antimicrobial, antioxidant, cytotoxic, anticancer, and others demonstrated during experiments conducted on this species. This plant is used for treating various ailments in Iranian traditional medicine. This research has focused on the analyzing the phenolic compounds and radical scavenging activities of hexane, acetone, and methanol extracts of *S. mirzayanii*. The antioxidant activities of these extracts were assessed using three standard methods: DPPH, FRAP, and ABTS. Among all these extracts, hexane extract showed the highest Trolox-equivalent Ferric reducing activity  $12.72 \pm 0.03 \mu\text{mol/g}$ . The hexane extract also recorded the highest activity in the ABTS assay  $11.86 \pm 0.05$ . On the opposite side, the acetone extract of *S. mirzayanii* had the highest total phenolic content  $71.91 \pm 0.03 \text{ GAE/g}$ , and with methanol extract put the total to  $54.75 \pm 0.01 \text{ mg GAE/g}$ . In addition, *S. mirzayanii* exhibited the highest total flavonoid content  $156.40 \pm 0.02 \text{ mg quercetin equivalents/g}$ . These results reveal that *S. mirzayanii* possesses potent antioxidant activities, indicating its potential usefulness in the food industry and pharmaceutical applications.

**Keywords:** *Salvia mirzayanii*, FRAP, ABTS, DPPH, Phenolic content

## How to cite this paper

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## INTRODUCTION

Free radicals are defined as molecules or molecular fragments that contain one or more unpaired electrons in their outermost valence shell. In the body, these unpaired electrons are typically found in reactive species such as reactive oxygen species (ROS), nitrogen (RNS), and reactive sulfur species (RSS). There is a common belief that free radicals can be both beneficial and harmful at the same time [1-3]. Moreover, these multitude of substances operate at small or moderate concentrations. Also, they assist with physiological functions, but when their level is unreasonably high or low-due to an increase in the level of the free radical itself or a decrease in the concentration of antioxidants-oxidative stress occurs that thwarts the normal activities of cells, thus causing structural and subsequently functional damage followed by the disease itself.

Free radicals can originate from internal and external sources. They are produced through metabolic activities within the body such as the oxidation of carbohydrates, fats, and proteins, which occurs in both anaerobic and aerobic pathways. External sources in the production of free radicals include lead toxicity, pesticides, cadmium, ionizing radiation, alcohol, cigarette smoke, ultraviolet light, and pollutants [1]. The imbalance between antioxidants and oxidants leads to free radical accumulation within cells, resulting in oxidative stress. These free radicals cause extensive damage to nucleic acids, proteins, and lipids. Some diseases associated with oxidative stress include diabetes, some neurological disorders such as Parkinson's disease, Alzheimer's disease, and multiple sclerosis,

cardiovascular diseases like atherosclerosis and hypertension, respiratory diseases, cataracts, arthritis, and many types of cancer, including colon, prostate, breast, lung, and bladder cancers [2]. Antioxidants counteract free radicals by inducing them with their electrons. Such substances help retard and minimize damage to the targeted molecules. They can actually do a lot of things in the body as cleaners to prevent all sorts of damage caused by free radicals. Antioxidants can be effective inhibitors of oxidation processes even at low concentrations in different physiological processes [3]. Nowadays, the emphasis is placed upon the identification of antioxidant compounds derived from plant sources because those are pharmacologically potent and have less or no side effects [1]. Plant antioxidants encompass a range of beneficial compounds, including vitamins E and C, carotenoids such as xanthophylls and carotenes, as well as polyphenols, which include phenolic acids, flavonoids, anthocyanins, and lignans. Additionally, ethynes are also recognized for their antioxidant properties. Antioxidants play a crucial role in combating various health issues by targeting inflammation, bacteria, viruses, the aging process, and even cancer. Their multifaceted effects contribute to overall well-being and disease prevention [4]. Phenolic is one of the secondary metabolites of plant compounds. Those compounds tend to be categorized into various classes depending on their structures, hydrophobic group positions, and different substituent positions [5]. The dominant protective phenolic compounds of plants can be classified into four main groups, which are phenolic acids – gallic, protocatechuic, caffeic, and rosmarinic acids; phenolic diterpenes – carnosol and

