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Chemometrics-based analysis of the phytochemical profile and antioxidant activity of *Salvia* species from Iran

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In recent years, the exploration of the therapeutic potential of *Salvia* has gained considerable attention, leading to a growing number of scientific studies emphasizing its pharmacological properties. Despite this, therapeutic applications of *Salvia* remain underexploited, requiring further investigation. Iran is a major center for sage diversity in Asia, boasting 60 *Salvia* species, 17 of which are unique to the area. This study aimed to comprehensively explore and compare the extracts of 102 *Salvia* samples belonging to 20 distinct *Salvia* species from Iran, providing a deeper understanding of their specific polyphenol content and, consequently, their antioxidant capabilities and potential therapeutic uses. All samples were analyzed to determine the contents of total phenolics, total flavonoids, total tannin, photosynthetic pigments, and ascorbic acid, along with their antioxidant activity. These data were then combined with the forty distinct chemical fingerprints identified by ultrafast high-pressure liquid chromatography coupled with high-resolution mass spectrometry. Multivariate data analysis was employed to find correlations and differences among the huge number of data obtained and to identify *Salvia* species with similar phytochemical and/or antioxidant properties. The results show that each *Salvia* species is characterized by a distinct class of polyphenols recognized for their antidiabetic, anti-inflammatory, cardioprotective and neuroprotective properties. Overall, our findings reveal the potential of some *Salvia* species for targeted therapeutic applications and provide a rational basis for the development of *Salvia*-derived nutraceuticals, ultimately improving the prospects for the use of *Salvia* in medicine.

Keywords *Salvia*, Antioxidants, Polyphenolic composition, UHPLC-HRMS, Principal component analysis

The Lamiaceae family is composed of approximately 250 genera and 7000 species worldwide. *Salvia* is one of the largest and most valuable genera of the Lamiaceae family, with over 1000 species distributed worldwide^{1,2}. The *Salvia* genus (sage) is widely recognized as one of the most important and widespread medicinal and aromatic groups within the Lamiaceae family. Sages have traditionally been used for various medicinal purposes, including as spasmolytics, antiseptics, antihydrotics, anti-inflammatories, and in the treatment of mental conditions and nervous disorders^{3,4}.

Phytochemical analysis of *Salvia* species has revealed the presence of various compounds with a significant antioxidant role, as reported by Kahnmoeei et al.⁵ and Kan et al.⁶. Major phytochemicals identified within *Salvia* species include flavonoids, anthocyanins, phenolic acids, phenolic glycosides, polysaccharides, terpenoids, coumarins, and essential oils^{7,8}. Among these, terpenoids and polyphenols stand out as the most prominent secondary metabolites in *Salvia* species. In sage plants, among flavonoids, flavones like luteolin and apigenin, along with their corresponding flavonols and 6-hydroxylated derivatives, represent the most abundant compounds. Caffeic acid, found in various *Salvia* species, serves as a fundamental component for various metabolites, spanning from simple monomers to oligomers. Notably, rosmarinic acid emerges as the predominant dimer of caffeic acid identified in sage plants⁶. Additionally, trimers derived from caffeic acid, such as salvianolic, lithospermic, and yunnaneic acids, as well as sage-coumarin also exhibits a significant presence^{9–11}.

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A positive correlation was observed between phenolic content and antioxidant activity, as reported by Kamatou et al.¹². Notably, species with high phenolic content demonstrated the most promising antioxidant activity¹². Phenolic compounds are extensively distributed in *Salvia* species¹³, and studies by Kamatou et al.¹², Farhat et al.¹⁴, and Tepe et al.¹⁵ have reported the potent radical scavenging abilities of various *Salvia* species.

Salvia species are known to contain a wide range of terpenoid compounds. As an example, borneol, camphor, caryophyllene, and α - and β -thujone are widely mentioned in essential oil of *Salvia* species, and other characteristic diterpenoid quinones have been found in *Salvia* extracts, including tanshinone I, tanshinone IIA, cryptotanshinone and miltirone. These compounds, together with polyphenolics (such as rosmarinic acid, caffeic acid and its metabolites, yunnaneic and salvianolic acids) and flavonoids (like luteolin, apigenin, and kaempferol), contribute to a variety of bioactivities. Studies by Wu et al.⁸, Lu and Foo⁹, Ghorbani and Esmailzadeh¹⁶, Jassbi et al.¹⁷, Sharifi-Rad et al.¹⁸, Topçu¹⁹ and Fierascu²⁰ confirm the presence of various bioactive compounds in *Salvia* plants.

However, the specific types and concentrations of phytochemicals can vary significantly among different species of *Salvia*, contributing to their diverse pharmacological properties, which include antioxidant, anti-inflammatory, antidiabetic, and antimicrobial effects, among others. Moreover, the qualitative and quantitative composition of phytochemicals in sage plants, which is crucial for its beneficial properties, is strongly influenced by a multitude of factors, including genetics and specie variations, environmental factors, cultivation techniques, and post-harvest processing. Environmental factors, like temperature and rainfall, play a significant role in the biosynthesis of phenolic compounds in sage leaves. Changes in these environmental conditions can impact the metabolic pathways within the plant, influencing the production of phenolic compounds. For instance, certain phenolic compounds might be produced in higher quantities in response to specific temperature ranges. Rainfall or moisture levels in the soil can also impact the plant's stress levels, triggering the production of secondary metabolites, like phenolic compounds, as a defense mechanism. Therefore, understanding how environmental factors impact the growth, yield, and physiological processes of alternative crops like *Salvia*, is crucial for effective cultivation and optimizing the production of beneficial phytochemicals. The studies by Cardile et al.²¹, Mancini et al.²², Canzoneri et al.²³, and Tenore et al.²⁴ have explored these impacts in detail.

Investigating the phytochemical profile and antioxidant activity of *Salvia* is of paramount importance due to the growing interest in natural antioxidants and their potential health benefits. In the present study, 102 *Salvia* samples belonging to 20 different species (Fig. 1, Table 1), collected from various locations in the Iranian province of the West Azerbaijan (Fig. 2) were examined and their phytochemical properties were compared to select the superior species based on phytochemical markers. For this purpose, the contents of total phenolics, total flavonoids, total tannin, photosynthetic pigments, and ascorbic acid were analyzed, along with the antioxidant activity. These data were combined with the chemical fingerprints determined by ultrafast high-pressure liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) and then analyzed by employing multivariate data analysis. This approach enables simultaneous analysis of multiple variables, providing a more comprehensive understanding of the complex relationships within the data. Unlike many existing studies in the field, our research takes a broader approach by examining correlations and differences between a wide range of phytochemical and antioxidant properties among various *Salvia* species. Rather than quantifying individual phytochemicals, our goal is to identify similarities and differences between species. This holistic approach not only provides valuable insights into the intricate interplay of phytochemicals, but also holds promise for guiding the selection of *Salvia* species for targeted therapeutic applications. Through its comprehensive analysis and nuanced understanding of phytochemical profiles, this study paves the way for more effective and tailored applications of *Salvia* in medicine. Furthermore, the regional significance of this study is crucial. *Salvia* species are an integral part of Iran's rich botanical heritage, underscoring the importance of conserving and utilizing these plants for their medicinal properties. By specifically examining *Salvia* species from Iran, we offer valuable insights into the diversity and bioactivity of the flora in this region.

Results and discussion

Total phenolic content (TPC)

Due to the multitude of potential health advantages and industrial applications, total phenolic content serves as a valuable parameter in various fields, including food science, nutrition, and pharmaceuticals²⁵. TPC is extensively used in scientific research to study the bioactivity and potential health benefits of natural compounds and extracts²⁶. In fact, phenolic compounds are renowned for their antioxidant properties, reducing cellular oxidative stress and lowering risks linked to chronic diseases like cardiovascular diseases and certain cancers²⁷. Consequently, TPC proves instrumental in assessing the potential health advantages of herbal medicines and natural remedies.

In this study, TPC levels of the aerial parts extracts of *Salvia* plants were determined using the Folin-Ciocalteu method. Significant differences in total phenolic content were observed among the extracts ($p < 0.01$). Table 2 shows the TPC values for the 102 *Salvia* plants, ranging from 12.67 to 62.46 mg GAE/g DW, with *S. ceratophylla* and *S. limbata* exhibiting the highest and lowest total phenol content, respectively. Previous studies showed that the total phenolic content of *S. officinalis* ranged from 2.02 to 184 mg GAE/g DW. Alizadeh and Shaabani reported a TPC content of 25.13 mg GAE/g DW for Iranian *S. officinalis*²⁸. Similar ranges of phenolic amounts were obtained for some *Salvia* species such as *S. lanceolata*, *S. dolomitica*, and *S. garepensis* (54.2, 53.0, and 45.6 mg GAE/g DW, respectively)¹². TPC variations within species suggest that weather, geographic location, and genetic factors may influence total phenol content. Previous studies have also demonstrated the crucial role of intrinsic and extrinsic factors, such as genotype, climatic conditions, growing location, developmental stage, and extraction or calibration methods used in determining the TPC^{29,30}. In these literature studies, by carefully considering sample size and representativeness, it was possible to obtain accurate insights into the phytochemical diversity of

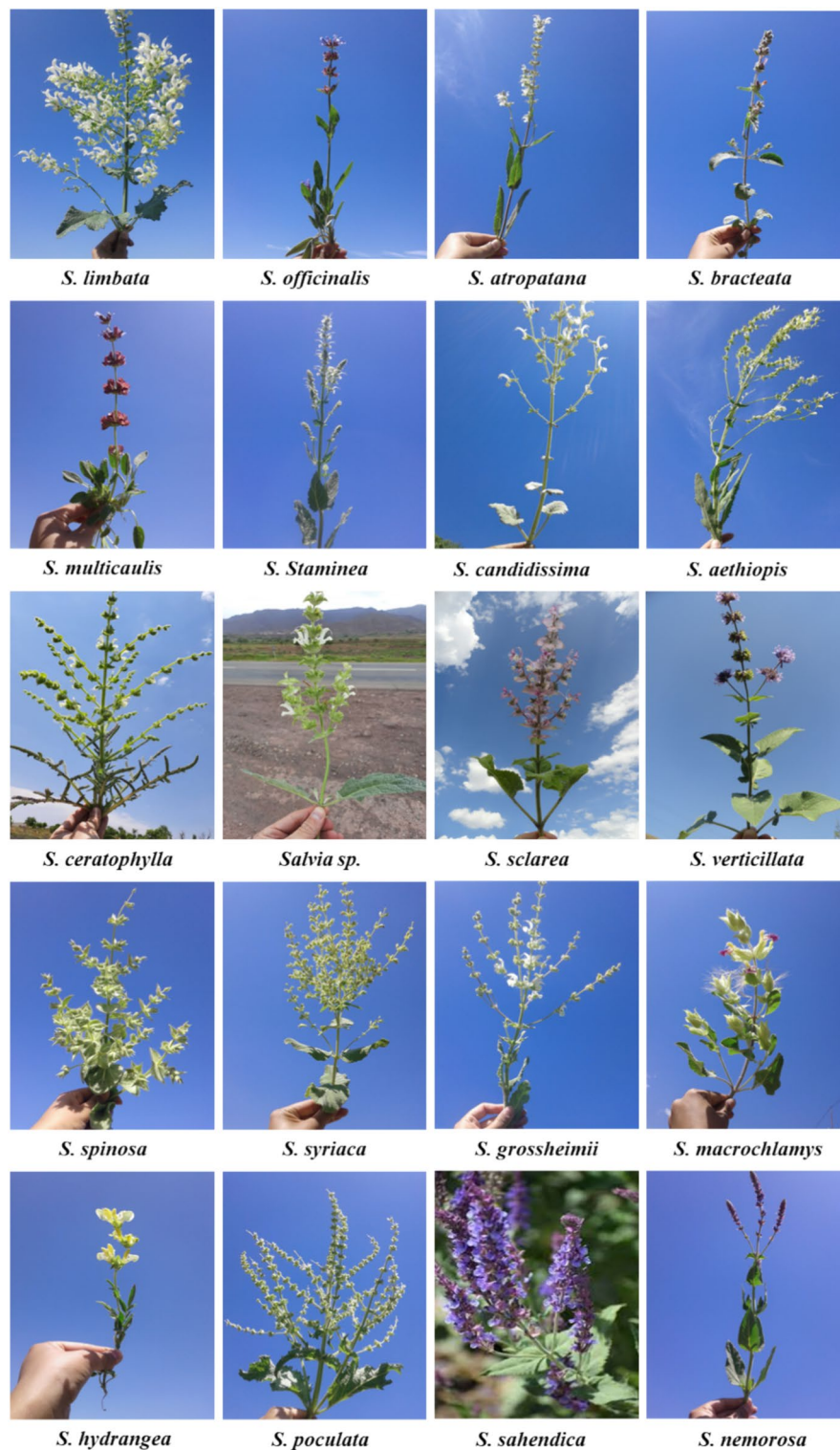


Figure 1. Pictures of the 20 *Salvia* species investigated in this study (all pictures were taken by A. Moshari-Nasirkandi and A. Alirezalu).

Salvia populations. This is essential for various purposes such as conservation, medicinal research, and breeding programs. Therefore, conducting multi-site studies encompassing different ecological conditions and habitats could provide a more comprehensive understanding distribution and characteristics of *Salvia* species within Iran.

