Research Article

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Phytochemical screening, antioxidant properties, and photo-protective activities of *Salvia balansae* de Noé ex Coss

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Abstract: The present study evaluated the potential of *Salvia balansae* from the Aures Mountains as a source of natural bioactive compounds. Leaves, flowers, and stems were extracted separately using methanol, ethanol, and acetone. Phenolic compounds were quantified colorimetrically and identified using liquid chromatography coupled with mass spectrometry (LC-MS). Antioxidant capacity was assessed using six different *in vitro* assays, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, 1,1-diphenyl-1-picrylhydrazyl, cupric reducing antioxidant capacity, ferric reducing antioxidant power, ferrous ion chelating, and phenanthroline assay, while the photo-protective capacity was evaluated using the sun protective factor. The methanolic flower extracts revealed the highest O-diphenol and phenolic levels (287.9 ± 0.50 and 147.87 ± 0.21 µg GAE/mg, respectively), whereas the acetonic and ethanolic leaf extracts contained the highest flavonoid (72.17 ± 0.12 µg QE/mg) and flavonol (35.28 ± 0.05 µg QE/mg) levels, respectively. LC-MS was used to identify 18 phenolics, including quinic acid (5.051–69.69 ppm), luteolin-7-*o*-glucoside (7.802–44.917 ppm), apigenin-7-*o*-glucoside (3.751–68.507 ppm), and cirsiliol (2.081–15.608 ppm), distinguishing this Aures taxon. Principal component analysis and unweighted pair-group method with arithmetic mean revealed variability in phytochemicals, antioxidant properties, and photo-protective activity influenced by biological activities and the compound content. Overall, *S. balansae* demonstrated promising photo-protective capacity, the presence of key bioactive compounds, and wide-ranging antioxidant potential, presenting this endemic plant as a valuable source of natural antioxidants and photo-protective agents with pharmaceutical and cosmetic applications.

Keywords: *Salvia balansae*, Aures mountains, phenolics, LC-MS, antioxidants

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1 Introduction

Throughout millennia, plants have been extensively utilized in the field of medicine and have constituted a significant component of the human diet. They serve as the primary reservoir for the development of novel medications and healthcare products [1]. Recently, an increasing amount of research has been dedicated to determining and identifying phenolic chemicals in plant raw materials. Consequently, herbal medicines have gained popularity and are being used more frequently due to their lack of adverse effects [2]. Over 80% of the global population continues to utilize medicinal herbs for health-related reasons. Furthermore, almost a quarter of contemporary medications incorporate at least one active component derived from plants [3].

Over time, the skin is exposed to multiple external damaging factors, such as ultraviolet (UV) radiation, pollution, and lifestyle variables that can accelerate the aging process, induce inflammation, and increase the risk of developing skin cancers by producing reactive oxygen species in cells that cause oxidative reactions and damage skin in connective tissue [4,5]. Plant secondary metabolites, such as phenolic compounds and flavonoids, have gained attention as promising photo-protective agents due to their high photostability, antioxidant effects, and low phototoxicity [6,7]. These natural compounds can absorb UV radiation, making them valuable additions to natural sunscreen formulations [6].

Salvia, commonly known as sage, is one of the largest genera of the Lamiaceae family, consisting of more than 980 species [8], distributed in the temperate, subtropical, and tropical regions all over the world [9]. Twenty-three *Salvia* species exist in Algeria, five of which are endemic to the North African region namely: *S. jaminiana S. pseudojaminiana*, *S. algeriensis*, *S. balansae,* and *S. chudaei*. Among these, only *S. jaminiana* and *S. balansae* are restricted to Algeria [10].

Salvia balansae Noë ex Coss. is an endemic perennial medicinal plant growing in two very distinct localities from Algeria, Mostaganem, on the northwest coast and Aures mountains on the east [10], which is more than 500 km east and 1,000 m higher than the first locality. The only shared characteristic between these two sites is the semi-arid bioclimate. However, one site is located in a maritime region with temperate winters, while the other is situated in a region with colder winters [11]. The plants from the two sites differ not only in the color of the flowers but also in the morphology of the leaves [12]. Consequently, the Aures taxon is exclusively found at this specific location, making it an endemic species.

Sage species are predominantly characterized by their richness in flavonoids and terpenoids [13]. These compounds are key contributors to numerous biological activities, including anti-inflammatory, anticancer, antibacterial, and, most importantly, antioxidant activity [14,15].

However, in contrast to sage species that are found worldwide or have a widespread distribution, the endemic taxa are still not well understood, especially in terms of their phytochemical and pharmacological characteristics [3]. Regarding *S. balansae*, we have only come across a limited number of modest studies that provide descriptions of certain chemical and pharmacological features. Only three investigations of this species are known to us. The first study examines the therapeutic impact of the species on metabolic disorders and testicular dysfunction caused by a high-fat diet in Wistar rats [16]. The second study involves the extraction and identification of phenolic compounds using high-performance liquid chromatography with diode array detection (HPLC-DAD), and the evaluation of the antioxidant activity of the *S. balansae* extract [17]. Finally, the third paper was published by Mokhtar et al. [3], and deals with the phytochemical screening, mineral contents, HPLC-DAD analysis, HPLC-mass spectrometry (HPLC-MS) analysis, and fatty acid analysis by gas chromatography-mass spectrometry (GC–MS). Additionally, it includes an evaluation of the antioxidant, enzyme inhibition, and antimicrobial activities of this species. These studies represent the initial contributions to the understanding of the chemical composition and biological activity of *S. balansae*. The commonality among them is the locality of plant collection, which is the Chelf Valley in the Mostaganem department. Thus, the plant of the Aures Mountains, which is probably a different species and endemic to this region, has not been the subject of any study until now.

It is proposed in this research that the effect of solvent and plant organs of *S. balansae* collected from Aures Mountains on the chemical composition of the crude extracts and their antioxidant and photo-protective activities be studied for the first time.

In the present work, the maceration technique was used for the extraction of chemical constituents from different plant parts, such as leaves, flowers, and stems of *S. balansae* collected from the Aures Mountains. Various mixtures of organic solvents (ethanol/water, methanol/water, and acetone/water) were used, and the total phenolic compounds, *ortho*-diphenol, flavonoid, and flavonol content in *S. balansae* crude extracts (SBCEs) were determined using UV–Vis. Their chemical composition was identified using liquid chromatography coupled with mass spectrometry (LC-MS). Subsequently, antioxidant activity was assessed through various *in vitro* tests, including 1,1-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ferrous ion chelating (FIC), ferric reducing antioxidant power (FRAP), phenanthroline, and cupric reducing antioxidant capacity (CUPRAC) assays. Additionally, principal component analysis (PCA) and unweighted pair-group method with arithmetic mean (UPGMA) were applied to detect similarities and dissimilarities among the nine extracts obtained in terms of chemical composition, secondary metabolite content, properties, and photo-protective activity.