carnosic acid; flavonoids – quercetin and catechins; and volatile oils – eugenol, carvacrol, thymol, and menthol [6]. Flavonoids represent the most extensive diversity within the polyphenol category. The prominent functions of phenolic compounds are primarily attributed to their antioxidant properties. The efficacy of these antioxidants is significantly influenced by the stability of the compound, the presence of hydroxyl groups, and their capacity to form hydrogen bonds with water molecules. Phenols have antioxidant activity far exceeding that of vitamins or carotenoids [5]. The hypothesis of action of The hypothesis of the action of antioxidant compounds includes the inhibition of hydrogen atom abstraction, chelation of transition metal ions, and the decomposition of free radicals [7]. Herbal-based therapies are generally considered safer than synthetic products due to their better compatibility with human physiology and fewer side effects. These compounds offer an important source of antioxidants, protecting the body from damage caused by free radicals. A significant amount of phenolic compounds is found within the Lamiaceae family, which serves as a rich source of natural antioxidants [8]. The Lamiaceae family comprises 7000 species and 236 genera; *Salvia* is the biggest genus with about 900 species. Fifty-six species have been identified in the Flora of Iran, one-third of all of which are indigenous. *S. mirzayanii*, a well-known native species, has traditionally been used in the treatment of various diseases, including diabetes and convulsions [9]. *S. mirzayanii* is a species in the genus *Salvia* and is an herbaceous biennial or perennial plant. It is native to the south of Khorasan Razavi and

Semnan provinces in Iran. The main constituents of the essential oil of *S. mirzayanii* indicated by experiments are: Linalyl acetate (11.8%) then linalool (11.8%),  $\alpha$ -terpinyl acetate (11%), and 1, 8 cineole (8.7%) [8]. Moreover, various pharmacological effects of *S. mirzayanii* have been reported in different assays, including antibacterial, antioxidant, cytotoxic, and anticancer activities [10]. Given the increasing recognition of plant-derived antioxidants and their broad therapeutic potential, it is essential to study and characterize such compounds for their role in combatting oxidative stress-related diseases. The antioxidant activity of phenolic compounds- particularly those found in Lamiaceae- offers promise in addressing a wide range of conditions involving oxidative damage, such as diabetes, neurological disorders, cardiovascular diseases, and cancer [8]. Among these, *S. mirzayanii* is unique because of abundant bioactivity-like content comprising substantial levels of linalyl acetate, linalool and  $\alpha$ -terpinyl acetate which are known as a strong antioxidant by definition. With these results in mind, it becomes crucial to elucidate the full pharmacological profile of *S. mirzayanii*, specifically of her potential as a non-chemical source of natural antioxidants (Fig. 1). This research aims to expand our understanding of the antioxidant potential of *S. mirzayanii*, contributing to the growing body of evidence supporting the therapeutic use of plant-derived compounds in managing oxidative stress and associated diseases. Hence this study aims to investigate the effect of *S. mirzayanii* as a natural antioxidant in new generations for medicinal applications.