Species	Number of samples	Collecting areas
<i>S. multicaulis</i>	14	Silvana; Mavana; Aghbolagh; Hesarlu; Saqqez; Qasemlu; Tekab; Urmia-Tabriz; Nokhtalu; Khalifatan; Khaneqah Sorkh; Marmisho; Marmisho-Serow; Oshnavieh-Piranshahr
<i>S. limbata</i>	15	Talebin village; Aghbolagh; Bukan; Qasemlu; Marmisho; Serow; Qurdik-Khoy; Shahindej-Tekab; Takhte-Soleiman; Gareziaedin; Nokhtalu; Daryan; Bukan-Mahabad; Mahabad-Miandoab; Chaldoran
<i>S. syriaca</i>	16	Shiban village; Baruq town; Shahindej-Tekab; Naghadeh-Piranshahr; Saqqez; Qushchi; Qasemlu; Marmisho; Serow; Takhte-Soleiman; Nokhtalu; Daryan; Bukan-Mahabad; Salmas-Khoy; Marmisho-Serow; Oshnavieh-Piranshahr
<i>S. verticillata</i>	6	Mavana; Qasemlu; Marmisho; Qurdik-Khoy; Daryan; Marmisho
<i>S. nemorosa</i>	17	Silvana; Aghbolagh; Mahabad-Urmia; Miandoab; Utmish village; Noshin Shahr; Qasemlu; Qasemlu; Anhar road; Marmisho; Serow; Khoy; Daryan; Tasuj; Maku; Piranshahr-Sardasht; Noshin Shahr
<i>S. officinalis</i>	2	Urmia university
<i>S. atropatana</i>	4	Sirdaghi; Pardanan jungle; Qushchi; Mishodagh
<i>S. sclarea</i>	3	Urmia university; Marmisho; Piranshahr-Sardasht
<i>S. bracteata</i>	2	Pardanan jungle; Piranshahr-Sardasht
<i>S. spinosa</i>	6	Bukan; Saqqez; Mahabad-miandoab; Qushchi; Urmia-Tabriz; Cheshmeh Kanan
<i>S. Staminea</i>	1	Qahreman Kandi
<i>S. ceratophylla</i>	5	Qushchi; Daryan; Bukan road; Khalifatan; Marmisho-Serow
<i>S. macrochlamys</i>	2	Marmisho; Marmisho-Serow
<i>S. candidissima</i>	1	Marmisho
<i>S. aethiopsis</i>	2	Dalamber; Khoy
<i>S. sahendica</i>	1	Tabriz
<i>Salvia sp.</i>	1	Tupchi
<i>S. grossheimii</i>	1	Khaneqah Sorkh
<i>S. poculata</i>	1	Qushchi
<i>S. hydrangea</i>	2	Qushchi; Marmisho-Serow

Table 1. List of species investigated along with the relative number of samples and collecting areas.

Total flavonoid content (TFC)

Total flavonoid content holds significance across various fields due to the potential health benefits of flavonoids, wide industrial applications, and roles in diet and environmental analysis³¹. Flavonoids are among the most diverse and widespread natural compounds and considered the most important among natural phenolics. These compounds exhibit a wide spectrum of chemical and biological activities³¹. Flavonoids are renowned for their antioxidant properties. Indeed, they are capable of neutralizing free radicals and reducing oxidative stress, potentially decreasing the risk of chronic diseases. Some flavonoids also exhibit anti-inflammatory properties and the potential to inhibit cancer cell proliferation⁹. Furthermore, consuming flavonoid-rich foods is linked to improved cardiovascular health as it lowers blood pressure and reduces the risk of heart disease. Certain flavonoids also demonstrate neuroprotective properties, beneficial for conditions such as Alzheimer's disease.

Therefore, identifying sage species with high TFC is crucial for developing nutraceuticals to prevent and support treatment for various health issues and metabolic disorders. Significant variation in TFC ($p < 0.01$) was observed among the studied plants. TFC ranged from 9.58 to 18.66 mg QUE/g DW (Table 2), with the highest and lowest levels exhibited by *S. syriaca* and *S. limbata*, respectively. A previous study identified the three *Salvia* species with the highest total flavonoids content as *S. hierosolymitana* (770.85 mg QUE/g DW), *S. eigii* (520.60 mg QUE/g DW), and *S. viridis* (311.36 mg QUE/g DW)³². While the amount of flavonoid content in methanolic extracts of *S. chudaei* and *S. officinalis* L. was reported as 4.68 and 9.1 mg QUE/g, respectively^{33,34}. The variations in TFC may be due to seasonal changes. This and several other studies have not taken this effect in account. Different seasons and years can influence the synthesis and accumulation of secondary metabolites in plants. To mitigate this limitation, future research could consider conducting multiple sampling rounds across different seasons to capture seasonal variations in plant chemistry and antioxidant activity.

Total tannin content (TTC)

A statistically significantly difference in total tannin content (TTC) was also observed between the extracts ($p < 0.01$). TTC ranged from 7.60 to 245.02 mg TAE/100 g DW (Table 2), with *S. limbata* and *S. verticillata* extracts showing the highest and lowest values, respectively. In a previous study, the TTC content ranged from 0.33 to 6.49 mg TAE/100 g DW, with *S. macrochlamys* and *S. verticillata* displaying the highest and lowest values, respectively³⁵. Variations in species, extraction methods, solvents, and analytical techniques can lead to differences in the measured TTC.

The higher content of TTC compared to TPC may be due to factors related to the chemical nature and measurement methods of tannins and phenolics. For instance, the Folin-Ciocalteu method for TPC is known to not react equally with all phenolics. TTC measurements, specifically targeting tannins, may be more sensitive. In addition, different solvents and extraction methods affect yields, and tannins, with multiple phenolic groups per molecule, may exhibit higher TTC, even if fewer in number. Sample matrix interferences could also reduce TPC more than TTC. Phenolics are prone to degradation, reducing TPC, while larger, more complex tannins are more stable, leading to higher TTC.

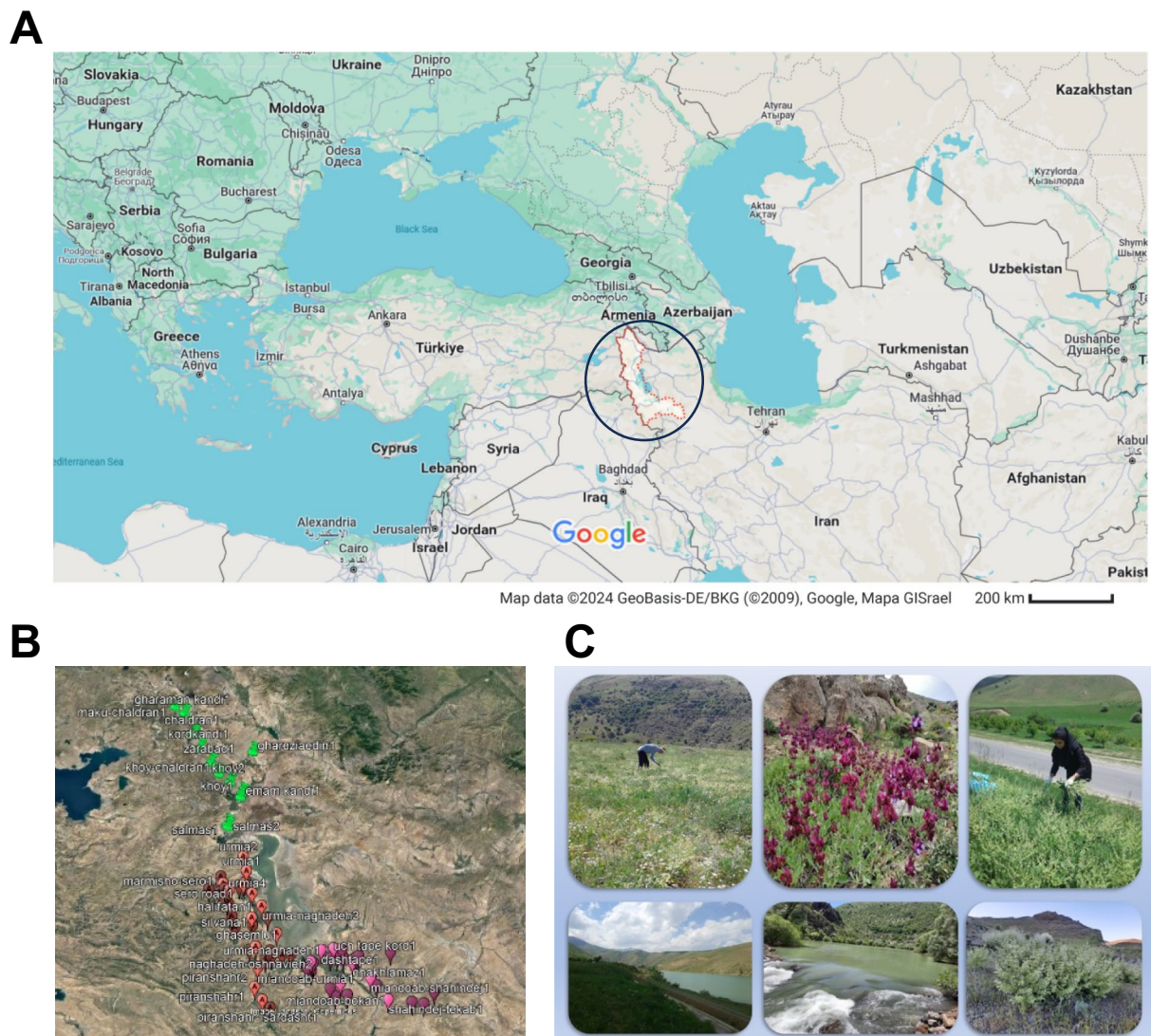


Figure 2. (A) Geographical location of West Azerbaijan. (B) Satellite image of West Azerbaijan province showing identification of 3 distinct collection areas where *Salvia* samples were picked. (C) Illustrative shots of both the handpicking process of sampling and the different collecting landscapes. Images in (A,B) were extracted from Google Earth Pro V 7.3.6.9796. Pictures in (C) were taken by A. Moshari-Nasirkandi and A. Alirezalu.

Tannins are polyphenolic substances with different molecular weights and complexity^{36,37}. Tannins are secondary metabolites that play a primary role in plant defense mechanisms³⁸. Tannins possess various properties, including anti-inflammatory, regeneration, anticatarrhal, antimicrobial and soothing effects³⁹. Tannins have been used in traditional medicines to treat various diseases. Epidemiological data suggest that tannin intake may inhibit the onset of chronic diseases⁴⁰. Tannins are also renowned to possess antimicrobial and antioxidant properties that are significant in addressing skin disorders and wounds⁴¹. Traditional medicines rich in tannins have been reported to be used for treating wounds, burns, inflammation, and other medical ailments⁴². Tannins can generate smaller phenolic compounds, such as pyrogallol, catechol, and ellagic acid, which are known for their bactericidal activity. Tannins also perform many other biologically significant functions, including protection against oxidative stress and degenerative diseases^{39,42}.

Ascorbic acid content (AAC)

Significant differences in ascorbic acid content were observed among the investigated samples ($p < 0.01$), as shown in Table 2. *Salvia multicaulis* extracts showed the highest AAC value, while *S. nemorosa* extracts showed the lowest (49.70 and 36.61 mg AA/100 g DW, respectively). In the investigation of the amount of ascorbic acid in 20 different species of the Lamiaceae family, values ranged from 0.58 to 1.15 mg AA/g DW, with sage and *Stachys* sp. showing the highest and lowest amount of ascorbic acid, respectively³⁵. Ascorbic acid is susceptible to rapid decomposition, and improper storage and processing of plant samples can result in the degradation of phytochemicals and antioxidants, thus affecting the accuracy of analysis. Establishing standardized protocols for sample handling and storage is crucial to mitigate these issues.