The purpose of this research is to conduct a thorough evaluation of the antioxidant and sun-protective properties of *S. balansae*, a plant native to the Aures Mountains in Algeria. It aims to explore how different solvents and plant parts affect their chemical makeup, identify active compounds, assess antioxidant capabilities through various

laboratory tests, and measure their sun protective factor (SPF). This study intends to reveal the untapped potential of this species for diverse applications.

2 Materials and methods

2.1 Chemicals and reagents

All solvents used in this present study (methanol, ethanol, acetone, chloroform) were of analytical grade obtained from Honeywell. DPPH, trichloroacetic acid (TCA), ferric chloride ($FeCl_3$), Folin–Ciocalteu reagent, aluminum chloride ($AlCl_3$ $6H_2O$), sodium carbonate (Na_2CO_3), ferrozine, potassium ferricyanide, ammonium molybdate (NH4)6Mo7O24 4H2O, $ABTS^{\cdot+}$, sodium molybdate (Na_2MoO_4), sodium acetate ($C_2H_3NaO_2$), copper(II) chloride dehydrate ($CuCl_2$ × $2H_2O$), neocuproine ($C1_4H1_2N_2$), and phosphate ($Na_3PO_4{\cdot}12H_2O$) were purchased from Sigma Chemical Co. (Sigma-Aldrich GmbH, Stern-heim, Germany). The reference standards were obtained from EXTRASYNTHESE (Genay Cedex, France). These standards included quercetin ($C_{15}H_{10}O_7$), butylated hydroxytoluene (BHT) ($C_{15}H_{24}O$), gallic acid ($C_7H_6O_5$), butylated hydroxyanisole (BHA) ($C_{11}H_{16}O_2$), ascorbic acid ($C_6H_8O_6$), EDTA, and α-tocopherol.

2.2 Collection of plant samples and preparation of extracts

Arial parts of *Salvia balansae* de Noé were collected during the flowering stage in June 2019 from Ichemoul (1,350 m of altitude), Department of Batna in Algeria, at 35.2721 (latitude in decimal degrees) and 6.4629 (longitude in decimal degrees), with a supra-Mediterranean climate and precipitations superior to 450 mm. The species was identified by Dr. A. Zeraib, a lecturer at Abbes Laghrour University. A voucher specimen has been deposited under the code ZA-SB-003-6-2019, in the Herbarium of Laboratory of Biotechnology, Water, Environment and Health, University of Abbes Laghrour, Khenchela, Algeria.

The fresh plant samples were washed and dried separately (flower, leaf, and stem) for 3 weeks at room temperature in a well-ventilated room. The dried plant materials were then separately ground to powder, packed in paper bags, and sealed in a dark container at room temperature, until use.

Fifteen grams of the dry powder of *S. balansae* leaves, flowers, and stems were individually macerated in 500 ml of methanol/distilled water (8:2, v/v) (MW), ethanol/distilled water (7:3, v/v) (EW), and acetone/distilled water (6:4, v/v) (CW) at room temperature for 24 h with mechanical stirring and then filtered. This procedure was repeated three times until complete extraction. Finally, the filtrates were combined and concentrated on a rotary evaporator under reduced pressure at 40°C. The obtained extracts were stored in amber glass bottles in a refrigerator at 4°C until the performance of the analysis.

The percentage of yield of the crude extract was calculated using the following equation:

$$\% \text{ Yield} = (\text{Weight of dried crude extracts(g)} / \text{Weight of dried plant sample taken(g)}) \times 100.$$

2.3 Quantitative estimation of chemical contents

2.3.1 Estimation of total phenolic contents (TPCs)

The TPCs of leaves, flowers, and stems were determined using the Folin–Ciocalteu colorimetric method as described by Singleton and Rossi [18]. Briefly, 20 µl of crude extracts (0.5 mg/ml) were added to 100 µl of Folin–Ciocalteu reagent (ten-fold diluted); after 4 min, 80 µl of Na_2CO_3 was added with shaking. The solution mixture was allowed for 2 h in darkness at room temperature before UV–Vis measurement at 765 nm. The amount of phenolic compounds in plant samples was determined using the calibration curve obtained between concentration (0–100 µg/ml) and absorbance of gallic acid. The phenolic content was expressed as mg gallic acid equivalent per gram of dry weight basis of crude extracts (mg GAE/g extract) and ±SD (standard deviation) for three replicate analyses.

2.3.2 *Ortho*-diphenol (ODP) content

The content of ODPs in the SBCEs was determined by the colorimetric method described by Martins-Gomes et al. [19]. Two hundred microliters of 5% sodium molybdate solution were added to 40 µl of the sample extracts, and then the absorbance was read at 370 nm after 15 min of incubation at room temperature. A standard curve using gallic acid with different concentrations was built, and the results are expressed as mg of gallic acid equivalent (mg GAE/g extract).

2.3.3 Estimation of flavonoid contents

The total flavonoid content of *S. balansae* extracts was measured using the aluminum chloride colorimetric assay according to the method described by Mbaebie et al. [20]. One hundred microliters of the crude extracts at different concentrations were added to an equal volume of 2% ethanolic solution of aluminum chloride. The mixtures in the test tubes were incubated at room temperature for 1 h, and then the absorbance of the mixture was determined at 420 nm. The total flavonoid content was reported as milligrams of quercetin equivalents per 1 g of crude extract (mg QE/g extract).

2.3.4 Total flavonol contents

Total flavonols were determined as described by Benmeddour et al. [21] with slight modifications. Briefly, 50 µl of SBCEs was added to 50 µl of aluminum chloride (2%) and 150 µl of sodium acetate solutions (5%). The absorbance at 440 nm was recorded after 2.5 h at room temperature. Results are expressed as milligrams of quercetin equivalents per g of extract (mg QE/g extract).

2.4 LC-MS separation and identification of phenolic compounds

The *S. balansae* extracts (SBCEs) were analyzed using a Shimadzu UFLC XR system (Kyoto, Japan), equipped with a SIL-20AXR auto-sampler, a CTO-20 AC column oven, an LC-20ADXR binary pump, and a quadrupole 2020 detector system.

The chromatographic separation was performed on an Inertsil ODS-4 C18 column (L150 mm × 3.0 mm i.d. 3 µm particle size) maintained at 40°C with an injection volume of 20 µl and a flow rate of 0.5 ml/min. Mobile phases A and B included 0.2% acetic acid in 95% water and 5% methanol, and 0.2% acetic acid in 50% water and 50% acetonitrile, respectively. Compounds were eluted employing the following gradient: 0.01–14 min from 10 to 20% B; 14–27 min from 20 to 55% B; 27–37 min from 55 to 100% B; 37–45 min, 100% B; and 45–50 min 10% B.

The mass spectrometry settings were optimized for negative ion electrospray ionization (ESI): the settings included a capillary voltage of −3.52 V, nebulizing gas flow at 15 l/min, a dissolving line temperature set to 280°C, the block source temperature of 400°C and a detection voltage of 1.35 V. LC-ESI (−) MS mass spectra corresponding to the [M–H] deprotonated molecules were recorded using lab solution software.