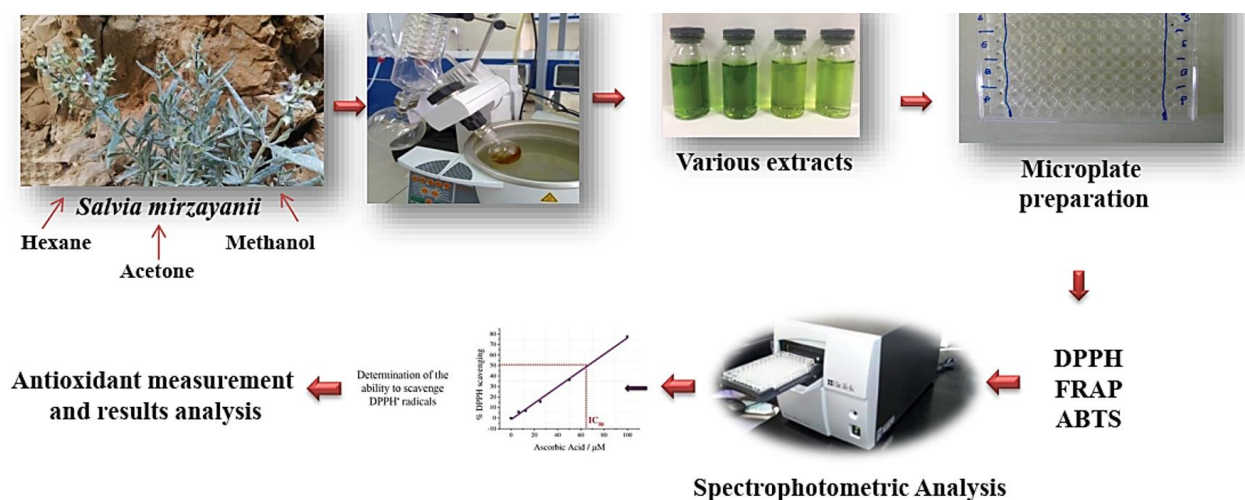


Fig. 1 Biochemical evaluation of *S. mirzayanii* extracts.

## MATERIALS AND METHODS

### Plant Preparation

The aboveground parts of *S. Mirzayanii* were retrieved from Genu Mountain region in the Bandar Abbas province of Iran. The plant specimen was taxonomically identified and authenticated by Dr. Mohammad Amin Soltanipoor at the Agricultural and Natural Resources Center of Hormozgan, Bandar Abbas, Iran. A voucher specimen (herbarium number ANRC-5322) was deposited and preserved in the herbarium of the same center for future reference.

### Plant Extracts

After the plant materials underwent a cleaning and drying process, they were air-dried for 14 days at 20 °C. The solution was prepared by soaking 20 grams of dried and crushed plant material in 250 milliliters of n-hexane, acetone, and methanol for 48 hours. In addition, the extraction was also done by shaking for approximately

48 hours. The extracts were filtered with No. 1 filter paper and the solvents were removed by a rotary evaporator set to 35°C.

### Chemicals

The following were obtained from Merck (Germany): Ethanol, sodium acetate, quercetin, n-hexane, butylated hydroxytoluene (BHT), Folin-Ciocalteu reagent, methanol, potassium persulfate, gallic acid, dimethyl sulfoxide, acetone, 2,2' diphenyl picrylhydrazyl (DPPH), and 2,2-azinobis (3-ethylbenothiazoline-6-sulphonic acid) diammonium salt (ABTS).

### Determination of Antioxidant Activity Using DPPH

The antioxidant activity of the extract was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, which measures the ability of antioxidants to donate hydrogen

atoms to neutralize DPPH radicals. According to the method described by Xu *et al.*, 50  $\mu$ L of the methanolic extract of the sample was mixed with 200  $\mu$ L of 100  $\mu$ M DPPH solution in methanol. The mixtures were vortexed and incubated at room temperature in the dark for 30 minutes. The absorbance was then measured at 517 nm using a BioTek XS2 microplate reader.

