

Total phenolic compounds and antioxidant potential of *Salvia nydeggeri* Hub.-Mor.: An endemic species of southwestern Türkiye

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Abstract: The genus *Salvia* constitutes one of the most prominent groups of medicinal and aromatic plants, with documented usage tracing back to antiquity. *Salvia nydeggeri* is an endemic species distributed in southwestern Türkiye. In this study, the antioxidant activity of this species was investigated. *S. nydeggeri* was collected from natural populations in Elmalı, Antalya. The samples were extracted using solvents of different polarities (*n*-hexane, chloroform, ethanol, methanol, and water). Using these extracts, the total phenolic and total flavonoid content, as well as the DPPH antioxidant capacity of *S. nydeggeri*, were assessed. The total phenolic content was evaluated using the Folin-Ciocalteu reagent with a gallic acid standard curve. The highest phenolic content was found in the water extract (110.489 ± 5.07 mg GAE/g) and the methanol extract (109.74 ± 2.25 mg GAE/g). The total flavonoid content in the extracts was determined using calibration curves for rutin hydrate and quercetin. The highest flavonoid content was found in the methanol extract (151.22 ± 2.64 mg RU/g and 96.00 ± 1.71 mg QE/g) and the chloroform extract (150.32 ± 3.15 mg RU/g and 95.41 ± 2.03 mg QE/g). The antioxidant activity of *S. nydeggeri* extracts in different solvents was studied using DPPH (1,1-diphenyl-2-picrylhydrazyl). The highest antioxidant capacity was observed in the methanol extract ($IC_{50}=12.66$ µg/mL) and the water extract ($IC_{50}=13.96$ µg/mL), while the lowest was in the *n*-hexane extract ($IC_{50}=498.964$ µg/mL). This study indicates that *S. nydeggeri* may be an important potential natural resource for developing pharmaceuticals, cosmetics, and nutraceuticals.

1. INTRODUCTION

Salvia L., often known as sage (Adaçayı in Turkish), is a genus found around the world and comprises about 1,000 species (Haq *et al.*, 2020). It is regarded as the largest genus in the Lamiaceae family, which consists of 7,133 species across 236 genera (Ghavam *et al.*, 2020). The Lamiaceae family comprises 48 genera and 782 taxa, with a 54% endemism rate in Türkiye (Celep & Dirmenci, 2017). According to Jenks *et al.* (2013), *Salvia* belongs to the subfamily Nepetoideae. In Türkiye, *Salvia* has 107 species, of which 58 are endemic (Celep & Dirmenci, 2017).

From ancient times, *Salvia* has been used as an aromatic and therapeutic herb (Sharma *et al.*, 2019). *Salvia* species have diverse applications, serving as herbal teas, sauces, spices, and culinary ingredients, while also being used medicinally to treat rheumatic conditions, stomach

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ailments, liver diseases, and wounds (Poulios *et al.*, 2020). Furthermore, it is used in infusions and decoctions to treat colds in many parts of the world (Li *et al.*, 2013). *Salvia* species have been reported to exhibit effective biological activity, whether used as a whole plant, extract, essential oil, or in other forms (Albayrak *et al.*, 2021; Emre *et al.*, 2021; Güzel *et al.*, 2020; Ivanitskikh & Tarakanov, 2014; Kaya *et al.*, 2017; Köse *et al.*, 2025; Semiz *et al.*, 2023). Many plants from various genera are used as sources of essential oils in southern Türkiye. According to Cinbilgel and Kurt (2019), the taxa most frequently utilized as sources of essential oils belong to the Lamiaceae family.

Salvia nydeggeri Hub.-Mor. is an endemic species of Southwest Anatolia (Türkiye) and consists of perennial, suffrutescent, and procumbent plants. The calyx is tridentate, with an acute upper lip, the corolla is yellow, and the plant is annulate (Celep & Doğan, 2023; Huber-Morath, 1982; Thiambeng, 2015). There are no ethnobotanical records for this species. However, studies on different solvents have investigated their bioactivity and essential oil content. In their research, Şenol *et al.* (2010) performed acetylcholinesterase inhibitory and antioxidant activity tests on 55 *Salvia* taxa distributed in Türkiye. The DPPH antioxidant capacities of the dichloromethane, ethyl acetate, and methanol extracts of *S. nydeggeri*, one of the study materials, were determined to be 11.77-13.15%, 87.66-90.99%, and 87.66-90.99%, respectively. Kıvrak *et al.* (2018) investigated the phenolic profiles and antioxidant activities of three *Salvia* species, one of which is *S. nydeggeri*. The total phenolic content was found to be between 36.3-55.4 (µg PEs /mg of extract), and the total flavonoid content was found to be between 29.1-198.4 (µg QEs /mg of extract).