The corresponding compounds were identified and quantified by matching the relative retention time and the UV spectra to reference standards, as described by Mahmoudi et al. [22].

2.5 Antioxidant assays

Antioxidant and free radical scavenging activities of SBCEs were assayed by using different *in vitro* assays like DPPH, ABTS, FIC, FRAP, phenanthroline, and CUPRAC. Absorbance was measured against a blank solution containing the extract or standards but without the reagents. A control test was performed without the extract or standards. Percentage scavenging and IC50 values ± SD were calculated.

2.5.1 DPPH radical scavenging assay

The free radical scavenging activity of SBCE was determined according to the method based on the reduction of the stable free radical 1,1-diphenyl-2-picrylhyrazyl by the addition of a radical species or an antioxidant, which is accompanied by the discoloration of the purple DPPH solution, as described by Blois [23] and reported by Burits and Bucar [24]. Using 96-well micro-plate reader, 160 µl of a DPPH solution (0.1 mM) was added to 40 µl of each extract at various concentrations (6.25–400 µg/ml). The mixture was incubated in obscurity at room temperature for 30 min; the absorbance was measured at 517 nm against a blank. BHA, BHT, α-tocopherol, and ascorbic acid were used as positive controls.

The percentage of free radical scavenging activity of the crude extracts was calculated using the following equation:

$$\% \text{ of free radicular activity} = (A_c - A_s)/A_c \times 100,$$

where A_c is the absorbance of the control and A_s is the absorbance of samples.

2.5.2 ABTS radical scavenging assay

The ABTS radical scavenging ability of *S. balansae* extracts was assessed spectrophotometrically using the slightly modified method described by Re et al. [25]. The ($ABTS^+$) radical cations were generated by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate. The mixture was allowed

to stand in the dark at room temperature for 12–16 h prior to use, and the standard solution of ABTS was diluted to an absorbance of 0.700 ± 0.02 at 734 nm. Then, to 40 µl of each crude extract with different concentrations (12.5–800 µg/ml), 160 µl of ABTS solution was added using a 96-well microplate. The mixture was allowed to stand at room temperature for 10 min, and its absorbance was measured at 734 nm. BHA, BHT, α-tocopherol, and ascorbic acid were used as positive controls. The inhibition percentage of the crude extracts was estimated in terms of the ABTS radical cation decolorization according to the following formula: inhibition % = $[(A_c - A_s)/A_c] \times 100$.

2.5.3 FIC activity

The evaluation of the capability of the tested compounds on chelating ferrous ions was conducted in terms of inhibition of the generation of the Fe^{2+}-ferrozine complex using the ferrozine method as proposed by Decker and Welch [26]. The reaction mixture contained 40 µl of each extract with different concentrations (12.5 to 800 µg/ml) of *S. balansae*, 40 µl of methanol, and 40 µl of 0.2 mM $FeCl_2$. The reaction was then initiated by adding 80 µl of ferrozine. The absorbance of the mixture was red against a blank after 10 min of incubation at 593 nm. The chelating percentage of Fe^{2+} was calculated using the following formula:

$$Fe^{2+} \text{ chelating percentage} = (A_c - A_s)/A_c \times 100,$$

where A_c is the absorbance of the control and A_s is the absorbance of samples.

The results are expressed as IC_{50} (µg/ml). The antioxidant capability of the extracts was compared to EDTA and α-tocopherol, which served as a standard.

2.5.4 FRAP assay

The ability of each extract to reduce ferric ions (Fe^{3+}) to ferrous ones was investigated by following the ferric-reducing power assay as reported by Oyaizu [27]. Ten microliters at different concentrations (3.125–200 µg/ml) of SBCEs were allowed to react with 40 µl of phosphate buffer (0.2 M, pH 6.6) and 50 µl of 1% potassium ferricyanide solution. The reaction mixture was incubated for 20 min at 50°C, and after cooling, 50 µl of 10% TCA was added to the mixture to terminate the reaction, followed by 40 µl of distilled water and 10 µl of 0.1% $FeCl_3$ solution. The absorbance was read at 700 nm against a blank. The results were calculated as $A_{0.5}$, referring to the tested extract concentration providing 0.5 of absorbance, and compared to BHA, BHT, ascorbic acid, and α-tocopherol.

2.5.5 Phenanthroline assay

The antioxidant capacity of the crude extracts was investigated using the reduction activity by the phenanthroline method suggested by Szydłowska-Czerniak et al. [28]. In the presence of chelating compounds, the formation of the O-phenanthroline-Fe^{+2} is interrupted, and the extract capable of interfering with such complex is suggested to have metal chelating activity. In a 96-well microplate, 10 µl of each extract at various concentrations (3.125–200 µg/ml), 50 µl of 0.2% $FeCl_3$ solution, 30 µl of 1,10-phenanthroline (0.5 %), and 110 µl of methanol were mixed and incubated at 20°C for 10 min, and the absorbance was recorded at 510 nm against a blank. The results are expressed as $A_{0.5}$ (µg/ml), indicating the concentration of extracts giving an absorbance of 0.50. BHA, BHT, and ascorbic acid were used as standards.

2.5.6 CUPRAC

The reduction of cupric ions by the samples was exploited to determine the antioxidant capacity of each extract using the CUPRAC method reported by Apak et al. [29]. Briefly, 50 µl of 10 mM copper (II) chloride solution, 50 µl of 7.5 mM neocuproine solution, and 60 µl of ammonium acetate buffer solution (1 M, pH = 7) were transferred into a 96-well microplate, and then 40 µl of each extract with different concentrations (12.5–800 µg/ml) were added to the previous mixture. After 1 h of incubation in darkness at room temperature, the absorbance was red at 450 nm. The results are expressed as $A_{0.5}$ (µg/ml), indicating the concentration of extracts giving an absorbance of 0.50. BHA, BHT, ascorbic acid, EDTA, and α-tocopherol were used as standards.

2.6 Photo-protective activity

The SPF was employed to assess the *in vitro* photo-protective capacity of the samples by measuring their UV absorption ability, as described by Mansur et al. [30]. An aliquot of the sample solution was prepared, and absorbance readings were taken within a wavelength range of 290–320 nm, with a 5 nm interval.

The SPF was determined using the following equation:

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda),$$

where CF is the correction factor (=10); EE is the erythemogenic effect of radiation at wavelength λ; I is the solar

Table 1: Correlation between the erythemogenic effect of radiation at a wavelength EE and the solar intensity spectrum I at each wavelength [31]

λ (nm)	EE(λ) × I(λ)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

intensity spectrum; Abs is the absorbance of the sunscreen product; EE × I are constant values previously determined by Sayre et al. [31], as shown in Table 1.