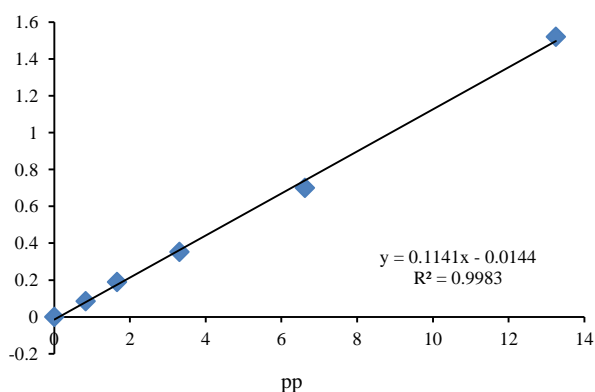
Butylated hydroxytoluene (BHT) was used as the positive control. A blank was prepared using methanol alone, without the sample extract. All measurements were performed in triplicate. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\% \text{ inhibition} = [1 - (A_s - A_b)/A_c] \times 100$$

$A_b$  is the term used for the term relating to the absorbance value of the blank sample,  $A_c$  is the absorbance value of the control, and  $A_s$  defines the absorbance of the sample mixture. About DPPH free radical scavengers, there was a dose-response dependence in which the quantity of additives inversely corresponded to the inhibition of the DPPH free radical. The more negative the amount of additive absorption, the stronger the DPPH inhibition. The  $IC_{50}$  value ( $\mu$ g/mL), representing the concentration required to scavenge 50% of DPPH radicals, was calculated from the regression analysis of the dose-response curve.

#### Trolox Equivalent Antioxidant Capacity Assay (TEAC)

The TEAC test measures the rate of neutralization of ABTS radicals to determine the extracts' sensitivity towards antioxidants. This procedure analyzes hydrophilic and lipophilic fractions separately. The ABTS radical cation is generated by mixing a 7 mM ABTS solution in ethanol with 2.45 mM potassium persulfate at a ratio of 1:1. The sample was left to react for at least one day to make sure the reaction was complete [12–15]. The ABTS radical cation solution was prepared and its absorbance adjusted to  $0.04 \pm 0.70$  m at 734 nm. The dilution with the ABTS solution was achieved by dispensing 100  $\mu$ L of plant extracts within a time window of 45 seconds and photometric measurements were performed one minute after the dispensing at 734 nm. The quantity of antioxidant was determined from the absorption spectrum at 834 nm. In this experiment standard component of the sample is Trolox. The data are presented in micromoles of Trolox equivalents per gram of dry weight of the plant material.



**Fig. 2** The calibration curve of ethanol solutions containing different iron (II) concentrations.

#### Ferric Reducing Antioxidant Power Assay (FRAP)

The FRAP technique was employed based on the methodology developed by Benzie and Strains in 1996 [16]. The new FRAP agent was synthesized by the reaction of 2.5 ml 10 mM TPTz solution mixed with 40 mM HCl, 2.5 ml 20 mM  $FeCl_3$ , and 25 ml of 0.3 molar acetate buffer (pH 3.6). FRAP agent was used to estimate the antioxidant capacity of the samples. A volume of 1.8

ml of FRAP agent was added to 0.2 ml of extract. The calibration curve was created using ethanol solutions containing different concentrations of iron (II) (Fig. 2). Ethanol solutions were prepared by diluting a 100 mM stock solution of iron(II) sulfate. The FRAP results were calculated from the difference in optical density measured at 593 nm between the test samples and the standard iron(II) solutions.

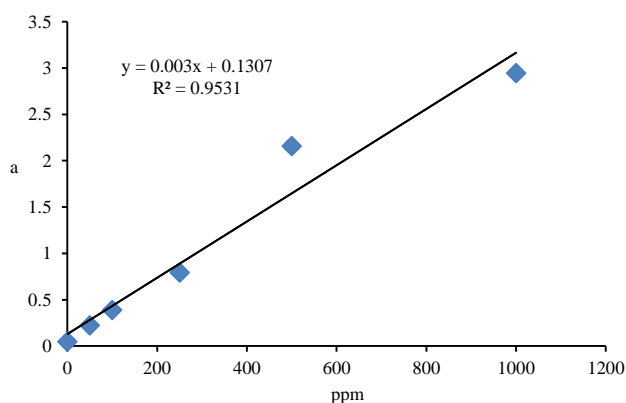
#### Assay for Total Phenolic Contents

According to the Folin-Ciocalteu method described by Zhou *et al.* (2004) [13], the total phenolic content of each extract was determined. A 5ml sample was taken and mixed with 7 percent sodium carbonate before being filled with distilled water to a final volume of 250 ml.