Sample code	Species	TPC (mg GAE/g DW)	TFC (mg QUE/g DW)	TTC (mg TAE/100 g DW)	AAC (mg AA/g DW)
S1	<i>S. multicaulis</i>	52.46±0.01	11.72±0.74	15.59±0.27	41.05±1.69
S2	<i>S. limbata</i>	17.72±0.04	9.58±0.13	12.62±0.20	39.96±1.24
S3	<i>S. syriaca</i>	42.81±0.81	13.18±0.63	18.83±0.17	41.97±1.60
S4	<i>S. multicaulis</i>	39.47±0.07	10.92±0.12	14.06±0.16	39.96±0.86
S5	<i>S. verticillata</i>	49.25±0.25	11.23±0.39	7.60±0.68	39.07±1.44
S6	<i>S. nemorosa</i>	49.70±1.70	11.96±0.85	24.22±0.65	37.38±0.38
S7	<i>S. officinalis</i>	54.31±2.31	13.63±0.37	123.87±5.87	40.47±0.41
S8	<i>S. officinalis</i>	57.55±4.55	11.75±0.50	45.74±1.60	37.76±0.26
S9	<i>S. atropatana</i>	48.97±0.22	11.87±0.74	57.50±0.16	41.02±1.85
S10	<i>S. sclarea</i>	52.26±0.06	11.82±0.27	85.43±2.15	40.44±1.85
S11	<i>S. syriaca</i>	48.91±0.01	12.24±0.49	46.80±2.34	40.76±1.66
S12	<i>S. multicaulis</i>	54.04±0.04	10.88±0.09	46.13±0.87	41.24±2.27
S13	<i>S. nemorosa</i>	32.44±1.22	10.10±0.21	9.04±0.03	41.59±0.83
S14	<i>S. limbata</i>	23.00±0.50	10.49±0.34	22.38±0.59	41.37±0.86
S15	<i>S. multicaulis</i>	41.20±0.20	11.63±0.41	27.97±0.97	41.37±2.14
S16	<i>S. syriaca</i>	40.29±0.03	11.59±0.94	46.80±2.10	43.83±1.91
S17	<i>S. nemorosa</i>	46.17±0.07	11.58±0.30	33.71±0.29	36.93±0.57
S18	<i>S. syriaca</i>	27.28±0.28	11.45±0.12	24.89±2.51	40.60±0.67
S19	<i>S. atropatana</i>	49.84±3.84	12.52±0.55	29.64±1.24	39.58±0.73
S20	<i>S. bracteata</i>	48.17±3.17	10.72±0.26	56.33±0.63	43.12±2.11
S21	<i>S. nemorosa</i>	51.57±1.07	11.49±0.73	51.80±1.40	39.36±0.96
S22	<i>S. bracteata</i>	38.19±1.00	10.31±0.31	46.60±2.40	40.44±3.45
S23	<i>S. nemorosa</i>	42.25±1.61	10.50±0.22	22.30±0.40	41.56±0.54
S24	<i>S. limbata</i>	30.09±1.09	12.07±0.78	138.06±5.03	44.02±0.57
S25	<i>S. spinosa</i>	42.56±0.56	12.43±0.12	56.76±3.32	44.40±0.26
S26	<i>S. spinosa</i>	20.61±0.61	10.30±0.22	16.41±0.41	39.90±0.10
S27	<i>S. syriaca</i>	45.10±0.90	13.53±0.53	38.32±1.60	39.33±3.22
S28	<i>S. Staminea</i>	34.51±1.00	11.15±0.06	56.84±4.18	41.18±0.03
S29	<i>S. multicaulis</i>	57.52±1.22	12.63±3.81	46.25±4.92	42.13±2.46
S30	<i>S. nemorosa</i>	30.63±0.63	10.11±0.17	24.18±0.35	42.13±0.35
S31	<i>S. spinosa</i>	32.81±0.70	11.47±0.05	21.41±1.72	40.60±0.99
S32	<i>S. spinosa</i>	23.60±0.20	9.96±0.16	8.93±0.53	40.44±0.57
S33	<i>S. syriaca</i>	35.72±0.02	12.96±0.93	29.53±0.53	40.25±0.26
S34	<i>S. ceratophylla</i>	62.46±0.03	16.04±0.86	66.76±1.76	41.18±0.61
S35	<i>S. atropatana</i>	28.44±0.44	10.35±0.14	38.63±1.63	41.66±1.40
S36	<i>S. nemorosa</i>	36.34±0.04	10.66±0.28	21.56±0.56	36.61±0.61
S37	<i>S. verticillata</i>	43.80±0.05	11.67±0.15	19.92±0.47	40.92±1.12
S38	<i>S. nemorosa</i>	35.55±0.15	10.35±0.18	21.13±0.13	42.29±1.66
S39	<i>S. limbata</i>	27.00±0.20	11.04±0.25	63.71±1.91	38.62±1.44
S40	<i>S. syriaca</i>	28.59±0.19	11.28±0.32	25.90±0.90	42.55±1.79
S41	<i>S. nemorosa</i>	31.87±0.04	11.16±0.10	31.25±0.45	39.61±1.91
S42	<i>S. multicaulis</i>	45.72±1.02	11.03±0.73	21.80±0.80	42.58±2.46
S43	<i>S. nemorosa</i>	54.51±0.49	11.99±0.19	39.18±0.18	41.62±1.76
S44	<i>S. sclarea</i>	32.93±0.07	10.06±0.01	26.91±0.66	40.41±0.35
S45	<i>S. macrochlamys</i>	39.13±0.13	10.91±0.44	121.86±3.14	40.67±0.41
S46	<i>S. syriaca</i>	36.76±0.20	12.17±0.72	13.63±0.42	41.91±1.40
S47	<i>S. verticillata</i>	48.36±0.03	10.77±0.23	10.02±0.53	40.16±2.20
S48	<i>S. nemorosa</i>	43.94±0.05	11.10±0.42	14.14±0.16	39.64±3.16
S49	<i>S. candidissima</i>	47.47±1.32	15.96±0.77	80.08±2.99	41.21±1.09
S50	<i>S. limbata</i>	18.76±0.76	9.95±0.14	14.57±0.57	39.64±2.07
S51	<i>S. syriaca</i>	22.76±0.04	11.05±0.23	15.70±0.82	40.92±2.27
S52	<i>S. limbata</i>	31.92±0.08	11.30±0.33	41.99±2.55	42.10±0.38
S53	<i>S. nemorosa</i>	33.28±0.28	9.80±0.11	11.95±0.93	41.50±1.63
S54	<i>S. verticillata</i>	44.76±0.76	10.54±0.51	9.34±0.12	41.88±0.35
S55	<i>S. limbata</i>	23.13±0.03	10.19±0.27	42.50±2.11	40.73±1.69
S56	<i>S. limbata</i>	12.67±1.67	9.72±0.16	27.87±0.98	44.05±1.18

Continued

Sample code	Species	TPC (mg GAE/g DW)	TFC (mg QUE/g DW)	TTC (mg TAE/100 g DW)	AAC (mg AA/g DW)
S57	<i>S. multicaulis</i>	53.66±0.2	12.40±0.94	31.45±1.37	49.70±4.09
S58	<i>S. syriaca</i>	36.93±0.93	11.92±0.1	43.91±3.13	41.40±1.02
S59	<i>S. nemorosa</i>	51.03±0.97	12.68±0.95	67.07±1.93	41.97±2.87
S60	<i>S. aethiopsis</i>	52.56±1.44	12.05±1.00	126.62±4.38	45.23±0.64
S61	<i>S. limbata</i>	32.07±2.04	11.29±0.55	120.61±2.88	40.76±0.06
S62	<i>S. sahendica</i>	33.38±0.30	11.02±0.29	59.80±0.98	41.97±0.26
S63	<i>S. nemorosa</i>	41.23±0.12	10.69±0.34	19.84±0.39	42.13±0.35
S64	<i>S. verticillata</i>	55.77±0.37	16.26±0.66	23.55±0.55	39.96±1.50
S65	<i>S. aethiopsis</i>	59.33±0.27	12.80±0.30	68.71±2.20	43.67±0.48
S66	<i>S. spinosa</i>	34.54±1.54	11.16±0.38	9.44±0.94	44.88±2.07
S67	<i>S. multicaulis</i>	56.14±0.14	11.99±0.42	32.85±0.20	43.60±1.63
S68	<i>S. limbata</i>	38.07±1.41	10.37±0.09	29.69±0.69	39.26±2.65
S69	<i>S. syriaca</i>	45.30±1.30	12.24±0.94	27.50±0.50	40.67±0.03
S70	<i>S. multicaulis</i>	50.09±0.09	10.72±0.14	13.48±0.20	42.74±1.09
S71	<i>S. limbata</i>	26.54±1.04	10.07±0.21	14.49±0.82	43.25±0.26
S72	<i>S. syriaca</i>	54.34±0.09	18.66±0.27	114.68±1.68	43.09±1.18
S73	<i>S. limbata</i>	56.86±0.86	13.61±0.61	245.02±4.12	43.76±2.11
S74	<i>S. ceratophylla</i>	45.23±0.23	11.64±0.20	34.88±2.07	42.87±1.53
S75	<i>S. spinosa</i>	53.13±0.13	12.08±0.10	31.60±0.37	44.81±2.78
S76	<i>Salvia</i> sp.	43.52±0.52	11.67±0.13	37.73±2.66	42.10±0.89
S77	<i>S. nemorosa</i>	56.91±0.61	12.34±0.81	37.81±0.93	41.85±0.83
S78	<i>S. ceratophylla</i>	34.02±0.24	10.14±0.22	18.59±0.41	43.41±1.56
S79	<i>S. syriaca</i>	23.10±0.52	10.98±0.14	23.52±0.52	41.37±0.86
S80	<i>S. limbata</i>	19.99±1.11	10.38±0.19	123.67±3.67	40.38±2.11
S81	<i>S. limbata</i>	21.10±1.10	10.91±0.59	134.73±4.73	41.81±0.73
S82	<i>S. poculata</i>	33.50±1.05	10.34±0.27	15.63±0.08	40.41±0.73
S83	<i>S. hydrangea</i>	54.88±2.38	12.06±0.57	38.24±0.54	40.16±2.65
S84	<i>S. syriaca</i>	33.67±1.14	11.71±0.09	31.68±0.68	44.27±0.57
S85	<i>S. ceratophylla</i>	53.77±3.77	13.63±0.63	48.98±1.19	41.02±1.02
S86	<i>S. limbata</i>	25.35±0.35	10.37±0.11	22.19±0.81	40.41±1.12
S87	<i>S. multicaulis</i>	52.86±2.85	12.21±0.21	33.83±0.93	43.28±4.12
S88	<i>S. grossheimii</i>	54.59±1.50	13.42±0.42	66.56±2.89	42.04±0.77
S89	<i>S. multicaulis</i>	53.30±0.08	12.24±0.50	24.53±0.53	45.55±0.77
S90	<i>S. multicaulis</i>	50.66±1.66	11.40±0.36	11.12±0.06	40.06±0.38
S91	<i>S. verticillata</i>	57.13±1.02	16.85±0.86	14.88±0.98	45.23±2.43
S92	<i>S. syriaca</i>	34.17±1.12	12.30±0.34	32.30±0.40	44.56±0.99
S93	<i>S. multicaulis</i>	46.71±1.01	12.19±0.93	16.25±0.02	46.73±1.63
S94	<i>S. aethiopsis</i>	30.36±0.36	10.53±0.28	23.13±0.31	44.97±1.34
S95	<i>S. hydrangea</i>	40.12±0.12	11.07±0.36	23.71±0.71	44.66±1.09
S96	<i>S. macrochlamys</i>	30.93±0.10	9.92±0.13	33.75±1.25	42.13±2.14
S97	<i>S. ceratophylla</i>	58.83±0.83	13.24±0.34	40.35±1.05	42.36±3.64
S98	<i>S. atropatana</i>	34.39±0.31	11.15±0.23	43.16±1.84	45.61±1.15
S99	<i>S. sclarea</i>	44.09±0.09	11.60±0.16	57.42±2.66	45.10±1.47
S100	<i>S. nemorosa</i>	45.45±1.11	10.21±0.19	15.70±0.28	38.78±1.15
S101	<i>S. syriaca</i>	29.08±0.31	11.45±0.46	20.08±0.23	41.88±1.50
S102	<i>S. multicaulis</i>	48.56±0.56	11.83±0.58	60.39±3.14	43.83±1.53
Significant levels		**	**	**	**

Table 2. Phytochemical content (TPC, TFC, TTC and AAC) determined for the 102 *Salvia* extracts studied. TPC total phenolic content, TFC total flavonoid content, TTC total tannin content, AAC ascorbic acid content. **Indicates significant difference at 1% confidence levels.

The action of ascorbic acid (AA) is essential for normal wound healing⁴³. Ascorbic acid acts as a hydrogen atom donor to lipid radicals, quenches singlet oxygen, and removes molecular oxygen⁴⁴. The biochemical function of AA is often associated with its redox potential^{45,46}. In other words, AA has the characteristic of being easily oxidized by releasing electrons in an aqueous solution, making it a powerful water-soluble antioxidant that reacts with reactive oxygen species (ROS) or free radicals⁴⁷.

Photosynthetic pigments content

Among the investigated plants, significant differences in the levels of chlorophyll a and b (C_a and C_b , respectively), total carotenoid concentration (C_{x+c}), and β -carotene were observed ($p < 0.01$). However, in this case it was not possible to identify a *Salvia* species with the highest and lowest pigment concentration (Table 3). The extracts from *S. spinosa* (S31) showed the highest content of C_a and C_b (41.78 mg/100 g DW and 18.25 mg/100 g DW, respectively), while *S. atropatana* (S35), showed the highest C_{x+c} and β -carotene content (38.13 mg/g DW and 2.98 mg/100 g DW, respectively). Conversely, the lowest values of C_a and C_b were displayed by *S. verticillata* (S64) and *S. multicaulis* (S42) (5.13 mg/100 g DW and 2.04 mg/100 g DW, respectively), while *S. spinosa* (S66) exhibited the lowest content of both C_{x+c} and β -carotene (5.25 mg/g DW and 0.40 mg/100 g DW, respectively). Previous studies reported a chlorophyll a content in *S. sclarea*, *Origanum vulgare* L., *Mentha* and *Thymus vulgaris* L. of 1.56, 10.42, 14.23 and 2.89 mg/g fresh weight (FW), respectively^{48–50}. Also, the content of chlorophyll b in *S. sclarea*, *Origanum vulgare* L., *Mentha* and *Thymus vulgaris* L. were reported as 0.43, 4.66, 5.78 and 1.38 mg/g FW, respectively^{48,49}. The content of total carotenoids in *S. sclarea* and *Thymus vulgaris* L. were reported as 0.45 and 0.56 mg/g FW, respectively^{48,49}. Various factors, including light conditions, ozone stress, drought, salinity, and temperature, can help to explain some of the observed variations among the samples. In fact, these factors are known to influence carotenoid content in sage^{51–53}. Carotenoids are antioxidants that function to protect membranes against damage from free radicals and play a significant role in plant reproduction. Along with phenolic compounds, they are responsible for the vibrant colors of plants⁵⁴. Carotenoids play a role in supporting cellular structures by dissipating excess energy that reaches the chloroplast and inhibiting the formation and/or scavenging of singlet oxygen, which can lead to lipid peroxidation in photosynthetic membranes⁵⁵.