2.7 Statistical analysis

The results were expressed as mean ± standard deviation (SD) of three parallel and independent analyses. After performing ANOVA tests, significant differences between mean values were identified by conducting a Tukey HSD (honest significant difference) multiple comparison test using STISTICA 10.0 software (StatSoft Inc., USA). The level of significance was set at 5%; thus, values less than 0.05 were considered significant. PCA and UPGMA were employed to estimate SBCEs similarity based on their phenolic compounds and biological activities, and the relationships between them were determined using Pearson's correlation method at a 95.0% confidence level.

3 Results and discussion

3.1 Phytochemical study

3.1.1 Yield of extraction

Conventional extraction was carried out using three different types of solvents: methanol, ethanol, and acetone to extract phenolic compounds from the leaves, flowers, and stems of *S. balansae* (Figure 1). The results showed that the flowers are richer in phenolic compounds, followed by the leaves and stems. Among the leaves and flowers, ethanol extracts contained the highest proportion of phenolic content, followed by acetone and methanol extracts. In stems, the highest proportion of phenolic content was recorded with the methanol extract, followed by acetone and ethanol extracts.

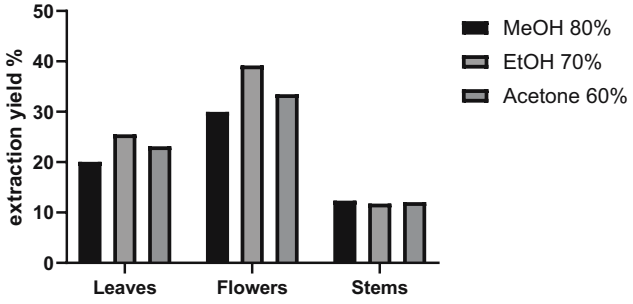

Figure 1: Extraction yield (%) of different extracts of *S. balansae*.

3.1.2 Quantitative analysis

The determination of TPC, ODPs, total flavonoid content (TFC), and flavonol (FOL) contained in SBCE isolated from flowers, leaves, and stems was carried out using colorimetric methods. The findings are illustrated in Table 2.

3.1.2.1 TPC

Phenolic compounds are one of the most important secondary metabolites that possess an important antioxidant power due essentially to the presence of hydroxyl groups, their position, and molecular size [32]. Table 2 presents the findings of the colorimetric examination of total phenolic compounds, obtained by comparing the absorbance values of SBCE solutions reacting with Folin–Ciocalteu reagent to the equivalents of gallic acid solutions.

The TPC in MeOH, EtOH, and Ac extracts ranged from 99.85 to 147.86 mg GAE/g, 60.79 to 83.55 mg GAE/g, and 114.30 to 134.19 mg GAE/g, respectively, showing the following order of concentrations: flowers > leaves > stems in MeOH, EtOH extracts, while for the Ac extracts, the TPC showed the following order: stem > leaves > flowers. The MeOH extract of flowers contained the highest amount of TPC, followed by the Ac extract of stems. Meanwhile, the EtOH extract of flowers and the other organs exhibited the lowest amount of phenolic components.

This variation in TPC distribution might be attributed to several factors, with the polarity and type of the solvent used playing a major role in the selectivity and solubility of the phenolic compounds [33]. Therefore, the combination of water and solvent is more effective for extracting bioactive compounds than using a pure solvent, considering that

Table 2: Secondary metabolite contents of SBCEs

SBCE		TPC (µg GAE/mg)	ODP (µg GAE/mg)	TFC (µg QE/mg)	FOL (µg QE/mg)
Solvents	**Organs**				
MeOH	Leaves	113.12 ± 0.38^{d}	158.57 ± 0.76^{d}	60.22 ± 0.40^{b}	30.93 ± 0.33^{b}
	Flowers	147.87 ± 0.21^{a}	287.9 ± 0.50^{a}	55.47 ± 0.24^{c}	21.19 ± 0.29^{d}
	Stems	99.85 ± 0.10^{e}	97.87 ± 0.53^{f}	17.23 ± 0.06^{f}	14.80 ± 0.41^{e}
EtOH	Leaves	82.61 ± 0.38^{f}	143 ± 0.58^{e}	61.79 ± 0.63^{b}	35.28 ± 0.05^{a}
	Flowers	83.56 ± 0.10^{f}	57.53 ± 0.59^{h}	34.70 ± 0.06^{e}	13.94 ± 0.16^{e}
	Stems	60.80 ± 0.42^{j}	25.63 ± 0.56^{i}	17.58 ± 0.35^{f}	15.12 ± 0.19^{e}
AC	Leaves	129.89 ± 0.27^{c}	257.5 ± 0.32^{b}	72.17 ± 0.12^{a}	33.77 ± 0.21^{a}
	Flowers	114.31 ± 0.49^{d}	173.87 ± 0.53^{c}	36.80 ± 0.60^{d}	24.04 ± 0.57^{c}
	Stems	134.2 ± 0.96^{b}	87.47 ± 0.34^{j}	16.67 ± 0.20^{f}	9.42 ± 0.14^{f}

Mean values that share the same superscript letters in the specified column are not significantly different, whereas those with different superscript letters are significantly ($P < 0.05$) different, as assessed by Tukey's HSD post-hoc test. SBCEs, *Salvia balansae* crude extracts; TPC, total phenolic content; ODPs, *ortho*-diphenols; TFC, total flavonoid content; FOL, flavonol content. GAE, gallic acid equivalent; QE, quercetin equivalent.

it allows molecular diffusion through high mass transfer, enhancing cellular porosity [34].

Therefore, combining acetone and methanol with water allows for better selectivity and extraction of phenolic constituents, as indicated by the data from this research. This finding aligns with numerous previous studies [32,33,35–38]. However, other researchers reported contradictory results [34,39], who found that ethanol contained higher phenolic levels than methanol and acetone.

Our results showed that flowers have a higher abundance of polyphenols compared to leaves and stems. These findings could be explained by the storage of bioactive compounds in the flowers to attract potential pollinators through the influence of visual characteristics [40].

3.1.2.2 ODP content

ODPs are a group of phenolic compounds characterized by having two groups in adjacent positions on an aromatic ring; this specific catechol arrangement enables these compounds to function as potent antioxidants [41]. The ODP content in SBCEs was determined using a colorimetric assay; the results are expressed in Table 2 as milligram gallic acid equivalent per gram (mg GAE/g).

The ODP content in SBCEs ranged from 25.63 ± 0.56 to 287.9 ± 0.5 mg GAE/g across the different extracts. According to our findings, leaves had the highest amount of ODP in EtOH and AC extracts compared to the other organs, showing the following order: leaves > flowers > stems. However, MeOH extracts exhibited a different range, with flowers > leaves > stems.

To the best of our knowledge, no prior research has characterized ODP in this Algerian endemic Salvia species until this report. However, another study conducted on *Salvia tebesana* found similar results, where MeOH was more effective in extracting ODP compared to water and EtOH, while leaves proved to be the richest compared to stems [42].

The high accumulation of ODP in the different organs of *S. balansae* suggests that the Aures mountain environment and climate induce high production of these secondary metabolites. However, stressors such as high UV exposure and low rainfalls may stimulate an enhanced biosynthesis of these photo-protective and redox-regulating compounds [43].