Fifty microliters (50  $\mu$ L) of the Folin-Ciocalteu reagent was added the flask and the mixture was kept at room temperature for 30 minutes. It was then analyzed using a microliter reader to measure absorbance. A blank reading was initially taken at 765 nm, and subsequently, gallic acid standards were used with the same wavelength as the reference. All of the aforementioned procedures were conducted in three replications. The results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg Gae/g extract). Among the distinguishing important measurements of the concentration of gallic acid for every microliter of solution, the values 12.5, 25, 50, 100, and 200  $\mu$ g/mL.

#### Content of Flavonoids

To construct the calibration curve, standard solutions of gallic acid were prepared at concentrations of 12.5, 25, 50, 100, and 200 mcg/ml (Fig. 3). All steps were done in triplicates.



**Fig. 3** The calibration curve of quercetin.

#### Ethical Considerations

The Lorestan University of Medical Sciences Ethics Committee granted permission to conduct the study with an issued number IR.LUMS.REC.1403.299.

#### RESULTS AND DISCUSSIONS

Table 1 presents the various extracts of *S. mirzayanii* and their DPPH radical scavenging activity.

Among the samples analyzed, the methanolic extract of *S. mirzayanii* exhibited the lowest  $IC_{50}$  value of  $5.38 \pm 0.03$ , indicating stronger DPPH radical scavenging activity compared to BHT ( $IC_{50}$  value of  $14.50 \pm 1.20$   $\mu$ g/mL). The acetone and hexane extracts followed, with  $IC_{50}$  values of  $17.51 \pm 0.12$  and  $173.54 \pm 0.04$   $\mu$ g/mL. In two other studies using TEAC values to represent anti scavenging activity on the different extracts of the plant, the related findings together with  $IC_{50}$  values were:  $11.86 \pm 0.05$  g and  $15.90 \pm 0.06$  g. Concerning the dry weight of the plants, the highest

value from the experiments and its corresponding TEAC value recorded ranged between  $11.86 \pm 0.05$  g and  $12.72 \pm 0.03$  g. The determined ranges of phenolic content in Table 2 were  $51.41 \pm 0.02$  and  $71.91 \pm 0.03$  mg GAE/g. The strongest total phenolic content was recorded for the most powerful *S. mirzayanii* acetone extract at  $71.91 \pm 0.03$  GAE/g and the weakest methanol extract at  $54.75 \pm 0.01$  mg GAE/g. For the flavonoid analysis, quercetin was used as a standard. The total flavonoid content ranged from  $26.60 \pm 0.03$  to  $156.40 \pm 0.02$  mg quercetine equivalents per gram of dry extract. The acetone extract exhibited the highest flavonoid content when examined by the methods of extraction ( $156.40 \pm 0.02$  mg quercetine equivalents/g) among all tested extracts.

**Table 1** *S. mirzayanii* various extracts DPPH, ABTS and FRAP radical scavenging activity.

Samples	Extract	DPPH IC <sub>50</sub> (µg/mL)	ABTS (µmol Trolox/g)	FRAP (µmol Trolox /g)
<i>S. mirzayanii</i>	Hexane	$173.54 \pm 0.04$	$11.86 \pm 0.05$	$12.72 \pm 0.03$
	Acetone	$17.51 \pm 0.12$	$13.82 \pm 0.03$	$19.72 \pm 0.02$
	Methanol	$5.38 \pm 0.03$	$15.90 \pm 0.06$	$62.88 \pm 0.05$
BHT	-	$14.50 \pm 1.20$	-	-