Chlorophyll is typically synthesized and photo-oxidized in the presence of light. Rezai et al. investigated the carotenoid and chlorophyll contents in *S. officinalis* grown in a semi-arid region of Iran under different light conditions, by quantifying the effects of different shade levels (30%, 50%, or 70%) on plant morphology⁵⁶.

Our samples were collected from an environment with limited shade, which could explain the lower carotenoid content observed, primarily in high shade conditions.

Antioxidant activity

The antioxidant capacities of the 102 plant extracts studied were examined using two free radical scavenging methods represented by the DPPH and FRAP assays. Due to its simplicity, speed, reproducibility and low cost, the DPPH assay has been used most frequently to evaluate the antioxidant potential of hydrophilic extracts (alcoholic, hydroalcoholic) taken from the above-ground parts of a number of *Salvia* species, including two known medicinal species as *S. multiorrhiza* and *S. officinalis*^{57,58}. The DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical that can be reduced by transferring a hydrogen from other compounds. The FRAP assay is based on the presence of antioxidant substances in plant extracts, mainly polyphenolic compounds, which convert the ferric (Fe^{3+}) complex to the ferrous (Fe^{2+}) form. The decrease in Fe^{3+} in the solution indicates the potent reducing power of the plant extracts. Iron and copper ions are well known as effective pro-oxidant agents, and polyphenolic compounds can chelate metal ions, preventing free radical formation⁵⁹. Polyphenols are natural antioxidants and have a correlation with ROS scavenging capacity helping to reduce the oxidative stress at product^{60,61}.

In this study, a significant difference ($p < 0.01$) in antioxidant activity was observed among the investigated plants (Table 4). Antioxidant activity values, measured using the DPPH method, ranged from 4.58 to 68.93 μ g AAE/mL DW, with the highest and lowest values observed for *S. limbata* and *S. ceratophylla* extracts, respectively. Results from the FRAP assay indicate that the highest values were observed for a sample of *S. limbata* (S56) and one of *S. aethiopsis* (S60) (1090.55 and 1071.79 μ mol Fe^{2+} /g DW, respectively), while the lowest values were observed for another sample of *S. limbata* (S2) and one of *S. nemorosa* (S53) (64.28 and 73.06 μ mol Fe^{2+} /g DW, respectively). The differences observed in antioxidant activity could be attributed to the diverse methods used for evaluation⁶². Each method has distinct strengths and limitations. Depending on the assay chosen, some aspects of antioxidant capacity may be overlooked. To address this limitation, future research should consider employing multiple antioxidant assays, each targeting different mechanisms or aspects of antioxidant activity. This approach would provide a more comprehensive assessment of antioxidant capacity and improve understanding of the overall antioxidant potential of the compounds or samples studied.

Primary antioxidants found in sage plants, such as *Salvia* species, often include polyphenols, flavonoids, and other phytochemicals. Polyphenols represent potent antioxidants primarily due to the presence and specific positioning of hydroxyl ($-OH$) and carboxyl ($-COOH$) groups on their aromatic rings. These functional groups make polyphenols effective in scavenging free radicals and neutralizing oxidative reactions, which is why they are considered important contributors to the antioxidant properties of various foods, plants, and dietary compounds. On the other hand, the differences in the free radical scavenging activity of flavonoids can be attributed to their structural variations, as flavonoids typically possess three different rings, labeled as A, B, and C⁶³. The specific arrangement and types of substitutions on these rings can significantly impact their antioxidant properties and biological activities. Primary antioxidants also include hindered phenols and secondary aromatic amines⁶⁴, which play a crucial role in preventing the oxidation of molecules by scavenging free radicals and stabilizing reactive intermediates that are formed during oxidative reactions. Evidence supporting the role of these compounds as primary antioxidants in the aerial parts of *S. viridis* has been previously provided^{65,66}.

Identification of metabolites

UHPLC-HRMS was used to determine the chemical compounds present in the extracts of the 20 selected *Salvia* species. Representative chromatograms for a sample of each of the 20 species studied are included in Figs. S1–S20. The compounds identified in the samples mainly belong to phenolic and flavonoid groups, with one from the fatty acyl glycoside group, as listed in Table 5.

Sample code	Species	C _a (mg/100 g Dw)	C _b (mg/100 g Dw)	C _{tot} (TCC) (mg/g)	β-Carotene (mg/100 g Dw)
S1	<i>S. multicaulis</i>	9.88 ± 0.09	5.71 ± 0.05	9.54 ± 0.74	0.73 ± 0.02
S2	<i>S. limbata</i>	16.04 ± 1.04	5.52 ± 0.02	12.60 ± 0.26	0.94 ± 0.01
S3	<i>S. syriaca</i>	19.06 ± 1.41	5.41 ± 0.06	13.71 ± 0.03	1.07 ± 0.02
S4	<i>S. multicaulis</i>	25.09 ± 0.02	9.59 ± 0.24	22.12 ± 0.02	1.71 ± 0.02
S5	<i>S. verticillata</i>	25.75 ± 0.84	8.77 ± 0.07	22.43 ± 0.43	1.75 ± 0.05
S6	<i>S. nemorosa</i>	19.78 ± 0.88	6.73 ± 0.18	12.89 ± 0.89	0.97 ± 0.08
S7	<i>S. officinalis</i>	24.74 ± 0.07	9.78 ± 0.28	24.16 ± 0.16	1.88 ± 0.07
S8	<i>S. officinalis</i>	25.41 ± 0.94	11.00 ± 0.18	26.56 ± 0.16	2.07 ± 0.02
S9	<i>S. atropatana</i>	30.26 ± 0.09	11.40 ± 0.01	23.51 ± 0.24	1.80 ± 0.09
S10	<i>S. sclarea</i>	10.46 ± 0.12	5.16 ± 0.57	12.98 ± 0.03	1.02 ± 0.02
S11	<i>S. syriaca</i>	12.56 ± 0.86	4.17 ± 0.01	10.96 ± 0.96	0.86 ± 0.01
S12	<i>S. multicaulis</i>	26.63 ± 0.63	9.61 ± 0.61	20.52 ± 0.86	1.57 ± 0.10
S13	<i>S. nemorosa</i>	25.60 ± 0.01	9.99 ± 0.81	20.94 ± 0.94	1.60 ± 0.01
S14	<i>S. limbata</i>	11.77 ± 0.96	3.78 ± 0.30	7.45 ± 0.29	0.56 ± 0.03
S15	<i>S. multicaulis</i>	20.51 ± 0.17	7.45 ± 0.90	7.69 ± 0.17	0.80 ± 0.03
S16	<i>S. syriaca</i>	18.71 ± 0.09	5.22 ± 0.87	20.06 ± 0.50	1.31 ± 0.04
S17	<i>S. nemorosa</i>	28.24 ± 0.27	11.74 ± 0.31	20.87 ± 0.54	1.57 ± 0.02
S18	<i>S. syriaca</i>	21.35 ± 0.85	6.74 ± 0.25	17.01 ± 0.50	1.32 ± 0.05
S19	<i>S. atropatana</i>	39.88 ± 1.88	16.14 ± 0.14	33.94 ± 0.72	2.60 ± 0.09
S20	<i>S. bracteata</i>	9.25 ± 0.20	4.20 ± 0.09	10.28 ± 0.50	0.80 ± 0.04
S21	<i>S. nemorosa</i>	28.91 ± 0.10	12.49 ± 0.11	25.70 ± 0.90	1.97 ± 0.07
S22	<i>S. bracteata</i>	15.59 ± 0.46	6.70 ± 0.02	20.84 ± 0.7	1.34 ± 0.04
S23	<i>S. nemorosa</i>	18.46 ± 0.05	6.57 ± 0.07	18.41 ± 0.05	1.20 ± 0.03
S24	<i>S. limbata</i>	18.77 ± 0.97	6.43 ± 0.18	15.08 ± 0.34	1.17 ± 0.08
S25	<i>S. spinosa</i>	15.88 ± 0.63	3.23 ± 0.05	8.79 ± 0.19	0.85 ± 0.02
S26	<i>S. spinosa</i>	11.00 ± 0.19	4.29 ± 0.81	10.08 ± 0.34	0.78 ± 0.02
S27	<i>S. syriaca</i>	23.80 ± 1.15	7.34 ± 0.35	24.35 ± 0.03	1.94 ± 0.04
S28	<i>S. Staminea</i>	23.17 ± 0.37	8.13 ± 0.05	19.82 ± 0.37	1.54 ± 0.02
S29	<i>S. multicaulis</i>	28.19 ± 0.01	10.62 ± 0.62	20.10 ± 0.77	1.52 ± 0.03
S30	<i>S. nemorosa</i>	29.86 ± 0.48	11.66 ± 0.66	23.63 ± 0.63	1.80 ± 0.05
S31	<i>S. spinosa</i>	41.78 ± 0.50	18.25 ± 0.61	32.22 ± 0.75	2.48 ± 0.02
S32	<i>S. spinosa</i>	11.04 ± 0.34	4.28 ± 0.08	8.83 ± 0.09	0.67 ± 0.01
S33	<i>S. syriaca</i>	16.62 ± 0.05	4.81 ± 0.02	10.78 ± 0.16	0.83 ± 0.01
S34	<i>S. ceratophylla</i>	20.41 ± 0.03	7.70 ± 0.33	19.00 ± 0.09	1.48 ± 0.02
S35	<i>S. atropatana</i>	40.73 ± 0.02	14.61 ± 0.17	38.13 ± 0.63	2.98 ± 0.04
S36	<i>S. nemorosa</i>	26.37 ± 0.37	9.73 ± 0.96	22.85 ± 0.95	1.77 ± 0.08
S37	<i>S. verticillata</i>	25.14 ± 0.09	8.96 ± 1.97	18.95 ± 0.27	1.48 ± 0.07
S38	<i>S. nemorosa</i>	19.07 ± 1.08	6.83 ± 0.28	17.52 ± 0.25	1.13 ± 0.03
S39	<i>S. limbata</i>	16.11 ± 0.01	6.17 ± 0.20	15.00 ± 0.79	1.01 ± 0.01
S40	<i>S. syriaca</i>	17.50 ± 0.39	7.89 ± 0.17	15.19 ± 0.61	1.18 ± 0.02
S41	<i>S. nemorosa</i>	33.79 ± 0.79	12.35 ± 0.35	27.11 ± 0.11	2.08 ± 0.08
S42	<i>S. multicaulis</i>	5.19 ± 0.03	2.04 ± 0.48	6.76 ± 0.27	0.53 ± 0.03
S43	<i>S. nemorosa</i>	22.48 ± 0.86	8.53 ± 0.06	18.58 ± 0.70	1.42 ± 0.07
S44	<i>S. sclarea</i>	23.38 ± 0.58	10.01 ± 0.68	23.29 ± 0.81	1.80 ± 0.08
S45	<i>S. macrochlamys</i>	7.96 ± 0.37	3.09 ± 0.05	7.18 ± 0.02	0.55 ± 0.06
S46	<i>S. syriaca</i>	9.38 ± 0.14	3.38 ± 0.03	8.93 ± 0.03	0.70 ± 0.02
S47	<i>S. verticillata</i>	21.31 ± 0.51	7.77 ± 0.77	20.48 ± 0.74	1.60 ± 0.08
S48	<i>S. nemorosa</i>	12.22 ± 0.22	4.53 ± 0.00	10.08 ± 0.33	0.78 ± 0.02
S49	<i>S. candidissima</i>	12.48 ± 0.58	7.13 ± 0.13	20.51 ± 0.77	1.62 ± 0.04
S50	<i>S. limbata</i>	11.64 ± 0.12	4.47 ± 0.03	9.68 ± 0.45	0.73 ± 0.01
S51	<i>S. syriaca</i>	12.48 ± 0.68	4.61 ± 0.02	9.12 ± 0.10	0.69 ± 0.01
S52	<i>S. limbata</i>	28.14 ± 0.06	9.42 ± 0.04	21.66 ± 0.03	1.67 ± 0.02
S53	<i>S. nemorosa</i>	21.42 ± 1.09	8.14 ± 0.15	22.02 ± 0.05	1.73 ± 0.05
S54	<i>S. verticillata</i>	19.99 ± 1.11	8.19 ± 0.91	25.61 ± 0.06	2.03 ± 0.01
S55	<i>S. limbata</i>	20.04 ± 0.54	7.61 ± 0.61	16.87 ± 0.33	1.30 ± 0.02
S56	<i>S. limbata</i>	13.44 ± 0.70	6.09 ± 0.05	15.29 ± 0.10	1.19 ± 0.01