3.1.2.3 TFC and FOL content

In light of our findings, both the total flavonoid and total flavonol contents extracted from *S. balansae* are solvent-dependent. Acetone demonstrated excellent efficiency in extracting the maximum yield concentration of both groups, exhibiting the following consecutive arrangement acetone > ethanol > methanol.

Several studies previously substantiated these findings [35,44–46], which provided evidence supporting the suitability of acetone for the recovery of these metabolites. Acetone surpassed the performance of methanol and ethanol, aligning with our observations. Additionally, Wijekoon et al. [35] demonstrated that an acetone–water mixture with a 50% ratio outperformed pure acetone, water, and methanol at varying ratios (100, 90, and 50%) for the extraction of TFC.

In terms of the plant parts employed for extraction, the leaves exhibited the highest concentrations using methanol, ethanol, and acetone with 60.21–61.78–72.17 mg QE/g, respectively, for flavonoids, and 30.92–32.05–35.28 mg QE/g, for flavonols. This reveals the consecutive order regarding the

plant parts: leaves > flowers > stems, whereas the acetonic extraction yielded the highest concentration overall.

The highest concentration of flavonoids and flavonols in leaf extracts, compared to flowers and stems, can be attributed to their important physiological roles in plants after all, leaves are highly metabolically active tissues involved in photosynthesis, carbohydrate metabolism, secondary metabolite synthesis, defense compound production, and respiration [47–49]. Flavonoids contribute to these processes by modulating electron transport during photosynthesis, protecting leaf tissue from excessive UV radiation [50], and providing protection against biotic and abiotic stresses, whereas flavonoid biosynthesis has emerged as a nearly universal stress pathway triggered in response to a wide range of stress conditions [51] and participating in plant protection as phytoalexins in response to microbial attacks [52,53].

3.1.3 Polyphenol analysis by LC/MS

LC/MS was used to screen 33 compounds in SBCEs. Phenolic compounds in the extracts were identified by comparison of mass spectra and retention times with those of 33 standards available in the laboratory. In contrast, their amounts were determined using the equations obtained from standard calibration curves after making the necessary dilution of plant extract solutions if needed. In SBCEs, only 18 compounds were identified, eight phenolic acids and ten flavonoid compounds (Table 3).

Quinic acid (from 5.051 ppm in acetone extract of leaves to 69.69 ppm in stem ethanol extract) and rosmarinic acid (from 2.881 ppm in ethanol extract of flowers to 32.914 ppm in methanol extract of flowers) were classified as the most abundant phenolic acids. Three distinct flavonoid compounds were identified as the major ones in SBCEs: luteolin-7-*o*-glucoside (from 7.802 ppm in stem ethanol extract to 44.917 ppm in acetone extract of flowers), apegenin-7-*o*-glucoside (from 3.751 ppm in stem ethanol extract to 68.507 ppm in stem ethanol extract), and cirsiliol (from 2.081 ppm in ethanol extract of leaves to 15.608 ppm in stem acetone extract). All these compounds, along with the other identified components, are commonly found in the genus *Salvia* [54,55].

Compared to the *S. balansae* collected from Chelf Valley in the Mostaganem department, which is probably a different species than our samples, our findings confirm the distinction between them. To the best of our knowledge, only two recent studies investigated the qualitative and quantitative analysis of phenolic compounds of *S. balansae* extracts using HPLC [3,17]. In the study conducted by Mokhtar et al. [3], nine phenolic constituents were

Table 3: Identification and quantification of phenolic compounds in different crude extracts of *S. balansae* by LC/MS analysis

No.	Phenolic compounds	Chemical class	Phenolic compounds quantity (ppm)								
			MeOH extracts			EtOH extracts			Ac extracts		
			L	F	S	L	F	S	L	F	S
1	Quinic acid	Phenolic acid	40.122	40.313	59.394	16.927	47.509	69.69	5.051	19.55	22.498
2	Gallic acid	Phenolic acid	0.115	0.02	0.083	0.114	0.031	0.07	0.037	0.045	0.018
3	Protocachuic acid	Phenolic acid	1.026	0.27	ND	1.057	0.637	ND	0.597	1.126	0.568
4	Caffeic acid	Phenolic acid	1.231	1.563	1.873	1.085	2.508	1.876	0.772	2.945	2.42
5	Syringic acid	Phenolic acid	ND	ND	ND	ND	ND	ND	ND	ND	1.15
6	*p*-Coumaric acid	Phenolic acid	0.348	0.267	0.087	0.39	0.099	0.127	0.644	0.48	0.312
7	*trans*-frulic acid	Phenolic acid	0.146	0.571	0.078	0.161	1.656	0.047	0.252	2.068	0.134
8	Hyperoside (quercetin-3-*O*-galactoside)	Flavonol	0.586	0.307	ND	0.504	0.344	0.408	0.365	0.507	0.635
9	Luteolin-7-*O*-glucoside	Flavone	14.328	40.985	13.683	11.96	29.641	7.802	8.822	44.917	13.501
10	Naringin	Flavanone	3.839	2.248	ND	2.697	2.098	1.23	2.109	2.55	2.052
11	Apegenin-7-*O*-glucoside	Flavonol	6.592	46.672	6.943	4.956	49.549	3.751	4.538	68.507	9.225
12	Rosmarinic acid	Phenolic acid	18.741	32.914	26.614	3.389	2.881	6.39	3.907	6.61	14.712
13	Quercetin	Flavonol	ND	ND	ND	ND	ND	0.068	ND	0.054	0.094
14	Naringenin	Flavonone	0.305	ND	ND	0.109	ND	0.172	0.409	ND	ND
15	Apegenin	Flavone	0.445	6.416	0.565	0.72	3.142	0.73	0.858	5.565	0.946
16	Cirsiliol	Flavone	2.378	3.81	8.362	2.081	2.828	10.575	4.6	3.285	15.608
17	Cirsilineol	Flavone	0.381	ND	ND	0.38	ND	ND	0.353	ND	ND
18	Acacetin	Flavone	0.767	0.497	0.229	0.712	0.705	0.176	1.226	0.242	0.199

MeOH, methanol; EtOH, ethanol; Ac, acetone; L, leaves; F, flowers; S, stems.

quantified by HPLC-MS analysis; catechin (72.5%) was the main compound, followed by myricetin (21.7%), while epicatechin (1.3%) and BHA (1.1%) were present in lower amounts. The research conducted by Mahdjoub et al. [17] highlighted the significant presence of two main classes, phenolic acids and flavonoids, characterized by substantial quantities of tannic acid, benzoic acid, gallic acid, and ascorbic acid. The minor chemicals found include the flavonoids myricetin and catechin, along with their derivatives.

Regarding the major phenolic compounds in other *Salvia* species, rosmarinic acid was the abundant compound in *Salvia lavandulifolia* Vahl. [56], luteolin, rosmarinic acid, and caffeic acid in *Salvia officinalis* and *Salvia verticillata* L. [14,57,58] and *p*-coumaric acid, gallic acid, and syringic acid in *Salvia bicolor* [59].