The results of the DPPH antioxidant test known that methanolic extracts of *S. mirzayanii* possess significant antioxidant properties, likely due to the presence of flavonoids and phenolic compounds. The observed activities in the DPPH, ABTS, FRAP, and total phenolic content assays suggest that these phenolic constituents may be primarily responsible for the reductive effects on oxidants present in the samples. This research demonstrated that as the concentration of phenolic compounds increased, the IC<sub>50</sub> values are decreased, thereby indicating stronger antioxidant activity. Concerning the activity and the shifts of IC<sub>50</sub> these extracts exhibit, they are all positively correlated with the extract's phenol concentration. The highest amount of phenolic compounds was recorded in the acetone extract at  $71.91 \pm 0.03$  mg GAE/g dry extract, while the methanol extract came second at  $54.75 \pm 0.01$  mg GAE/g dry extract. Acetone enclosed middle values of flavonoids at  $156.40 \pm 0.02$  mg QE/g, while lower values were recorded in hexane extract at  $26.60 \pm 0.03$  mg QE/g. These results further confirm that the antioxidant activities of the acetone extracts are the result of *S. mirzayanii* flavonoids and phenolic compounds.

**Table 2** *S. mirzayanii* extracts total phenol and flavonoid content.

Samples	Total phenol content (mg GAE/g)	Flavonoid content (mg QE/g)
Hexane	$51.41 \pm 0.02$	$26.60 \pm 0.03$
Acetone	$71.91 \pm 0.03$	$156.40 \pm 0.02$
Methanol	$54.75 \pm 0.01$	$40.00 \pm 0.01$

The antioxidant activities of the three extracts from moderate to strong, with IC<sub>50</sub> values between  $5.38 \pm 0.03$  and  $173.54 \pm 0.04$  µg/mL. All of the extracts demonstrated remarkable FRAP and ABTS radical scavenging activities.

Statistically significant correlations were observed between ABTS and total flavonoids, ABTS and total phenolic content, and DPPH as well as between total flavonoids. However, the correlation between DPPH was less pronounced, suggesting that both the phenolic and flavonoid compounds contribute to the observed antioxidant capacity, but their relative impact may vary across different assays.

The hexane extract exhibited the weakest antioxidant activity, with an IC<sub>50</sub> of  $173.54 \pm 0.04$  µg/mL, significantly higher than that of the methanol extract. This finding highlights the importance of solvent polarity in the extraction process. Methanol, being a polar solvent, is more efficient at extracting phenolic and flavonoid compounds, which are known to be responsible for high antioxidant activity in plant materials such as *S. mirzayanii*. Despite its lower DPPH radical scavenging activity, the hexane extract exhibited moderate performance in ABTS and FRAP assays, suggesting the presence of non-polar antioxidant compounds that may also play a role in oxidative reduction.

The hexane extract demonstrates the highest value in terms of Trolox Equivalent Antioxidant Capacity. It had an astounding  $11.86 \pm 0.05$  and is a very good ABTS radical cation scavenger as shown by the ABTS assay. The FRAP assay further corroborates the earlier findings, demonstrating that the hexane extract has a FRAP value of  $12.72 \pm 0.03$ . This value indicates a characteristic transformation in the Trolox form associated with the hexane extract. This signified a fair ferric ion-reducing capacity, which is within the scope of antioxidant activities. The phenolic content of *S. mirzayanii* extracts was determined using the Folin-Ciocalteu method. The findings indicated that the acetone extracts exhibited the highest total phenolic content, measuring  $71.91 \pm 0.03$  mg GAE/g of dry weight. This was followed by the methanol extracts, which demonstrated a total phenolic content of  $54.75 \pm 0.01$  mg GAE/g dry weight. Among the various categories of antioxidants, phenolic compounds- particularly phenolic acids- are recognized for their significant health benefits. These compounds function as antioxidants by effectively reducing free radicals through the donation of electrons or hydrogen atoms, thereby preventing oxidative damage induced by free radicals. The pronounced antioxidant activity observed in the acetone extract of *S. mirzayanii* can be attributed to its substantial phenolic content, indicating a rich presence of phenolic compounds. The flavonoid contents of the extracts were estimated based on the above concerns. The acetone extract had the most pronounced flavonoid content,  $156.40 \pm 0.02$  mg QE/g dry weight, followed by the methanol extract. Flavonoids are a group of plant polyphenolic compounds with free radical scavenging and antioxidant properties. Since polyphenols, particularly flavonoids and stilbenes, are present in high amounts in the extracts, the strong antioxidant potential of the acetone extract is likely due to its high flavonoid content. The hexane extract with the lowest concentration of flavonoids ( $26.60 \pm 0.03$  mg QE/g) demonstrated reduced potency in antioxidant activities. This observation underscores the significant role that flavonoids play in the antioxidant efficacy of the extracts. The study demonstrated a significant correlation between total antioxidant activity and the content of total phenolics and flavonoids. Notably, optimal synergistic activity was observed across the DPPH, ABTS, and FRAP assays. These relations imply that *S. mirzayanii*'s antioxidants mainly originate from the phenolic and flavonoid compounds [14].