Continued

Sample code	Species	C _a (mg/100 g Dw)	C _b (mg/100 g Dw)	C _{x+c} (TCC) (mg/g)	β-Carotene (mg/100 g Dw)
S57	<i>S. multicaulis</i>	16.60 ± 1.16	6.20 ± 0.53	13.96 ± 0.33	1.08 ± 0.03
S58	<i>S. syriaca</i>	31.64 ± 0.03	10.77 ± 0.04	25.95 ± 0.01	2.02 ± 0.02
S59	<i>S. nemorosa</i>	22.16 ± 0.51	8.50 ± 0.09	20.95 ± 0.46	1.63 ± 0.01
S60	<i>S. aethiopsis</i>	30.80 ± 0.80	14.53 ± 0.42	28.78 ± 0.30	2.19 ± 0.05
S61	<i>S. limbata</i>	24.27 ± 0.53	8.18 ± 0.14	19.19 ± 0.29	1.48 ± 0.08
S62	<i>S. sahendica</i>	17.59 ± 0.41	6.44 ± 0.07	14.22 ± 0.45	0.96 ± 0.06
S63	<i>S. nemorosa</i>	27.29 ± 0.31	11.13 ± 0.44	25.26 ± 0.78	1.95 ± 0.06
S64	<i>S. verticillata</i>	5.13 ± 0.03	2.48 ± 0.26	5.92 ± 0.06	0.46 ± 0.08
S65	<i>S. aethiopsis</i>	20.67 ± 0.20	8.18 ± 0.12	18.73 ± 0.73	1.45 ± 0.09
S66	<i>S. spinosa</i>	5.36 ± 0.04	2.34 ± 0.60	5.25 ± 0.01	0.40 ± 0.02
S67	<i>S. multicaulis</i>	23.86 ± 0.86	8.46 ± 0.56	18.01 ± 0.53	1.38 ± 0.05
S68	<i>S. limbata</i>	13.36 ± 0.43	4.74 ± 0.21	13.84 ± 0.84	1.09 ± 0.09
S69	<i>S. syriaca</i>	21.04 ± 0.36	6.45 ± 0.95	14.30 ± 0.45	1.10 ± 0.09
S70	<i>S. multicaulis</i>	21.27 ± 0.55	6.94 ± 0.03	14.54 ± 0.04	1.11 ± 0.02
S71	<i>S. limbata</i>	11.66 ± 0.85	4.11 ± 0.01	8.04 ± 0.10	0.61 ± 0.02
S72	<i>S. syriaca</i>	38.24 ± 1.00	14.30 ± 0.94	28.22 ± 0.07	2.14 ± 0.01
S73	<i>S. limbata</i>	12.59 ± 0.02	4.70 ± 0.22	9.37 ± 0.07	0.71 ± 0.02
S74	<i>S. ceratophylla</i>	14.49 ± 0.58	5.61 ± 0.03	12.00 ± 0.34	0.92 ± 0.03
S75	<i>S. spinosa</i>	23.97 ± 0.97	7.89 ± 0.92	19.40 ± 0.91	1.51 ± 0.06
S76	<i>Slvia</i> sp.	20.21 ± 0.04	10.26 ± 0.06	23.14 ± 0.57	1.78 ± 0.08
S77	<i>S. nemorosa</i>	22.14 ± 0.59	7.60 ± 0.07	16.53 ± 0.53	1.27 ± 0.09
S78	<i>S. ceratophylla</i>	13.99 ± 0.22	6.83 ± 0.07	20.61 ± 0.02	1.63 ± 0.02
S79	<i>S. syriaca</i>	28.61 ± 1.58	9.55 ± 0.84	20.65 ± 0.29	1.58 ± 0.01
S80	<i>S. limbata</i>	6.72 ± 0.05	3.03 ± 0.19	6.71 ± 0.07	0.51 ± 0.02
S81	<i>S. limbata</i>	14.08 ± 0.08	5.18 ± 0.18	10.22 ± 0.02	0.77 ± 0.02
S82	<i>S. pocolata</i>	30.78 ± 0.47	11.85 ± 0.85	26.75 ± 0.05	2.07 ± 0.07
S83	<i>S. hydrangea</i>	13.54 ± 0.73	5.31 ± 0.18	12.69 ± 0.02	0.98 ± 0.02
S84	<i>S. syriaca</i>	22.93 ± 0.07	6.92 ± 0.01	16.93 ± 0.06	1.31 ± 0.02
S85	<i>S. ceratophylla</i>	15.70 ± 0.07	7.18 ± 0.33	21.20 ± 0.58	1.68 ± 0.04
S86	<i>S. limbata</i>	20.78 ± 0.13	6.93 ± 0.93	19.25 ± 0.25	1.50 ± 0.01
S87	<i>S. multicaulis</i>	30.25 ± 0.42	11.76 ± 0.06	25.03 ± 0.05	1.92 ± 0.07
S88	<i>S. grossheimii</i>	20.00 ± 1.01	7.18 ± 0.18	17.06 ± 0.02	1.32 ± 0.02
S89	<i>S. multicaulis</i>	15.60 ± 0.73	5.56 ± 0.56	13.86 ± 0.02	1.08 ± 0.02
S90	<i>S. multicaulis</i>	13.30 ± 0.80	5.18 ± 0.91	12.37 ± 0.63	0.96 ± 0.07
S91	<i>S. verticillata</i>	18.81 ± 0.27	7.02 ± 0.02	18.54 ± 0.09	1.45 ± 0.03
S92	<i>S. syriaca</i>	12.66 ± 0.67	4.34 ± 0.04	11.73 ± 0.81	0.92 ± 0.05
S93	<i>S. multicaulis</i>	17.81 ± 0.69	6.76 ± 0.20	16.67 ± 0.67	1.30 ± 0.10
S94	<i>S. aethiopsis</i>	25.49 ± 0.77	8.99 ± 0.14	18.37 ± 0.96	1.40 ± 0.08
S95	<i>S. hydrangea</i>	29.56 ± 0.65	12.35 ± 0.71	27.09 ± 0.79	2.09 ± 0.06
S96	<i>S. macrochlamys</i>	11.90 ± 0.03	4.24 ± 0.22	9.48 ± 0.18	0.72 ± 0.02
S97	<i>S. ceratophylla</i>	12.61 ± 0.61	4.60 ± 0.55	15.40 ± 0.66	1.23 ± 0.06
S98	<i>S. atropatana</i>	39.01 ± 0.14	13.58 ± 0.67	28.37 ± 0.89	2.17 ± 0.10
S99	<i>S. sclarea</i>	15.50 ± 0.16	6.05 ± 0.18	13.38 ± 0.75	1.03 ± 0.06
S100	<i>S. nemorosa</i>	23.51 ± 0.51	8.58 ± 0.25	18.57 ± 0.57	1.43 ± 0.10
S101	<i>S. syriaca</i>	14.19 ± 0.28	5.86 ± 0.21	11.13 ± 0.13	0.84 ± 0.05
S102	<i>S. multicaulis</i>	9.05 ± 0.15	3.67 ± 0.25	8.05 ± 0.28	0.62 ± 0.02
Significant levels		**	**	**	**

Table 3. Content of photosynthetic pigments for the 102 *Salvia* species studied. *Ca* Chlorophyll a content, *Cb* Chlorophyll b content, *Cx + c* Carotenoid content, *β-Carotene* β-Carotene content. **Indicates significant difference at 1% confidence levels.

The identified compounds were classified into nine other subgroups (Table 6) to better represent their structural heterogeneity. These compounds are known to have different antioxidant activities and pharmacological properties.

Sample code	Species	Antioxidant activity (DPPH) ($\mu\text{g AAE/mL}$)	Antioxidant activity (FRAP) ($\mu\text{mol Fe}^{2+}/\text{g DW}$)
S1	<i>S. multicaulis</i>	66.63 ± 0.72	524.35 ± 6.26
S2	<i>S. limbata</i>	35.72 ± 1.52	64.28 ± 3.40
S3	<i>S. syriaca</i>	48.29 ± 2.99	231.59 ± 4.83
S4	<i>S. multicaulis</i>	60.86 ± 0.99	165.08 ± 1.11
S5	<i>S. verticillata</i>	67.73 ± 0.39	178.73 ± 6.16
S6	<i>S. nemorosa</i>	67.85 ± 0.21	274.80 ± 5.68
S7	<i>S. officinalis</i>	67.85 ± 0.75	997.09 ± 5.27
S8	<i>S. officinalis</i>	67.46 ± 0.06	375.98 ± 5.88
S9	<i>S. atropatana</i>	66.96 ± 0.27	252.63 ± 2.63
S10	<i>S. sclarea</i>	67.70 ± 0.48	395.31 ± 5.22
S11	<i>S. syriaca</i>	54.77 ± 3.61	258.88 ± 4.11
S12	<i>S. multicaulis</i>	66.90 ± 0.87	839.85 ± 2.52
S13	<i>S. nemorosa</i>	57.40 ± 0.51	81.51 ± 1.51
S14	<i>S. limbata</i>	38.56 ± 1.91	124.62 ± 1.01
S15	<i>S. multicaulis</i>	63.79 ± 0.57	290.15 ± 2.05
S16	<i>S. syriaca</i>	15.95 ± 1.10	382.39 ± 3.73
S17	<i>S. nemorosa</i>	66.06 ± 0.87	234.44 ± 2.00
S18	<i>S. syriaca</i>	39.75 ± 2.81	206.01 ± 3.00
S19	<i>S. atropatana</i>	67.70 ± 0.90	361.77 ± 4.10
S20	<i>S. bracteata</i>	67.79 ± 0.45	196.92 ± 3.92
S21	<i>S. nemorosa</i>	66.39 ± 0.54	224.20 ± 6.20
S22	<i>S. bracteata</i>	63.25 ± 0.75	236.14 ± 2.02
S23	<i>S. nemorosa</i>	63.31 ± 0.99	95.42 ± 4.42
S24	<i>S. limbata</i>	40.59 ± 1.13	1090.55 ± 3.55
S25	<i>S. spinosa</i>	60.30 ± 0.12	689.78 ± 1.78
S26	<i>S. spinosa</i>	41.37 ± 0.96	255.47 ± 5.47
S27	<i>S. syriaca</i>	50.59 ± 5.35	349.83 ± 5.18
S28	<i>S. Staminea</i>	44.11 ± 0.18	497.14 ± 5.06
S29	<i>S. multicaulis</i>	66.99 ± 0.54	713.08 ± 3.08
S30	<i>S. nemorosa</i>	58.03 ± 1.91	104.06 ± 4.06
S31	<i>S. spinosa</i>	46.86 ± 2.98	365.18 ± 4.18
S32	<i>S. spinosa</i>	46.53 ± 0.33	97.70 ± 4.70
S33	<i>S. syriaca</i>	45.10 ± 0.75	211.70 ± 1.70
S34	<i>S. ceratophylla</i>	68.93 ± 0.57	544.25 ± 6.25
S35	<i>S. atropatana</i>	49.64 ± 1.16	345.29 ± 4.19
S36	<i>S. nemorosa</i>	63.22 ± 2.21	167.93 ± 1.93
S37	<i>S. verticillata</i>	68.54 ± 0.06	260.02 ± 5.14
S38	<i>S. nemorosa</i>	57.70 ± 0.51	180.43 ± 3.38
S39	<i>S. limbata</i>	39.66 ± 3.79	414.07 ± 2.41
S40	<i>S. syriaca</i>	37.63 ± 1.64	220.79 ± 0.89
S41	<i>S. nemorosa</i>	63.49 ± 0.93	119.68 ± 1.68
S42	<i>S. multicaulis</i>	64.03 ± 1.88	287.30 ± 3.30
S43	<i>S. nemorosa</i>	67.17 ± 0.02	238.98 ± 3.98
S44	<i>S. sclarea</i>	53.07 ± 0.78	81.61 ± 1.61
S45	<i>S. macrochlamys</i>	65.49 ± 1.31	411.80 ± 4.72
S46	<i>S. syriaca</i>	47.28 ± 1.19	188.39 ± 1.85
S47	<i>S. verticillata</i>	68.12 ± 1.61	278.21 ± 1.21
S48	<i>S. nemorosa</i>	66.72 ± 0.45	200.90 ± 1.90
S49	<i>S. candidissima</i>	68.09 ± 0.21	810.86 ± 1.86
S50	<i>S. limbata</i>	40.35 ± 3.11	114.97 ± 1.97
S51	<i>S. syriaca</i>	34.32 ± 0.42	90.69 ± 1.71
S52	<i>S. limbata</i>	48.35 ± 1.55	406.11 ± 6.11
S53	<i>S. nemorosa</i>	58.80 ± 1.85	73.06 ± 1.86
S54	<i>S. verticillata</i>	67.67 ± 0.51	120.86 ± 1.98
S55	<i>S. limbata</i>	37.24 ± 0.02	205.44 ± 5.44
S56	<i>S. limbata</i>	4.58 ± 1.08	237.28 ± 4.28