Reactive oxygen species (ROS) are generated by the body's various enzymatic processes when oxygen is consumed. Examples of ROS include hydrogen peroxide, superoxide anion radical, and hydroxyl radical. These ROS can function as growth regulators (Hancock, 2001) and signal transducers in small doses (Finkel, 1998). However, because ROS can damage various molecules, including proteins and lipids, they may be produced in significant quantities under oxidative stress and can be harmful (Halliwell *et al.*, 1992). In fact, it has been shown that ROS significantly influence mutagenesis, cellular aging, and coronary heart disease by oxidizing low-density lipoproteins (LDL), damaging DNA, and destabilizing membranes. The cell can utilize either an exogenous system that includes antioxidants like vitamin C and α -tocopherol, or an endogenous system that involves enzymes such as catalase and superoxide dismutase to mitigate the effects of ROS (Tosun *et al.*, 2009).

Plant extracts have been used as antioxidants in the food industry in recent years. According to research, several of these substances have antioxidant capacities that are equal to, or occasionally higher than, those of synthetic antioxidants (Cuvelier *et al.*, 1990; Pokorny, 1991). Specifically, a wide variety of plants in the Lamiaceae family are well recognized for their antioxidant properties. Sage is one such plant, extensively used and primarily known for its antioxidant components. It is widely understood that phenolic compounds are mainly responsible for the antioxidant properties of sage (Pokorny, 1991; Das & Pereira, 1990; Schwarz & Ternes, 1992).

This study aims to estimate the phenolic compound content and evaluate the antioxidant activities of extracts obtained from *S. nydeggeri* collected in Elmalı, Antalya, Türkiye.

2. METHOD

2.1. Plant Material and Preparation of Extracts

Samples of *S. nydeggeri* were collected during the flowering period from Elmalı, Antalya (1170 m) in June 2022 and were identified by the authors (Figure 1). The plants were then dried at room temperature. Herbarium samples were stored at the Chemical Ecology Laboratory Herbarium at Pamukkale University (GSE 2485).

Extraction of the samples was performed using solvents of different polarities (*n*-hexane, chloroform, ethanol, methanol, and distilled water). A total of 100 mL of solvent was added to

the powdered samples (10 g). The samples were incubated in a water bath at 50 °C for 24 hours. Following incubation, the solvent was filtered, and fresh solvent was added to the remaining plant material. This extraction process was repeated three times to ensure complete extraction. Water extracts were subsequently dried using a lyophilizer, while organic solvent extracts were concentrated using a rotary evaporator. The resulting dry extracts were transferred to storage tubes and maintained at +4 °C until analysis.



Figure 1. Habit and flower of *S. nydeggeri*.

2.2. Total Phenolic and Total Flavonoid Content

The total phenolic content in *S. nydeggeri* extracts was determined using the Folin-Ciocalteu reagent (Singleton *et al.*, 1965), while the total flavonoid content was assessed using aluminum chloride reagent (Lamaison and Carnat, 1990), following a modified method from Özgün-Acar *et al.* (2022). In this study, gallic acid was used as the standard phenolic compound, and rutin hydrate and quercetin were used as standard flavonoids. Samples were analyzed spectrophotometrically at 765 nm and 415 nm, respectively. The total phenolic and flavonoid content was calculated using the formula below (C = total phenolic content mg GAE/g dry extract, c = concentration of gallic acid obtained from calibration curve in mg/mL, V = volume of extract in mL, m = mass of extract in gram.).

$$C=(c \times V)/m$$

2.3. DPPH Free Radical Scavenging Activity

The antioxidant activity of *S. nydeggeri* extracts was determined using DPPH (2,2-Diphenyl-1-picrylhydrazyl), with quercetin used as a positive control. Each extract and quercetin were dissolved in methanol at different concentrations. All samples were analyzed spectrophotometrically at 517 nm against a blank. The percentage of inhibition of the extracts was calculated using the formula below (A_{control} : Absorbance of control samples, A_{samples} : Absorbance of extracts).

$$\text{Inhibition(\%)}=100 \times (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}$$

3. RESULTS

3.1. Total Phenolic Content

Total phenolic content (TPC) of *S. nydeggeri* extracts was determined using a gallic acid standard curve (Figure 2-A). The formula of the graph was calculated as $y=7.965x+0.1016$, while an R^2 value was calculated as 0.9939. The results are summarized in Table 1.