These results substantiate reports of other *Salvia* species wherein a similar link between antioxidant activity and polyphenolic content has been shown. The hexane extract, although its phenolics and flavonoid contents were the lowest, had moderate antioxidant activity.

This suggests that although phenolic and flavonoid compounds are the main sources of antioxidant activity, other bioactive compounds of the hexane extract like terpenoids or any other non-polar compounds may also contribute to the plant's ability to scavenge free radicals. This verifies the claim that which broad range of phytochemicals are responsible for the antioxidant potential in other species of plants. Our research indicates that the findings align closely with those of related studies, particularly regarding *S. officinalis* and *S. miltiorrhiza*. These species are known to contain significant amounts of polyphenolic compounds, which contribute to their notable antioxidant capacity. It is highly probable that *S. mirzayanii*, similar to numerous other plant species, exhibits significant antioxidant activity. This property suggests its potential utility in addressing oxidative stress-related conditions, including cardiovascular diseases, cancer, and neurodegenerative disorders.

Several articles focus on the physiological activities of polyphenolic compounds derived from the *Salvia* genus. The results of our study confirm those of other studies concerning the phytochemistry of selected *Salvia* spp. Najafi *et al.* studied the antioxidant activities of *S. certophylla*, *S. chloroleuca*, *S. macrosiphon*, *S. virgate*, *S. chorassanica*, and *S. leriifolia*. The total phenolic contents (TPC) of extracts made using various solvents ranged from (mg GAE/g) 11.28 to 23.82. The scavenging potency of the extracts, as determined by IC<sub>50</sub> values, ranged from 27.38 to 78.469 µg/ml. The highest TPC (23.82 ± 0.16 mg GAE/g) was found in the methanolic extract, while it was found that the n-hexane extract had the highest IC<sub>50</sub> value (469.78 ± 5.97 µg/ml). Based on these findings, these plants demonstrate significant antioxidant attributes [15].