Continued

Sample code	Species	Antioxidant activity (DPPH) ($\mu\text{g AAE/mL}$)	Antioxidant activity (FRAP) ($\mu\text{mol Fe}^{2+}/\text{g DW}$)
S57	<i>S. multicaulis</i>	46.89 \pm 2.36	559.60 \pm 1.60
S58	<i>S. syriaca</i>	17.92 \pm 0.09	129.95 \pm 2.95
S59	<i>S. nemorosa</i>	68.12 \pm 0.42	398.72 \pm 0.72
S60	<i>S. aethiopsis</i>	66.84 \pm 0.33	1071.79 \pm 4.79
S61	<i>S. limbata</i>	20.67 \pm 0.15	910.91 \pm 1.91
S62	<i>S. sahendica</i>	21.63 \pm 1.46	313.45 \pm 0.68
S63	<i>S. nemorosa</i>	60.60 \pm 0.18	210.56 \pm 3.57
S64	<i>S. verticillata</i>	68.21 \pm 0.21	747.76 \pm 3.42
S65	<i>S. aethiopsis</i>	67.20 \pm 0.22	571.61 \pm 2.97
S66	<i>S. spinosa</i>	47.82 \pm 0.36	312.69 \pm 1.67
S67	<i>S. multicaulis</i>	64.81 \pm 0.81	296.40 \pm 3.40
S68	<i>S. limbata</i>	45.70 \pm 0.45	82.20 \pm 1.23
S69	<i>S. syriaca</i>	45.16 \pm 3.08	265.21 \pm 3.86
S70	<i>S. multicaulis</i>	59.49 \pm 0.63	186.12 \pm 0.56
S71	<i>S. limbata</i>	34.71 \pm 0.15	79.96 \pm 2.42
S72	<i>S. syriaca</i>	60.84 \pm 1.73	453.86 \pm 4.86
S73	<i>S. limbata</i>	65.67 \pm 0.54	995.02 \pm 4.24
S74	<i>S. ceratophylla</i>	63.13 \pm 0.63	257.17 \pm 2.17
S75	<i>S. spinosa</i>	62.87 \pm 0.24	473.19 \pm 3.19
S76	<i>Salvia</i> sp.	56.48 \pm 0.54	173.04 \pm 2.38
S77	<i>S. nemorosa</i>	64.84 \pm 0.48	382.24 \pm 2.24
S78	<i>S. ceratophylla</i>	43.25 \pm 0.09	161.67 \pm 5.78
S79	<i>S. syriaca</i>	18.70 \pm 0.39	102.07 \pm 3.73
S80	<i>S. limbata</i>	25.90 \pm 0.90	690.35 \pm 1.35
S81	<i>S. limbata</i>	23.21 \pm 0.22	780.16 \pm 1.01
S82	<i>S. pocolata</i>	39.66 \pm 0.99	93.41 \pm 1.52
S83	<i>S. hydrangea</i>	64.24 \pm 0.48	376.55 \pm 2.55
S84	<i>S. syriaca</i>	28.05 \pm 1.13	219.66 \pm 5.16
S85	<i>S. ceratophylla</i>	65.82 \pm 0.39	559.03 \pm 0.94
S86	<i>S. limbata</i>	31.96 \pm 0.45	88.11 \pm 3.11
S87	<i>S. multicaulis</i>	63.82 \pm 0.60	439.65 \pm 1.76
S88	<i>S. grossheimii</i>	64.12 \pm 0.84	700.58 \pm 6.25
S89	<i>S. multicaulis</i>	61.55 \pm 1.02	298.10 \pm 0.10
S90	<i>S. multicaulis</i>	60.27 \pm 0.63	320.84 \pm 2.84
S91	<i>S. verticillata</i>	67.08 \pm 0.87	374.85 \pm 4.85
S92	<i>S. syriaca</i>	32.56 \pm 0.21	225.34 \pm 5.56
S93	<i>S. multicaulis</i>	60.27 \pm 0.69	494.23 \pm 4.23
S94	<i>S. aethiopsis</i>	41.93 \pm 0.93	183.27 \pm 5.62
S95	<i>S. hydrangea</i>	40.29 \pm 0.06	280.48 \pm 2.93
S96	<i>S. macrochlamys</i>	40.83 \pm 2.15	179.86 \pm 3.41
S97	<i>S. ceratophylla</i>	66.03 \pm 0.54	451.59 \pm 6.04
S98	<i>S. atropatana</i>	43.52 \pm 0.66	287.87 \pm 6.22
S99	<i>S. sclarea</i>	56.95 \pm 0.90	346.42 \pm 0.92
S100	<i>S. nemorosa</i>	48.59 \pm 0.42	158.26 \pm 4.60
S101	<i>S. syriaca</i>	19.45 \pm 0.18	95.36 \pm 2.36
S102	<i>S. multicaulis</i>	59.19 \pm 0.33	719.91 \pm 1.03
Significant levels		**	**

Table 4. Antioxidant activity determined for the 102 *Salvia* species by DPPH and FRAP assays. AAE ascorbic acid equivalents. **Indicates significant difference at 1% confidence levels.

Previous studies have already revealed the presence of both flavonoids and phenolic acids in *Salvia* species⁵⁷. Flavonoids are categorized into six subclasses including flavones, flavonols, isoflavones, flavanols, flavanones, and anthocyanins⁹. Phenolic acids have diverse structures, ranging from monomers to dimers, trimers, tetramers, and multimers, and are present in high concentrations in sage, with significant taxonomic implications as observed for Chinese *Salvia* species⁶⁸. Phenolic acids have been shown to play a central role in mediating the major pharmacological activities of Chinese *Salvia* species, particularly their antioxidant effects⁶⁸. Several

Phenolic acids	Flavonoids	Fatty acyl glycosides
Syringic acid	Myricitrin	Tuberonic acid glucosides
Caffeic acid hexosides	Eriodictyol-O-glucuronide	
Yunnaneic acid F	Hyperoside	
Isoverbascoside	6-Hydroxyluteolin 7-O-glucuronide	
Yunnaneic acid E	Lipidoside A	
Rosmarinic acid	Luteolin-glucoside	
Sagerinic acid	Luteolin-glucuronide	
Salvianolic acid K	Luteolin	
Martynoside/isomartynoside	Apigenin 7-O-glucoside	
Caffeoyl malic acid	Genistein	
Sagecoumarin	Hispidulin	
Salvianolic acid A	Hispidulin glucuronide	
Salvianolic acid B	Kaempferol-glucoside	
Salvianolic acid C	Kaempferol-glucuronide	
Salvianolic acid F isomers	Apigenin acetyl glucoside	
Ferulic acid	Luteolin acetyl glucoside	
Lityhospermic acid isomers	Kaempferol	
Caffeoylquinic acids	Apigenin	
Verbascoside	Kaempferide	
Forsythoside A		

Table 5. General classification of the chemical compounds identified by UHPLC-HRMS.

Phenolic acids		
2-Arylbenzofuran propanoids	Phenyletanoids	Hydroxycinnamic acids
Salvianolic acid isomers	Isoverbascoside	Caffeic acid hexosides
Salvianolic acid A	Verbascoside	Ferulic acid
Salvianolic acid B	Martynoside	Rosmarinic acid
Salvianolic acid C	Phenylpropanoids	Caffeoyl malic acid
Lityhospermic acid isomers	Caffeoyl quinic acids	
Benzoic acids	Coumarin	Lignans
Syringic acid	Sagecoumarin	Yunnaneic acid F
Flavonoids		Yunnaneic acid E
Myricitrin	Genistein	Sagerinic acid
Forsythoside A	Hispidulin	Salvianolic acid K
Eriodictyol-O-glucuronide	Hispidulin-glucuronide	
Hyperoside	Kaempferol-glucoside	
6-Hydroxyluteolin-7-O-glucuronide	Kaempferol-glucuronide	Fatty acyl glycoside
Lipidoside_A	Apigenin acetyl glucoside	Tuberonic acid glucosides
Luteolin-glucoside	Luteolin acetyl glucoside	
Luteolin-glucuronide	Kaempferol	
Luteolin	Apigenin	
Apigenin-7-O-glucoside	Kaempferide	

Table 6. Detailed classification of compounds identified by UHPLC-HRMS.

studies have indicated that *S. officinalis* and other plants exhibit strong antioxidant and free radical scavenging activities, including the study by Nickavar et al.⁶⁹. The ethanol extract of *S. officinalis* contains both phenolic compounds and terpenoids, such as carnosic acid (phenolic diterpene), carnosol (ortho-diphenolic diterpene) and an oxidative derivative of carnosic acid), rosmarinic acid (phenolic compound), rosmanol (phenolic diterpene), rosmadial (diterpene lactone), methyl carnosate (diterpene), epirosmanol (diterpene lactone), luteolin-7-O-beta-glucopyranoside (flavonoid)⁷⁰, and caffeic acid (polyphenol)^{71,72}. Aqueous extracts of *Salvia africana*, *Salvia officinalis* 'Icterina', and *Salvia mexicana* were found to contain distinct phenolic compounds, with *S. africana* exhibiting the highest total phenol content level (231.6 mg GAE/g DW) in agreement with its potent antioxidant potential, as also reported by Afonso et al.⁷³. Various phenolic compounds, such as caffeic, chlorogenic and rosmarinic acids, as well as flavonoid compounds such as luteolin (flavone), apigenin (flavone) and their glycosides

were identified as the most important polyphenols in hydroalcoholic extracts of *S. hortensis*²⁰. Rosmarinic acid, which is one of the most abundant phenolic acids, is commonly found in many species of *Salvia*^{6,35}. Carnosic acid, caffeic acid, oleanolic acid (pentacyclic triterpenoid), ursolic acid (pentacyclic triterpenoid), kaempferol (flavonoid), and rosmarinic acid were identified in 17 extracts of indigenous *Salvia* species (obtained with a mixture of methanol/chloroform 1:1 v/v), as reported by Kamatou et al.⁷⁴. Around 160 polyphenolic compounds have been identified from sage leaves, encompassing a wide range of different flavonoids and phenolic acids. The six most significant flavonoids found in sage plants are luteolin, apigenin, hispidulin, 7-O-glucoside, kaempferol, cirsimaritin, and quercetin⁹. Numerous flavones were found in *S. disermas*, while a few were noted in *S. aurita*, *S. africana-caerulea*, *S. dolomitica*, *S. runcinata*, *S. stenophylla*, *S. garipensis*, *S. lanceolata*, *S. namaensis*, *S. chamelaeagnea*, *S. radula*, *S. schlechteri* and *S. repens*¹². Polyphenol analysis of *S. officinalis* leaf extracts has also revealed the presence of various phenolic compounds, such as salvianolic acids, caffeic acid and its derivatives, rosmarinic acid, lithospermic acids, sagerinic acid, yunnaneic acids and sage coumarin. Caffeic acid derivatives (trans-verbascoside, cis-verbascoside, leucosceptoside A, martynoside, caffeic acid, 6-O-caffeoyl-glucose, rosmarinic acid and salidroside) are considered as the primary phenolic acids in *Salvia* species, serving as building blocks for various plant metabolites. In the biochemistry of the *Lamiaceae* family, caffeic acid plays a central role and remains in subdued form as rosmarinic acid. According to a study by Skendi et al.⁷⁵ and Moshari Nasirkandi et al.⁷⁶, both rosmarinic acid and caffeic acid were identified as the main polyphenolic compounds in extracts obtained from plants of the *Lamiaceae* family.

Chemometric analysis

Principal Component Analysis (PCA) is one of the most commonly used statistical techniques in metabolomics^{77,78}. The primary goal of PCA is to reduce the dimensionality of a dataset consisting of many interrelated variables, thus providing a visual representation of the major variance in the data, emphasizing variation and bringing out strong patterns in a dataset. PCA achieves this by converting the original correlated variables into a smaller set of new orthogonal (uncorrelated) variables, called principal components (PCs). PCs can be considered as the axes of a new coordinate system, where the greatest variance lies on the first coordinate (principal component), the second greatest variance on the second coordinate, and so on. The PCs are displayed in two plots, called “scores plot”, where the samples appear close to each other when they are similar and distant when they are dissimilar, and “loadings plot”, which highlights the variables responsible for the separation of the samples along each PC. PCA is an unsupervised method commonly used in several research fields, including agricultural science^{79,80}. In the current study, PCA was employed due to the extensive volume of analyzed data/variables (102 samples \times 50 variables). The aim was to enhance result interpretation and find potential relationships between them. In this sense, PCA was performed using data from the 102 *Salvia* samples, i.e., the results of TPC, TFC, TTC, AAC, photosynthetic pigment content (chlorophyll a and b, total carotenoid concentration, and β -carotene), and antioxidant activity (FRAP and DPPH), combined with those from the quantification of 40 polyphenols via UHPLC-HRMS (for a total of 50 variables). In particular, a relative quantification of polyphenols was carried out, as it is commonly considered sufficient in metabolomics to explore similarities and differences among analyzed samples through Principal Component Analysis, which operates by comparing samples. Notably, a distinct separation was observed when samples were classified by species (Fig. 3), with the first two principal components (PCs) explaining 30.95% of the total variance (PC1: 18.45%, and PC2: 12.50%). These components were instrumental in visualizing the sample relationships. No significant separation was observed when samples were classified according to different area and altitude classes (Fig. S21).

The results of the PCA analysis revealed distinct characteristics among various *Salvia* species. For instance, the *S. multicaulis* group, positioned in the upper part of the scores plot (Fig. 3), showed high levels of the flavonoid 6-hydroxyluteolin-7-glucuronide. This group also contained notable quantities of several phenolic acids known for their potent antioxidant activity, such as salvianolic acid F isomer, rosmarinic and sagerinic acids, caffeic acid hexoside, caffeoyl quinic acid, and salvianolic acid B. Notably, these compounds exhibit connections; for instance, sagerinic acid is believed to be derived from rosmarinic acid through a photochemical cyclization and salvianolic acid B is a part of caffeic acid tetramers originating from the dimerization of rosmarinic acid^{81,82}.

The *S. nemorosa* samples, situated in the bottom right quadrant of the scores plot, showed higher levels of caffeic acid trimers, including salvianolic acid A and K, sagecoumarin, lityhospermic acid isomers, and yunnaneic acid E. These compounds correspondingly appear in the right section of the loadings plot. In contrast, the *S. macrochlamys* group, positioned in an opposing direction to *S. nemorosa*, displayed lower concentrations of these caffeic acid trimers but presented higher levels of various flavonoids: luteolin, apigenin, hispidulin, and hispidulin-glucuronide among the flavones and their glucuronides; kaempferol, kaempferide, myricitrin, kaempferol-glucoside and hyperoside among flavonols and their glycoside derivatives; genistein and eriodictyol-O-glucuronide among isoflavones and flavanone glucuronides.