The distilled water extract had the highest concentration of total phenolics (110.489 mg GAE/g), followed closely by the methanol extract (109.735 mg GAE/g). The extracts of *n*-hexane and chloroform did not contain any phenolic compounds.

Table 1. Total phenolic content of *S. nydeggeri* obtained from different solvents (ABS: Absorbance, SMP: Sample (mg GAE/g), SE: Standart Error, N/A: Not available).

Solvent	Formula	R^2	ABS1	ABS2	ABS3	SMP1	SMP2	SMP3	Mean	SE
Methanol	$y=7.965x+0.1016$	0.9939	0.937	0.977	1.013	104.88	109.90	114.42	109.735	2.76
Ethanol			0.480	0.422	0.455	47.505	40.223	44.366	55.301	2.11
<i>n</i> -hexane			0.068	0.056	0.076	N/A	N/A	N/A	N/A	N/A
Chloroform			0.016	0.022	0.096	N/A	N/A	N/A	N/A	N/A
Distilled water			0.914	0.953	1.078	101.99	106.89	122.58	110.489	6.21

3.2. Total Flavonoid Content

The total flavonoid content (TFC) in the extracts was determined using the calibration curve of rutin hydrate (see Figure 2-B) and quercetin (see Figure 2-C). The formulas of the graphs were determined as $y=18.888x+0.0224$ for rutin hydrate and $y=29.237x+0.072$ for quercetin, with R^2 values of 0.9943 for both. The results are summarized in Tables 2 and 3.

The total flavonoid content was highest in the methanol extract (151.22 mg RU/g and 96.00 mg QE/g), followed by the chloroform extract (150.32 mg RU/g and 95.41 mg QE/g). The lowest flavonoid levels were found in distilled water extracts (59.88 mg RU/g and 36.98 mg QE/g).

Table 2. Total flavonoid content of *S. nydeggeri* using rutin hydrate calibration curve obtained from different solvents (ABS: Absorbance, SMP: Sample (mg RU/g), SE: Standart Error, N/A: Not available).

Solvent	Formula	R^2	ABS1	ABS2	ABS3	SMP1	SMP2	SMP3	Mean	SE
Methanol	$y=18.888x+0.0224$	0.9943	2.831	3.000	2.805	148.70	157.65	147.32	151.22	3.24
Ethanol			1.183	1.164	1.147	61.45	60.44	56.54	60.48	1.50
<i>n</i> -hexane			1.740	1.385	1.291	90.94	72.14	67.17	76.75	7.24
Chloroform			2.753	3.000	2.832	144.57	157.65	148.75	150.32	3.86
Distilled water			1.155	1.346	0.959	59.97	70.08	49.59	59.88	5.92

Table 3. Total flavonoid content of *S. nydeggeri* using quercetin calibration curve obtained from different solvents (ABS: Absorbance, SMP: Sample (mg QE/g), SE: Standart Error, N/A: Not available).

Solvent	Formula	R^2	ABS1	ABS2	ABS3	SMP1	SMP2	SMP3	Mean	SE
Methanol	$y=29.237x+0.072$	0.9943	2.831	3.000	2.805	94.37	100.15	93.48	96.00	2.09
Ethanol			1.183	1.164	1.147	38.00	37.35	36.77	37.37	0.36
<i>n</i> -hexane			1.740	1.385	1.291	57.05	44.91	41.69	47.88	4.68
Chloroform			2.753	3.000	2.832	91.70	100.15	94.40	95.41	2.49
Distilled water			1.155	1.346	0.959	37.04	43.57	30.34	36.98	3.82

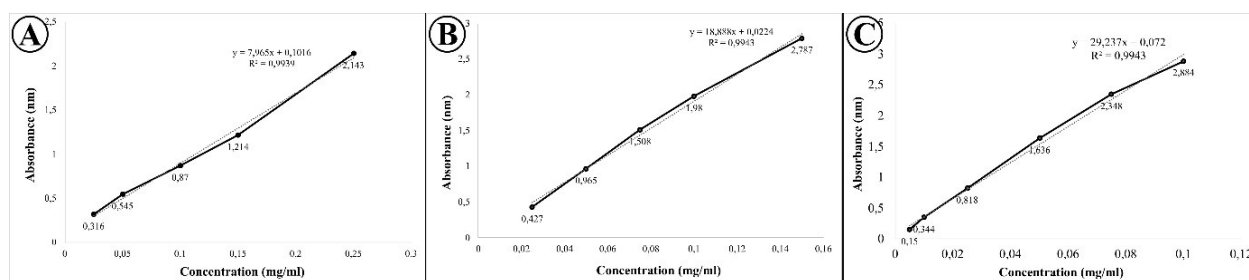


Figure 2. Calibration curves, A: Gallic acid curve for TPC, B: Rutin hydrate curve for TFC, C: Quercetin curve for TFC.