This variability in both types and quantities of phenolic compounds in *S. balansae* from different *Salvia* species was anticipated due to the complicated chemical profile exhibited by various *Salvia* species. Indeed, each species possesses a distinct and specific phenolic composition, as demonstrated by several studies [60–62]. Each species is adapted to its unique environment and distinct genetic characteristics [55].

PCA provided more detail about the relationships between SBCEs based on phenolic compound data. As shown in Figure 2, this analysis allowed the recognition of three distinct SBCEs types based on the content of quinic acid, cirsiliol, apegenin, luteolin-7-*o*-glucoside, naringin, apegenin-7-*o*-glucoside, protocachuic acid, p-coumaric acid, acacetin, circilineol, and naringenin.

The first group represented the flower extracts, characterized by high concentrations of luteolin-7-*o*-glucoside, apegenin-7-*o*-glucoside, and apegenin. The second group represented the stem extracts, which are characterized by a high content of quinic acid and cirsiliol. These groups are opposed to the third group formed by leaf extracts, which is characterized by the presence of cirsilineol, naringenin, protocachuic acid, *p*-coumaric acid, and acacetin with significant content compared to other extracts (Figure 1).

The same plant may synthesize and store many different compounds, which are expected in most plant organs but not evenly distributed. Indeed, various organs of the same plant produce different compounds at distinct concentrations. This variation can be explained by their gene expression, which is regulated by various biosynthesis/degradation procedures and transport processes. It also reveals strong inter-organ interactions, termed endogenous factors, which are influenced by exogenous agents [32,63], such as pedo-climatic conditions, biotic/abiotic stress, edaphic factors, microclimate type, and bioclimatic stages affecting the plant's growth [64].

3.2 Antioxidant activity

It is important to highlight that evaluating a plant's antioxidant potential should not rely only on a single method. Instead, various *in vitro* antioxidant assays should be conducted to assess the antioxidant potential of the sample

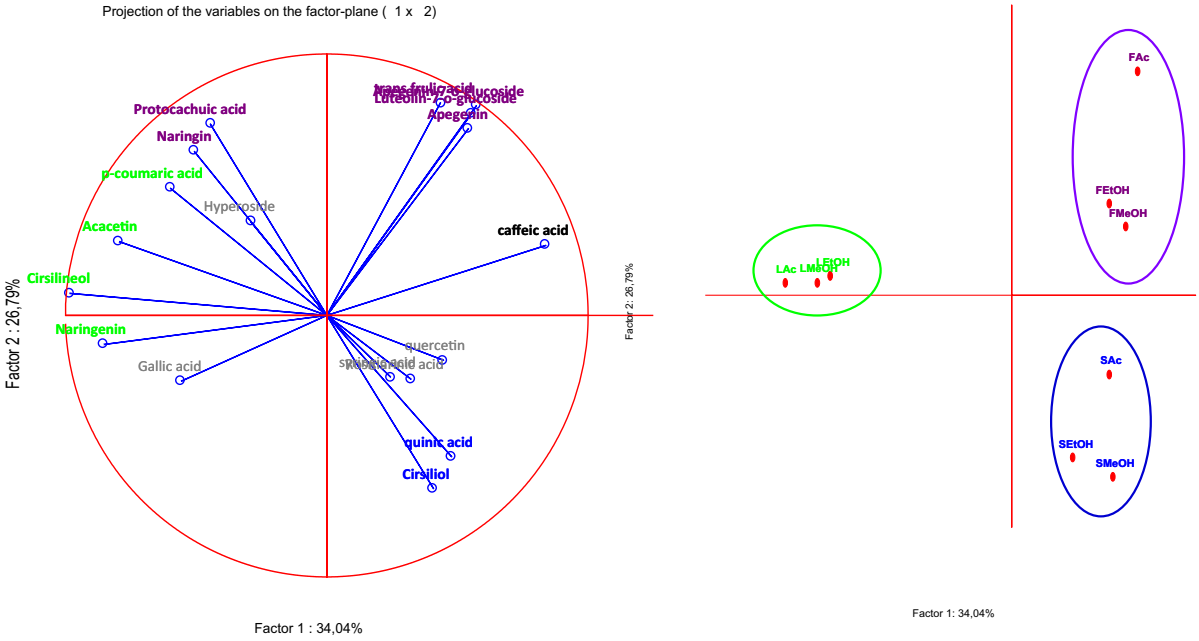

Figure 2: Chemical diversity of SBCEs using the PCA approach.

comprehensively [65]. Thus, to assess the efficacy of SBCEs, multiple analytical assays are employed to gain a comprehensive understanding of the antioxidant properties of various compounds. The results are shown as mean ± standard error of the mean in Figure 3.

3.2.1 DPPH free-radical scavenging

According to our results, the SBCEs exhibited variable free radical scavenging activity, with IC_{50}s ranging from 22.52 to 97.72 µg/ml (Figure 3a). The highest capacity to neutralize DPPH radicals was detected in MeOH followed by acetone extracts, particularly in the flower extracts, depicting an IC50 of 50.27 to 55.62 µg/ml, respectively. Meanwhile, the least effective DPPH scavenging activity was observed in the EtOH extract, especially in the extracts of stems, with an IC_{50} of 97.72 µg/ml.

The effect of antioxidants on DPPH radicals is driven by their hydrogen donation ability or radical scavenging activity [66,67], which suggests that this radical scavenging efficiency is highly associated with the chemical composition and structure of these compounds, known for the presence of phenolic hydroxyl groups capable of donating their hydrogen or electron, as proven by many previous studies [64,68,69].

As per our results, flowers revealed the best radical quenching capacity compared to the other organs used, which can be related to the variation in antioxidant distribution among the various plant organs [68]. These findings are consistent with previous studies [33,70–72].

3.2.2 ABTS radical scavenging assay

Besides DPPH activity, the quenching capacity of free radicals by SBCEs was evaluated using the ABTS assay, which relates to the ability of a substance to neutralize free radicals generated through the oxidation of ABTS. The IC_{50} of SBCEs recorded variable amounts ranging from 33.68 to 59.61 µg/ml, with the flower methanol extract (FMeOH) found to be the most efficient in quenching free radicals, followed by the leaf acetone extract (LAc) and flower ethanol extract (FEtOH). Meanwhile, the stem extracts were the least effective compared to other extracts (Figure 3b). These results align with findings from previous studies [73–75] that highlighted the efficacy of methanol in extracting antioxidants capable of neutralizing free radicals.

In light of our data, it seems that the IC_{50} values of ABTS were lower than for DPPH, which means that the extracts have more affinity for neutralizing the $ABTS^{+}$ radicals compared to the DPPH radicals. This is consistent with the findings from earlier studies [76,77]. These disparities can be attributed to the fact that ABTS radicals are capable of detecting the antioxidant potential of both hydrophobic and hydrophilic substances across a broad pH spectrum. In contrast, DPPH mainly detects hydrophobic antioxidants [77].