S. syriaca showed considerable differences from other sage species and was notably distanced from all other sample groups in the scores plot. The loadings plot attributes this to its high content of ferulic acid and martynoside. Conversely, species like *S. verticillata*, *S. bracteata*, and *S. officinalis*, located in the top right panel of the scores plot, had lower concentrations of these compounds. These species displayed increased levels of syringic acid, yunnaneic acid F, and salvianolic acid C, and higher values of TPC and DPPH. Additionally, the species belonging to *S. limbata*, *S. spinosa*, *S. staminea*, *S. aethiopsis*, and *S. atropatana* groups, clustered at the center of the graph, showed the highest levels of TTC and TFC. Among the latter, especially the flavonoid glycosides and glucuronides were notably abundant.

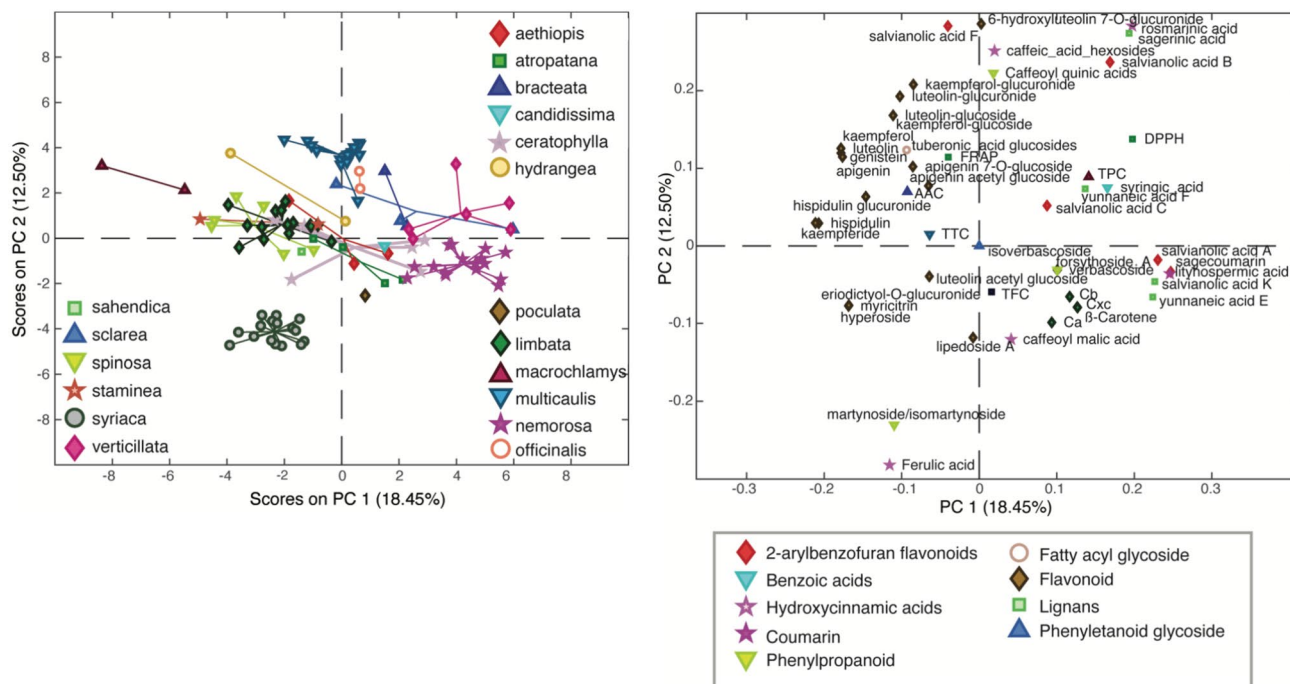


Figure 3. PCA scores plot (left) and loadings plot (right) results colored by species, obtained from the data of the 10 colorimetric determinations (TPC, TFC, TTC, AAC, FRAP, DPPH, Ca, Cb, Cxc, and β -carotene) and the 40 chemical compounds identified for the 102 *Salvia* extracts.

Potential applications of different *Salvia* species in treating different diseases

Considering that each *Salvia* species is distinguished by high levels of compounds of the same chemical class (Table 6) or sharing the same biological pathways, the observed separations suggest that each species serves as a distinct representative of a particular class of polyphenols. These differences in phenolic composition may hold potential for the treatment of various diseases. For instance, *S. verticillata* and *S. bracteata* species, characterized by increased antioxidant activity and a high content of polyphenols, including a high abundance of syringic acid, could face conditions that require robust free radical scavenging. Syringic acid, with its distinctive phenolic structure featuring methoxy moieties at positions 3 and 5, exhibits multifaceted properties (Fig. 4). Notably, it acts as a powerful antioxidant, helping to reduce oxidative stress and neuronal degeneration, while also contributing to cardioprotection by regulating blood pressure, lipid peroxides, and improving nitric oxide and antioxidant levels⁸³. Furthermore, syringic acid also possesses antimicrobial properties by inhibiting various bacteria, including drug-resistant strains of *Staphylococcus aureus* and *Salmonella typhi*⁸⁴. Moreover, it has been shown to induce apoptosis in tumor cells by modulating gene expression⁸⁵. Other wide-ranging effects of syringic acid encompass antidiabetic, hepatoprotective, antihyperlipidemic, and anti-inflammatory actions, which contribute to insulin regulation, liver health, lipid profiles, and immune responses^{83,86}.

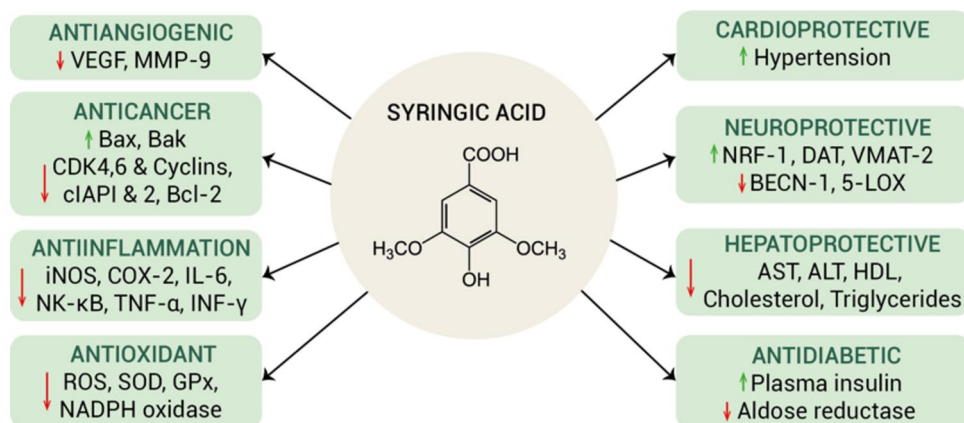


Figure 4. Biomedical applications of syringic acid.

On the contrary, the extracts of *S. nemorosa*, rich in salvianolic and lithospermic acids, exhibit promise in combating conditions related to excessive oxidative stress, such as endothelial dysfunction, pulmonary arterial hypertension, and cardiac fibrosis. These compounds, particularly salvianolic acid A, have shown chelating abilities, inhibiting Cu^{2+} -mediated oxidation and leading to the formation of oxidized LDL, associated with atherosclerosis⁸⁷. Their antioxidative effects are attributed to multiple pathways, involving the downregulation of NADPH oxidase 4 (NOX4) and upregulation of Nrf2/heme oxygenase-1 (HO-1) signaling pathway, significantly impacting vascular dysfunction and inflammation⁸⁷. Other evidence shows that salvianolic acids A, B and C, along with lithospermic and rosmarinic acids may have great potential for the treatment of Alzheimer's disease by inhibiting the glycogen synthase kinase 3 β (GSK-3 β), considered a strategic therapeutic target against Alzheimer's disease⁸⁸.

S. multicaulis and *S. officinalis* species, showing higher levels of salvianolic acid B, could also yield similar health benefits. Furthermore, the high sagerinic acid content observed for them holds promise due to its recognized anti-inflammatory and neuromodulatory properties, interacting with various families of proteins, including oxidoreductases, hydrolases, and enzymes like MAO-A, MAO-B, COX-2, tyrosinase, and cholinesterase⁸⁹.

Instead, *S. syriaca* stands out for its particularly high content of ferulic acid, renowned for its antioxidant role in the elimination of oxidative free radicals, inhibiting the generation of reactive oxygen species (ROS), and for its involvement in multiple signaling pathways⁹⁰. A detailed illustration of the molecular mechanisms underlying the antioxidant and anti-inflammatory activities of ferulic acid is reported in Fig. 5.

Ferulic acid has been reported to exert diverse effects such as reducing ROS production in glomerular podocytes, inhibiting advanced glycation end products and xanthine oxidase, and promoting glucose uptake by activating the PI3K/Akt signaling pathway (Fig. 5, left panel)⁹¹. It also exhibits anti-inflammatory properties in various diseases by suppressing the secretion and expression of inflammatory factors through multiple pathways (Fig. 5, right panel)^{90,91}. Moreover, ferulic acid is known for its cardioprotective, anti-fibrotic, anti-apoptotic, and anti-platelet activities. It helps regulate vascular function, endothelial cell health, and prevents excessive collagen accumulation, particularly beneficial in liver, kidney, and lung fibrosis^{90,91}.

Among the studied species, *S. limbata*, *S. spinosa*, and *S. staminea* exhibit the highest levels of flavonoid glycosides and glucuronides. Flavonoids possess a spectrum of biological activities, and O-glycosylation appears to enhance specific bioactivities, including anti-HIV, anti-rotavirus, anti-stress, anti-obesity, tyrosinase inhibition, anticholinesterase potential, anti-adipogenicity, anti-allergic effects, and utility for treatment of chronic kidney disease⁹². Flavonoid glycosides, especially administered orally, showed enhanced antidiabetic, anti-inflammatory, anti-degranulation, anti-stress, and anti-allergic activities compared to their aglycone counterparts⁹². Indeed, flavonoid glycosides exhibit prolonged presence in the bloodstream and higher plasma concentrations, also impacting cardiovascular health through metal chelation and reduction of oxidative processes^{92,93}. Flavonoid glycosides interact with enzymes like α -amylases and α -glucosidases, crucial in diabetes management. The glycosides of kaempferol and quercetin display higher inhibitory activity against α -glucosidase and aldose reductase^{92,94–96}. The latter, involved in the polyol pathway, has implications in diabetic complications by causing sorbitol accumulation in organs⁹⁷. Flavonoids are being explored for various diseases, including obesity, viral and bacterial infections, inflammatory conditions, neurodegenerative diseases, and cancer treatments^{92,98,99}.

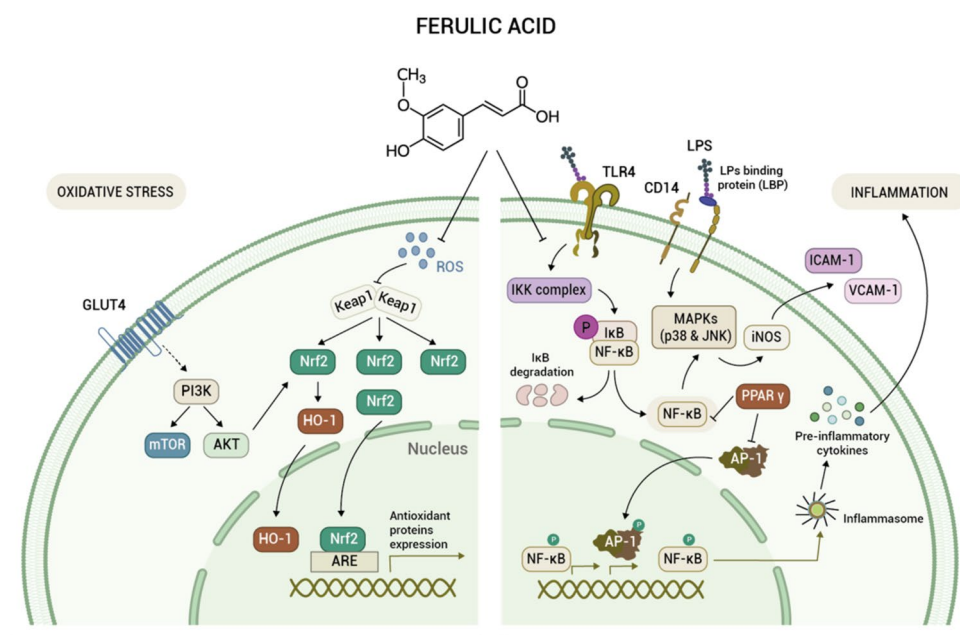


Figure 5. Molecular pathways involved in the antioxidant (left) and anti-inflammatory (right) activities of ferulic acid (adapted from Li et al.⁸¹).