3.3. DPPH Free Radical Scavenging Activity

The antioxidant activity of *S. nydeggeri* extracts in different solvents was studied using DPPH with quercetin used as a positive control. The results are summarized in Tables 4 and 5. IC50 values were calculated using the formulas of the graphs (see Figures 3 and 4). IC50 represents the amount of extract required to provide 50% inhibition of hydroxyl radicals, with lower IC50 values indicating a better protective effect of the extracts.

The best IC50 value was obtained from the methanol extract at 12.66 µg/mL. The distilled water extract also showed a high free radical-scavenging capacity, with an IC50 value of 13.96 µg/mL. Quercetin, the control, exhibited the lowest radical-scavenging capacity at 1.14 µg/mL among all extracts. The lowest IC50 values were detected in the *n*-hexane (498.96 µg/mL) and chloroform (216.60 µg/mL) extracts, respectively.

Table 4. DPPH antioxidant test results of *S. nydeggeri* extracts and Quercetin (Con: Concentration, Abs: Absorbance, Inh: Inhibition, A_{cont}: Absorbance of control, IC50: Half maximal inhibitory concentration).

Quercetin			Methanol			Distilled Water		
Con (µg/mL)	Abs	Inh (%)	Con (µg/mL)	Abs	Inh (%)	Con (µg/mL)	Abs	Inh (%)
0.25	0.498	22.36	37.5	0.049	89.18	25	0.102	83.84
0.5	0.469	26.89	12.5	0.183	59.23	12.5	0.284	54.80
1.25	0.331	48.36	10	0.214	52.69	10	0.419	33.39
5	0.050	92.2	5	0.307	32.23	5	0.485	22.89
			2.5	0.344	23.99	2.5	0.578	8.11
						1.25	0.611	2.86
A _{cont.}	0.641		A _{cont.}	0.453		A _{cont.}	0.629	
IC50	1.14		IC50	12.66		IC50	13.96	

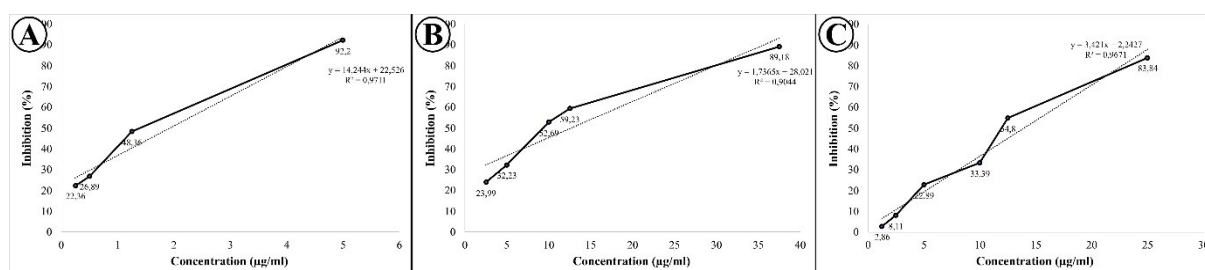
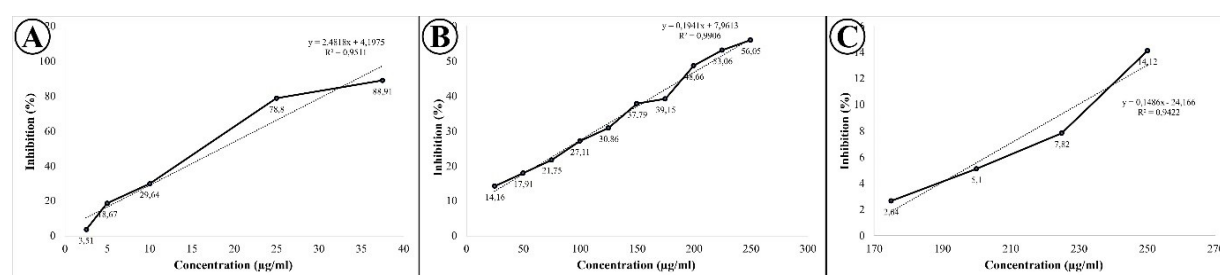


Figure 3. DPPH inhibition curves, A: Quercetin, B: Methanol extract, C: Distilled water extract.