3.2.3 CUPRAC

The results of *in vitro* CUPRAC assay are shown in Figure 3c. The acetone extracts demonstrated the highest antioxidant capacity, followed by the methanol extracts, while the ethanol extracts appeared to be the least effective, showing an $A_{0.5}$ = 38.17, 39.71, and 69.95 µg/ml, respectively. The effectiveness of both methanol and acetone as optimal extraction solvents for conducting the CUPRAC assay, as per our results, is in line with findings from previous studies [44,78,79]. The CUPRAC-reducing power of the different plant parts of *S. balansae* revealed that flowers recorded the best antioxidant activity, along with leaves. As can be seen, there is no significant difference between the reducing power of acetonic extracts of leaves and flowers, nor with flowers if methanolic extracts are considered. However, according to [80,81], flowers recorded the highest reducing power, while [82,83] indicated that leaves exhibited the best antioxidant capacity. Indeed, the strength of the same organ depends on the solvent used [84].

The CUPRAC method has demonstrated relatively high values when compared to the other two antioxidant assays based on electron transfer, namely DPPH and ABTS. This trend aligns with findings from prior research [85,86].

3.2.4 FRAP

The ferric reducing power of the SBCEs is indicated in Figure 3d as $A_{0.5}$, which represents the concentration in µg/ml at which the absorbance is 0.5 nm. The presented data of SBCEs indicate that among the different solvents used, methanol exhibited the highest reducing power compared to the other solvents, followed by ethanol and acetone. The antioxidant capacity according to solvents ranked as follows: methanol > ethanol > acetone. The ability of these solvents to reduce Fe^{3+} can indicate the potential antioxidant capacity, which is highly related to phenolic contents [87]. Several previous studies conducted on different plant species have highlighted the efficiency of methanol in reducing Fe^{3+}-ferricyanide [78,88–90].

Regarding the organs used, our results indicate that leaves and flowers have the best ferric-reducing power, while stems overall exhibited the most minor reductive capabilities. These findings indicate the presence of electron donor components in the different organs of *S. balansae*, which are capable of forming stable compounds by neutralizing free radicals. Previous studies have already proven the efficiency of leaves and flowers in reducing Fe^{3+}-ferricyanide [68,91,92].

3.2.5 O-Phenanthroline assay

The results of the reducing capacity by phenanthroline are displayed in Figure 3e. The antioxidant capacity is given in terms of $A_{0.5}$, where absorbance increases linearly with the increasing extract concentration. According to our results, the methanolic extracts demonstrated the highest capacity to reduce iron, followed by acetone, while ethanol exhibited the least antioxidant capacity. The highest $A_{0.5}$ was in methanolic extracts of flowers ($A_{0.5}$ = 17.71 µg/ml), while the lowest was in ethanolic extracts of stems ($A_{0.5}$ = 60.13 µg/ml).

The $A_{0.5}$ of the methanolic extracts ranged from 17.71 to 28.69 µg/ml, which was close to those manifested by the methanolic extracts of *Salvia aegeptiaca* ($A_{0.5}$ = 18.71) and *Salvia verbenaca* ($A_{0.5}$ = 27.03) as reported by Mamache et al. [93].

3.2.6 FIC activity

The FIC assay is a test used to determine the ability of a substance to bind with and neutralize ferrous ions (Fe^{2+}), thereby assessing its capacity to act as a chelating agent and mitigate oxidative reactions involving iron.

The results of the ferrous (Fe^{2+}) ion chelating capacity of the tested extracts are displayed in Figure 3f, and the chelating capacity is given in terms of IC_{50}, which ranged from 44.41 to 275.66 µg/ml. Among the solvents used, methanol proved to have a high chelating capacity, with an IC_{50} ranging from 44.41 to 90.24 µg/ml, indicating high amounts of antioxidants capable of inhibiting the formation of the ferrozine–iron complex.

According to these results, the highest chelating activity was observed in the extracts with polar solvents. Similar findings were reported by Chavan et al. [94] and Jaiswal et al. [95], who demonstrated the superiority of methanol over ethanol and acetone.

Compared to previous works, the methanolic extracts of *S. balansae* leaves exhibited a higher chelating capacity compared to those of *Salvia aegyptiaca* (IC_{50} = 67.99 µg/ml) and *Salvia verbenaca* (IC_{50} = 70.39 µg/ml) [93].

These findings clearly indicate that the extracts of *S. balansae* are capable of exerting a protective role against oxidative damage caused by metal-catalyzed Fenton reactions. Iron stimulates lipid damage through reactions like the Haber-Weiss and Fenton processes, generating aggressive hydroxyl radicals that harm lipids. Ferrous ions, found in food and known as pro-oxidants, exacerbate oxidative damage by initiating these reactions. This jeopardizes food quality and stability by inducing oxidative stress and lipid deterioration, underscoring the need to manage the presence of ferrous ions for food preservation [96].

Regarding the plant parts, it is evident that there is no consistent pattern in the effectiveness of different plant organs in metal chelation involving ferrous ions. This inconsistency suggests no predictable pattern in which parts consistently perform better in this metal-binding process. The variability in metal chelation activity among different plant organs could be attributed to various factors, such as the presence of different types and amounts of chelating compounds in each part or variations in the overall composition of these plant parts.

Usually, when comparing the IC_{50} of each extract with the reference standards α-tocopherol and EDTA, the extracts showed lower potency in all antioxidant tests. However, this does not necessarily imply weak antioxidant activity in our extracts. It is important to consider that these extracts consist of a multitude of components. Unlike the pure standards, the efficacy of our extracts could still be considered significant [68].

3.3 *In vitro* photo-protective assay

The photo-protective activity of *S. balansae*'s extracts was evaluated by calculating their SPF values using Mansur's equation [30]. The SPF values, determined across five concentrations, are shown in Figure 3. These values ranged from 26.64 to 46.34 at a 1 mg/ml concentration. According to the [97], SBCEs appear to possess a high SPF. Methanolic extracts displayed the highest SPF values, followed by acetonic extracts, while ethanol exhibited the lowest sun protection activity. Regarding different plant organs, flowers provided the highest level of protection. Notably, there was no significant difference in SPF values between leaves and flowers, except for those extracted by methanol. Interestingly, stems extracted by methanol showed a superior sun protection capacity compared to leaves.