The antioxidant, anticholinesterase, and antimicrobial properties of *S. ceratophylla* have been evaluated in several studies [16-18]. Investigations showed that the methanolic extract of *S. ceratophylla* overhead parts possesses antioxidant activity. Using the DPPH technique, we achieved an IC<sub>50</sub> value of 5.5 µg/ml, while the FRAP test yielded an extraction of 290.7 µmol Fe<sup>2+</sup> eq/g. The assessment of the dry extract revealed a total phenol content of 32.7 mg per gram of extract and a total flavonoid content of 27.0 mg per gram of extract, all of which are equivalent to gallic acid and (-)-catechin, respectively [19]. Sarroua *et al.* revealed the influence of climatic factors, their geography, and harvest season along with other genetic traits carried on the levels of various polyphenolic compounds. From the phenolic compounds studied, it was detected that the harvest of *S. fruticose* was most favorable at the beginning of summer since the greatest yield of oil was identified. The quantity of main obtained phenols changed from spring to autumn. The different periods for harvesting resulted in significant discrepancies in the DPPH and ABTS test outcomes. These discrepancies from the FRAP test results ranged from 31.83 to 202.93 as well as 60.94 to 242.3 M Trolox/g DW. The ABTS test results from contemplated and standard deviation were variable in measurement between 135.81 and 326.22 and 199.64 to 312.49 M Trolox/g DW. [20]. *S. glutinosa* had an aerial methanolic extract of IC<sub>50</sub> 3.2 during the DPPH test [19]. The FRAP test yielded a value of 422.0 µmol FeSO<sub>4</sub> eq/g. Conducting the phenolic content test on *S. hydrangea* revealed that this plant had 21.9 mg DPPH equivalent per gram of dry extract and an IC<sub>50</sub> of 5.3 µg/ml. Additionally, a phenolic content of 30.4 mg was reported in *S. sclarea* with an IC<sub>50</sub> of 4.8 µg/ml [21]. The essential oil from *S. sclarea* had an IC<sub>50</sub> value of 5.03 in the ABTS assay and 12.50 mg/ml in the DPPH assay. For the n-butanol fraction of *S. sclarea* root, DPPH results were IC<sub>50</sub> 12.9 µg/ml, and for ABTS – 1737.2 µmol T/g. The

chloroform root DPPH extract also showed an IC<sub>50</sub> value of 15.1 µg/ml. The most impressive characteristic of the *S. verbenaca* methanol extract is it's featuring phenolic diterpenes, which had values of 1056.90-1148.42 µg/g DW. Different stages of *S. verbenaca* from early to later phases showed a range of high activity with IC<sub>50</sub> DPPH value of 49.22 µg/ml and FRAP indicating maximum activity during the early stage of fruiting at 188.93 mM Fe(II)/mg. Numerous publications concerning different *Salvia* species described them as having a high content of phenolic compounds and active antioxidant properties. *S. mirzayanii* extracts showed decent antioxidant activities but were most prominent in methanol and acetone-extracted samples.

This activity is due to the presence of radical and oxidative stress scavengers such as phenolic and flavonoid compounds. This places *S. mirzayanii* in a good position as a potential source of natural antioxidants for the pharmaceutical, and nutraceutical industries as well as food products. This implies that further studies should be performed to identify and determine the structure of some of the compounds that were shown to possess such activities. At the same time, more clinical trials have to be conducted to confirm the antiviral effects of *S. mirzayanii* on patients with stress oxidative diseases. With *S. mirzayanii* being one of the most neglected species, this work is important to stimulate more research towards the medicinal qualities of *Salvia* species and to fill in the gaps of drug and functional food development and research.

## CONCLUSION

The findings of this study demonstrate that *S. mirzayanii* possesses remarkable antioxidant properties, making it a promising candidate for the development of pharmaceutical and nutraceutical products. Traditionally used in Iranian medicine to treat various ailments, this plant exhibited meaningful radical scavenging and ferric-reducing capacities, especially in its acetone and hexane extracts. Among the tested samples, the acetone extract showed the highest concentrations of phenolic and flavonoid compounds, which are known to contribute to mitigating oxidative stress and related pathologies. Given its strong antioxidant potential, *S. mirzayanii* holds promise for application in natural health products, herbal medicines, and dietary supplements. Furthermore, the results provide additional evidence supporting the therapeutic potential of this native medicinal plant and highlight the importance of continued phytochemical and pharmacological investigations. Finally, this work not only deepens our knowledge of *S. mirzayanii*'s biological properties because of the significance of native herbal plants, but it also draws attention to the need to harness available natural resources to improve health. We anticipate the findings of this specific research to enable further advances in the development and subsequent utilization of this plant for medicinal and industrial purposes while ensuring the responsible management of natural resources.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Not applicable.

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