Table 5. DPPH antioxidant test results of *S. nydeggeri* extracts (Con: Concentration, Abs: Absorbance, Inh: Inhibition, A_{cont}: Absorbance of control, IC₅₀: Half maximal inhibitory concentration).

Ethanol			Chloroform			<i>n</i> -hexane		
Con ($\mu\text{g/mL}$)	Abs	Inh (%)	Con ($\mu\text{g/mL}$)	Abs	Inh (%)	Con ($\mu\text{g/mL}$)	Abs	Inh (%)
37.5	0.060	88.91	250	0.290	56.05	250	0.505	14.12
25	0.115	78.80	225	0.309	53.06	225	0.542	7.82
10	0.381	29.64	200	0.338	48.66	200	0.558	5.10
5	0.440	18.67	175	0.401	39.15	175	0.573	2.64
2.5	0.522	3.51	150	0.410	37.79			
			125	0.456	30.86			
			100	0.480	27.11			
			75	0.516	21.75			
			50	0.541	17.91			
			25	0.566	14.16			
A _{cont.}	0.541		A _{cont.}	0.659		A _{cont.}	0.588	
IC ₅₀	18.46		IC ₅₀	216.60		IC ₅₀	498.96	

**Figure 4.** DPPH inhibition curves, A: Ethanol extract, B: Chloroform extract, C: *n*-hexane extract.

4. DISCUSSION and CONCLUSION

Many species of *Salvia* are utilized worldwide in the medicinal, cosmetic, and perfume industries, as well as in herbal teas and food condiments (Chalchat *et al.*, 1998). Additionally, this genus possesses significant characteristics that make it a valuable therapeutic plant for pharmaceutical applications (Jeshvaghani *et al.*, 2015).

Free radicals have garnered significant interest in recent years due to their role in the development of various diseases (Kamatou *et al.*, 2008). Antioxidants are essential for both the treatment and prevention of several diseases, as they chelate metals, react with free radicals, and scavenge oxygen (Shutava *et al.*, 2018). Natural antioxidants are far superior to synthetic antioxidant compounds when it comes to preventing the initial formation of free radicals, as they do not carry the same risks of cancer or liver and lung damage (Esmacili and Sonboli, 2010). Many studies have examined the antioxidant characteristics of various *Salvia* species native to different parts of the world (Asadi *et al.*, 2010, 2011; Esmacili & Sonboli, 2010; Orhan *et al.*, 2013; Stagos *et al.*, 2012; Şenol *et al.*, 2010; Torun, 2019; Tosun *et al.*, 2009). However, the antioxidant activity of *S. nydeggeri*, an endemic species of Türkiye, has not yet been reported.

In the current study, total phenolic content ranged from 44.031 mg GAE/g (in ethanol extract) to 110.489 mg GAE/g (in distilled water extract). Additionally, total flavonoid content ranged from 59.88 mg RU/g to 36.98 mg QE/g (in distilled water extract) and from 151.22 mg RU/g to 96.00 mg QE/g (in methanol extract). Tosun *et al.* (2009) reported that the total phenolic