Conclusions

In recent years, the exploration of the therapeutic potential of *Salvia* has gained considerable attention, leading to an increasing number of scientific studies emphasizing its pharmacological properties. Despite this, therapeutic applications of *Salvia* remain underexploited, necessitating further investigation. In this study, a comprehensive exploration and comparison of the extracts of 102 samples from 20 distinct *Salvia* species from Iran was performed, providing a deeper understanding of their specific polyphenol content and, consequently, their antioxidant capabilities and potential therapeutic uses. All samples were analyzed to determine the contents of total phenolics, total flavonoids, total tannin, photosynthetic pigments, and ascorbic acid, followed by the evaluation of their antioxidant activity in vitro. These data were then combined with those from the UHPLC-HRMS analysis, which identified forty distinct chemical compounds in the analyzed samples. The large number of data obtained was subjected to multivariate data analysis with the purpose of finding similarities and differences among the investigated species. Results indicate that each species represents a distinct class of polyphenols due to their unique phenolic compositions. The variations in phenolic profiles suggest diverse potential applications of different *Salvia* species in treating a wide array of diseases. More specifically, *S. verticillata* and *S. bracteata* stand out for their high syringic acid content, known for various activities like cardioprotection, hepatoprotection, antidiabetic, antimicrobial, anti-inflammatory, and pro-apoptotic effects, warranting further investigation in these fields. On the other hand, *S. nemorosa*, particularly rich in salvianolic and lithospermic acids, holds promise for treating endothelial dysfunction, pulmonary arterial hypertension, and cardiac fibrosis. Furthermore, combining extracts from species with a high content of specific compounds, like salvianolic acid A (abundant in *S. nemorosa*) and salvianolic acid B (in *S. multicaulis* and *S. officinalis*), along with rosmarinic acid and salvianolic acid C (in *S. verticillata* and *S. bracteata*) may have great potential for Alzheimer's treatment. The elevated ferulic acid content in the extracts of *S. syriaca* is also interesting for its cardioprotective, anti-fibrotic, anti-apoptotic, and anti-platelet activities. Extracts of species like *S. macrochlamys*, *S. staminea*, *S. limbata*, and *S. spinosa*, rich in flavonoid glycosides and glucuronides, could exhibit antidiabetic, anti-inflammatory, anti-stress, and antiallergic activities, and be potentially useful as anti-viral, anti-bacterial, and even as antiproliferative agents.

This study employed a targeted approach focused on the analysis of specific phytochemicals or antioxidant compounds. An untargeted approach, on the other hand, may offer a broader and more exploratory analysis, enabling the discovery of novel metabolites or unexpected chemical entities that may not have been previously characterized. Integrating both approaches in future studies will provide complementary insights into the metabolome of a sample, combining the specificity of targeted analysis with the exploratory nature of untargeted analysis to enhance metabolite discovery and characterization.

In conclusion, the *Salvia* species investigated in this study show significant potential for the development of innovative nutraceuticals, filling a gap in the current limited range of *Salvia*-derived products available on the market. Current offerings, often composed of individual *Salvia* species in forms like leaf extracts, tinctures, or tablets, address limited health concerns such as digestive issues or specific afflictions, such as headaches and rheumatism. Our findings highlight particularly effective species (*S. verticillata*, *S. bracteata*, *S. nemorosa*, *S. multicaulis*, *S. officinalis*, and *S. syriaca*), suggesting their collective use in the creation of new nutraceuticals. Combining two or more of these species could generate synergistic effects, broadening their medical applications with various health benefits.

Materials and methods

Chemicals and reagents

1,1-Diphenyl-2-picryl hydrazyl (DPPH), 2,4,6-tripyridyl-S-triazine (TPTZ), and quercetin were purchased from Sigma Chemical Co. (St., Louis, USA). Gallic acid, Folin Ciocalteu reagent, sulfuric acid (H₂SO₄), potassium hydroxide (KOH), potassium carbonate (K₂CO₃), sodium carbonate (Na₂CO₃), aluminum chloride (AlCl₃), sodium nitrite (NaNO₂), sodium hydroxide (NaOH), sodium acetate (CH₃COONa), formic acid, ethanol, hexane, acetone, acetonitrile, and methanol were all purchased from Merck & Co. Inc. (Rahway, New Jersey, USA).

Sampling of *Salvia* species

A total of 102 *Salvia* samples were handpicked between April and July 2020 from various regions in West Azerbaijan and identified by Dr. Shahram Bahadori of the Department of Biology, University of Tehran, Tehran (Iran). West Azerbaijan Province is recognized as one of the primary centers of genetic diversity of the Lamiaceae family in Iran. The 102 collected samples represent 20 different *Salvia* species (Fig. 1, Table 1), making them a comprehensive representation of the entire *Salvia* family. Moreover, even within the same *Salvia* species, the samples exhibit variations in collection location and altitude. The samples were collected in different cities within the Western Azerbaijan Province that can be categorized into three distinct collection areas (Fig. 2): the northern region extending from Maku to Salmas (highlighted in green), the central area from Urmia to Sardasht (in red), and the southern area from Miandoab to Takab (in purple). Furthermore, the samples were gathered at different altitudes, some at sea level, while others were collected in the mountains or near lakes. Consequently, even samples from the same species were exposed to different meteorological conditions. Table S1 provides details of the specific areas where the various samples of each of the 20 *Salvia* species were collected.

Preparation of methanolic extracts

The aerial parts of the selected plants were separated and then washed thoroughly with running water to remove surface dust. The collected samples were then air dried for few days, shrunken into powder, and stored in plastic bags for use. The plant powders (0.1 g) were placed in test tubes, and methanol (10 mL) was added to each tube to soak the plant powder, which was then shaken well. The solutions were then filtered by filter paper, and the resulting filtrates were collected separately and used for phytochemical analysis. Methanol was chosen as an

extraction solvent, based on the results of Bajkacz et al., who demonstrated that methanol outperformed other solvents in extracting polyphenols¹⁰⁰. Indeed, their comparative analysis revealed that methanol was the most effective solvent, followed by ethanol and its combinations.

Total phenolic content (TPC)

The Folin-Ciocalteu colorimetric method was employed to determine the total phenolic content in the methanolic extracts from the 102 *Salvia* samples, according to Ul-Haq et al.¹⁰¹. The concentration of the extracts was 20 µg/mL and their absorbances were measured at 765 nm using an HALO DB-20 UV-Vis spectrophotometer (Dynamica Scientific LTD, Livingston, UK). A standard curve was built using different concentrations of gallic acid (10–50 µg/mL). The total phenolic content for each sample was expressed in terms of gallic acid equivalents per gram of dry weight sample (mg of GAE/g of dry weight sample).

Total flavonoid content (TFC)

The total flavonoid content of each sample was determined using the aluminum chloride colorimetric assay¹⁰². Briefly, 0.15 mL of each diluted extract was separately mixed with 1.5 mL of methanol, 0.1 mL of potassium acetate (1.0 M), 0.1 mL of aluminum chloride (10%, w/v), and 2.8 mL of distilled water, and the mixture was incubated at room temperature for 35 min. Then, the absorbance of each sample was measured at 415 nm. A calibration curve using quercetin as standard (10–50 µg/mL) was drawn. The results of the TFC were expressed as mg of quercetin equivalent per gram of dry weight sample (mg QUE/g DW).

Total tannin content (TTC)

The amount of total tannin content for each extract was evaluated according to the Vanillin reagent method as reported by Bharath et al.¹⁰³. The reaction mixture consisted of 0.1 mL of extract, 2 mL of vanillin (4% w/v in methanol), and 1 mL of HCl (concentrated grade). The reaction mixture was shaken well and incubated for 30 min at 30 °C. The absorbance of each sample was measured at 500 nm. The total tannin content was evaluated using the linear regression equation obtained from the standard tannic acid and expressed in mg tannic acid equivalent per g of extract (mg TAE/g extract).

Ascorbic acid content (AAC)

The total concentration of ascorbic acid was determined using to the method of Klein and Perry¹⁰⁴. Briefly, 0.1 g of sample powder was extracted using 3 mL of a 1% metaphosphoric acid solution leaving the mixture at –4 °C for 35 min, followed by filtration at room temperature. The filtrate was mixed with 2,6-dichloroindophenol (2 mL) and the absorbance was recorded within 30 min at 520 nm against a blank. The content of ascorbic acid was assessed based on a standard calibration curve built using authentic L-ascorbic acid (10–50 mg/mL). Results were expressed as mg of ascorbic acid per gram dry weight (mg AA/g DW).

Antioxidant activity by DPPH assay

The free radical scavenging activity was measured using the elimination of DPPH radicals according to the method of Shimada et al.¹⁰⁵ with some modifications. Different concentrations of each extract were separately added to 4 mL of DPPH methanol solution (0.004%). The mixture was shaken and kept for 30 min at room temperature in the dark. The absorbance was measured at 517 nm. All determinations were performed in triplicate. The antioxidant activity was determined as the percent inhibition caused by the hydrogen donor activity of each sample according to the following equation: Inhibition (%) = (1 – absorbance of the sample/absorbance of the blank) × 100. The results are reported as micrograms of ascorbic acid equivalent per milliliter (µg AAE/mL). The percentage obtained for each sample was placed in the formula of the standard curve ($y = 1.5112x + 32.644$) of ascorbic acid and thus the amount of DPPH was obtained based on AAE.

Antioxidant activity by FRAP assay

FRAP activity assay was carried out according to the protocol of Miao et al.¹⁰⁶ with minor modifications. Briefly, the FRAP reagent consisted of 300 mmol/L acetate buffer (pH 3.6), 20 mmol/L ferric chloride (FeCl₃) solution, and 10 mmol/L 2,4,6-tripyridyls-triazine (TPTZ) solution, mixed in a 10:1:1 (v/v) ratio, respectively. The FRAP reagent was freshly prepared and warmed at 37 °C in a water bath before use. 100 µL of each plant extract was separately added to 3 mL of the FRAP reagent. The mixture was vortexed, and absorbance of the solution was evaluated at 593 nm after incubating at 37 °C for 35 min. Different concentrations (from 50 to 600 µmol/L) of Fe²⁺ solution were used to build the standard curve. Results were expressed in terms of micromoles of Fe²⁺ per gram of dry weight (µmol Fe²⁺/g DW).

Photosynthetic pigments content

Chlorophylls (C_a and C_b), total carotenoids (C_{x+c}), and β-carotene were measured spectrophotometrically using plant extracts prepared in 80% acetone/distilled water (v/v), following the methods outlined in the studies by Lichtenthaler¹⁰⁷ and Nagata et al.¹⁰⁸. The contents of these compounds were calculated for each extract using the following formulas:

$$C_a = 15.65 A_{662} - 7.340 A_{645},$$

$$C_b = 27.05 A_{645} - 11.21 A_{662},$$

$$C_{x+c} = 1000 A_{470} - 2.860 C_a - 129.2 C_b/245,$$

$$\beta - \text{Carotene} = 0.854 A_{470} - 0.312 A_{645} + 0.039 A_{662} - 0.005,$$

where A_{662} , A_{645} , and A_{470} are the absorbance values determined at the wavelengths of 662, 645, and 470 nm, respectively.

Identification of polyphenolic compounds by UHPLC-HRMS

Polyphenolic compounds' profiling was performed by using an UHPLC system (Dionex UltiMate 3000, Thermo Fisher Scientific, Waltham, MA, USA) coupled to a Q-Exactive Orbitrap mass spectrometer (UHPLC, Thermo Fisher Scientific, Waltham, MA, USA). A Luna Omega PS 1.6 μm column (50×2.1 mm, Phenomenex, Torrance, CA, USA) was employed for the chromatographic separation setting the temperature at 25 °C. The mobile phase was composed of solvent A (water containing 0.1% of formic acid), and solvent B (acetonitrile containing 0.1% of formic acid). Polyphenolic compounds were eluted using the following gradient program: 0–1 min, 0% B; 1–2 min, 0–95% B; 2–2.5 min, 95% B; 2.5–5 min, 95–75% B; 5–6 min, 75–60% B. Subsequently, the gradient returned to 0% B in 0.5 min and held for 2.5 min for column re-equilibration. The flow rate was 0.4 mL/min and the injection volume was 5 μL . The autosampler temperature was set at 10 °C.

The mass spectrometer was operated in negative ion mode (ESI^-) due to the acidic nature of the phenolic hydroxyl groups. In this mode, the molecules (M) lose a proton (H), forming anions (M–H)[−]. This results in higher sensitivity and better signal stability for polyphenols, as they tend to form stable negative ions. Two scan events (full ion MS and all ion fragmentation, AIF) were set up for all compounds of interest. Full scan data were acquired setting a resolving power of 35,000 FWHM (full width at half maximum) at m/z 200, whereas AIF scan events were acquired by setting a resolving power of 17,500 FWHM and collision energy values of 10, 20, and 45 eV. In both cases, the mass parameters were the following: Spray Voltage, −3.5 kV; Sheath Gas Flow Rate, 45 arbitrary units; Auxiliary Gas Flow Rate, 10 arbitrary units; Capillary Temperature, 275 °C; Auxiliary Gas Heater Temperature, 350 °C; S-lens RF level, 50; Scan Range m/z , 80–1200. Data acquisition and processing were performed with Quan/Qual Browser Xcalibur software, v. 3.1.66.10 (Xcalibur, Thermo Fisher Scientific, (Thermo Fisher Scientific, Waltham, MA, USA). Analyses were performed in triplicate.

Statistical analysis

Data were analyzed by one-way ANOVA using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). Means of three replicates were compared using the Duncan's Multiple Range Test (DMRT). For multivariate data analysis, raw data obtained from UHPLC-HRMS analysis were aligned based on the m/z value and retention time of the ion signals, then collected in a data matrix consisted of 102 rows (samples) and 40 columns (polyphenols data from UHPLC-HRMS analysis). Such data matrix was then imported into Matlab R2015b (The Mathworks Inc., Natick, MA, USA) to compute multivariate analysis. A principal component analysis (PCA) was performed using the PLS Toolbox 8.6.1 (Eigenvector Research Inc., Wenatchee, WA, USA) under Matlab environment. Before PCA, data was scaled to unit variance (autoscaling). Autoscaling employs both the standard deviation as a scaling factor, thus giving all metabolites the same chance to affect the model, and the mean-centering, which is needed to compute PCA.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding authors on reasonable request.

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Author contributions

Conceptualization: A.A. and J.A.; Methodology and investigation: A.M.N., N.I., F.R., G.G. and H.A.; Supervision: N.I., A.A., and J.A.; Manuscript writing: A.M.N., F.R., A.A., and J.A. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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