content of methanolic extracts from eight *Salvia* species in Türkiye ranged from 50.3 to 167.1 mg GAE/g. Giamperi *et al.* (2012) investigated the total phenolic contents of six *Salvia* species from Italy and found that the values ranged from 1.3 µg/mg to 74.0 µg/mg. Hamrouni-Sellami *et al.* (2013) investigated the effects of different drying methods on the total phenolic and flavonoid content of *Salvia officinalis* L. methanol extracts. According to the results, the total phenolic content ranged from 0.662 mg GAE/g to 1.035 mg GAE/g, while the total flavonoid content ranged from 0.361 mg QE/g to 0.923 mg QE/g. Orhan *et al.* (2013) investigated the bioactivities of dichloromethane, ethyl acetate, and ethanol extracts from the aerial parts and/or roots of fourteen *Salvia* species. In this study, the total phenolic content ranged from 3.09 ± 0.78 to 392.18 ± 4.70 mg/g⁻¹ extract, and the total flavonoid content ranged from 8.29 ± 0.12 to 206.23 ± 3.4 mg/g⁻¹ extract. Alimpić *et al.* (2014) examined the DPPH free radical-scavenging activity, as well as the total phenolic and flavonoid content of extracts from *Salvia amplexicaulis* Lam. According to their findings, the total phenolic content ranged from 112.42 ± 0.601 to 222.40 ± 0.643 mg, while the total flavonoid content ranged from 21.85 ± 1.846 to 49.81 ± 0.820 mg QE/g. Sharopov *et al.* (2018) worked on the bioactivity, total phenolic, and flavonoid contents of three *Salvia* species. The total phenolic content ranged from 658.3 to 1805.9 mg CAE, while the total flavonoid content ranged from 13.0 to 184.9 mg QE. Mokhtari *et al.* (2023) examined the antioxidant properties of sage (*Salvia officinalis*), thyme (*Thymus vulgaris* L.), and their mixtures. The total phenolic content of *S. officinalis* was found to be 6.43 mg GAE/g. Analyzing previous research reveals that these findings are consistent with those reported in the literature. In this paper, the IC₅₀ value of DPPH radical scavenging capacity of *S. nydeggeri* was found to range from 12.66 µg/mL (in methanol extract) to 498 µg/mL (in *n*-hexane extract). Previously, the IC₅₀ value of radical-scavenging capacity of *Salvia* species in Türkiye was reported to range from 18.00 to 1259 µg/mL (Tepe *et al.*, 2006; Tosun *et al.*, 2009; Ünal *et al.*, 2008; Yılmaz *et al.*, 2023). Our results are consistent with those of previous studies.

The primary source of oxidative stress is reactive oxygen species (ROS), which damage proteins, lipids, and DNA due to their high reactivity (Papaharalambus and Griendling, 2007). Under normal conditions, the production of ROS during aerobic metabolism and their scavenging by tissue antioxidant systems are balanced (Frei, 1994). However, when cardiovascular risk factors are present, this natural balance is disrupted, favoring oxidative stress (Dinh *et al.*, 2014; Margolis *et al.*, 1974; Tousoulis *et al.*, 2011). Studies have shown that *Salvia miltiorrhiza* species possess properties that scavenge ROS in cardiovascular diseases such as hypertension, hyperglycemia, hyperlipidemia, obesity, coronary artery disease, and ischemic heart disease. Molecules isolated from this species (lithospermic acid B, salvianolic acid A, salvianolic acid B, tanshinone IIA, etc.) have created a barrier against oxidative stress by affecting various pathways (e.g., MAPK signaling pathway, Bax/Bcl-2, caspase-3) involved in these diseases (Au-Yeung *et al.*, 2007; Chang *et al.*, 2016; Jia *et al.*, 2012; Karmin *et al.*, 2001; Lam *et al.*, 2006; Qian *et al.*, 2011; Qian *et al.*, 2012; Tang *et al.*, 2011; Yang *et al.*, 2015; Zhou *et al.*, 2005).

Salvia species (e.g., *Salvia officinalis*) are widely studied for their strong antioxidant activity. Their antioxidant potential is mainly attributed to polyphenols, flavonoids, phenolic acids, and diterpenoids present in various parts of the plant. The extracts show free radical scavenging, metal chelating, and lipid peroxidation inhibition activities (Işık, 2021). This translates into protective effects across multiple systems including neurological, cardiovascular, hepatic, and even anti-cancer benefits. These findings support the therapeutic potential of *Salvia* extracts for oxidative stress-related diseases.

In conclusion, the results of this study indicate that *S. nydeggeri* can serve as an important natural resource for the development of pharmaceuticals, cosmetics, and nutraceuticals, owing to its phenolic content, total flavonoid content, and antioxidant potential. *Salvia* species have promising antioxidant properties but require careful safety evaluation. Toxicity is generally low at therapeutic doses, but high doses or prolonged use may pose risks due to certain

phytochemicals. Standardized extracts, proper dosing, and thorough preclinical and clinical testing are essential to ensure safety in antioxidant applications. For this reason, further studies are necessary to gain a deeper understanding of the effects of its active ingredients in biological systems.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Asude Ersal: Investigation, resources, visualization, software, and formal analysis. **Batıkan Günal:** Methodology, supervision, and validation. **Gürkan Semiz:** Investigation, supervision, writing – original draft.

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