Compared to other studies, SBCEs exhibited a higher sun protection capacity at a concentration of 1 mg/ml than *Salvia officinalis* at a concentration of 2 mg/ml (SPF value =

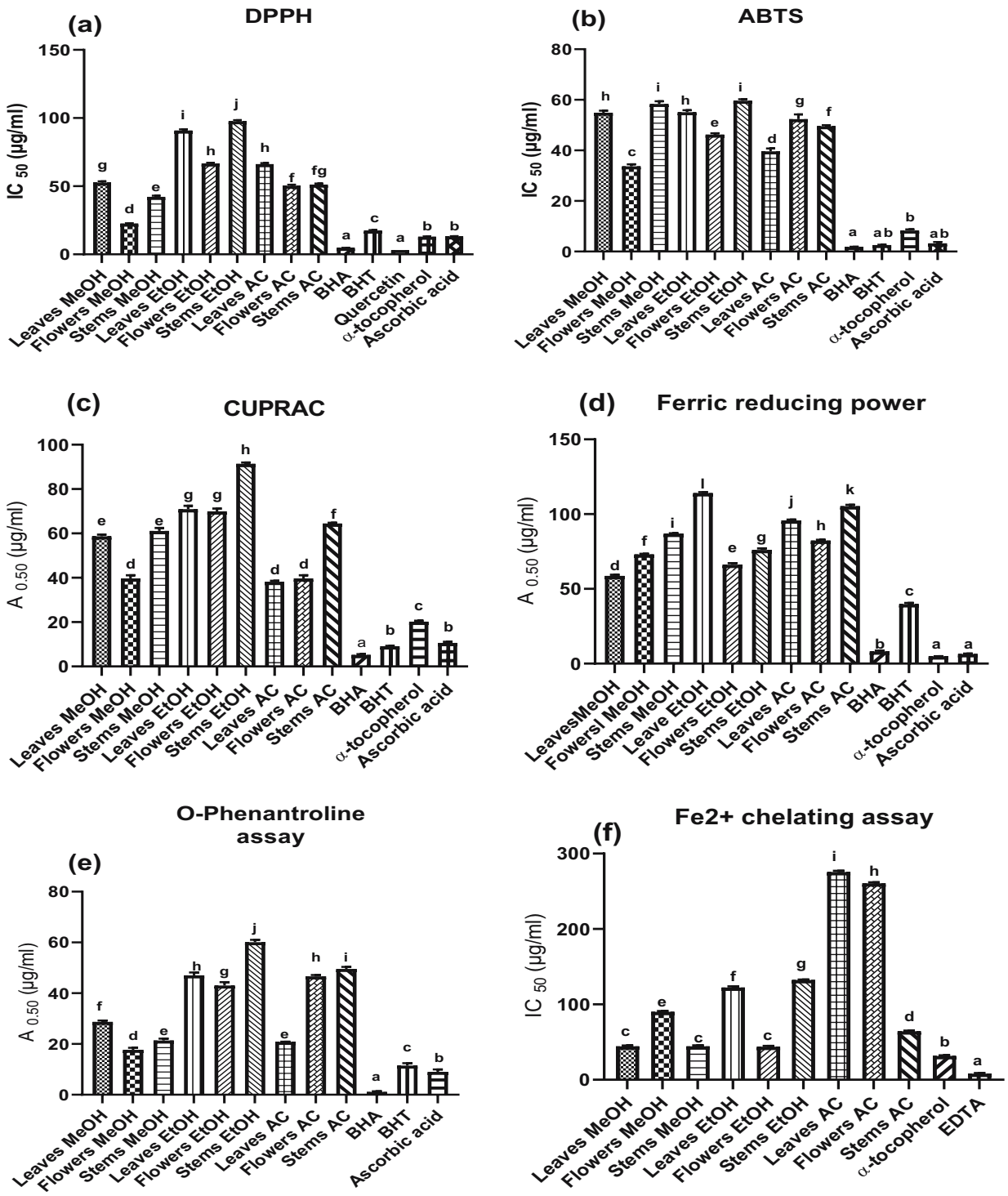

Figure 3: Six different *in vitro* antioxidant activities of Salvia balansae de Noé ex Coss extracts, (a) DPPH radical scavenging assay, (b) ABTS radical scavenging assay (c) FIC activity, (d) FRAP assay, (e) Phenanthroline assay, and (f) CUPRAC.

39) [98], and *Mentha pulegium*, which reveals an SPF value of 36.31 [99]. These findings underscore the substantial sun protection potential inherent in *S. balansae*.

Considering the high phenolic and flavonoid content in the SBCEs, the photo-protective capacity is not surprising since these compounds are considered a good source for agents with sunscreen properties due to their antioxidant capacity and UV absorption. Thus making them valuable compounds for natural defense against UV radiation [100]. In light of these facts, it has also been reported that

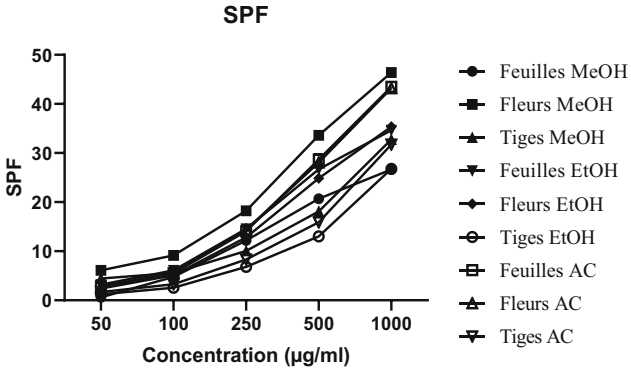

Figure 4: SPF determination from SBCEs.

assessing the most potent extracts for antioxidant activity would be crucial for the advancement of more effective sunscreens [7]. The capacity of these compounds to function as both direct and indirect antioxidants, along with their anti-inflammatory and immunomodulatory properties, offers promising avenues for the advancement of photoprotection [101].

3.4 Chemometric analysis

A data matrix plot, based on all the quantitative and qualitative phytochemical analyses, *in vitro* antioxidant tests, and photo-protective activity, was subjected to a second PCA to examine the relationships among the tested parameters and to determine whether *S. balansae* extracts could be classified according to their phenolic content and biological activities. The PCA, performed on the correlation matrix of the 33 variables, resulted in eight factors, of which the first two accounted for 52% of the variance among the nine SBCEs. As shown in Figure 4, SBCEs are divided into four distinct groups. The first group is represented by the Ac extract of the leaves, located on the positive part of axis one, and characterized by high contents of TPC, ODP, TFC, FOL, protocachuic acid, p-coumaric acid, and naringin. This group is opposed to the second group, formed by the *S. balansae* stem extracts, characterized by high concentrations of quinic acid, cirsiliol, ABTS, CUPRAC, and phenantroline. *S. balansae* flower extracts were placed in the third group due to phytochemicals such as luteolin-7-*o*-glucoside, apegenin-7-*o*-glucoside, caffeic acid, and apegenin. The methanol and ethanol extracts of *S. balansae* leaves constitute the fourth group located in the negative part of Axis Two, characterized by high concentrations of DPPH, SNP, TT, cirsilineol, naringenin, and gallic acid.

UPGMA analysis results (Figure 5) correspond to those obtained through PCA analysis. Two main clusters were obtained, and they were divided into a few sub-clusters at the linkage distance between 400 and 600. Extracts with lower phytochemical compounds and SPF activity are placed in the first cluster (LAc, FAc, and FMeOH), while

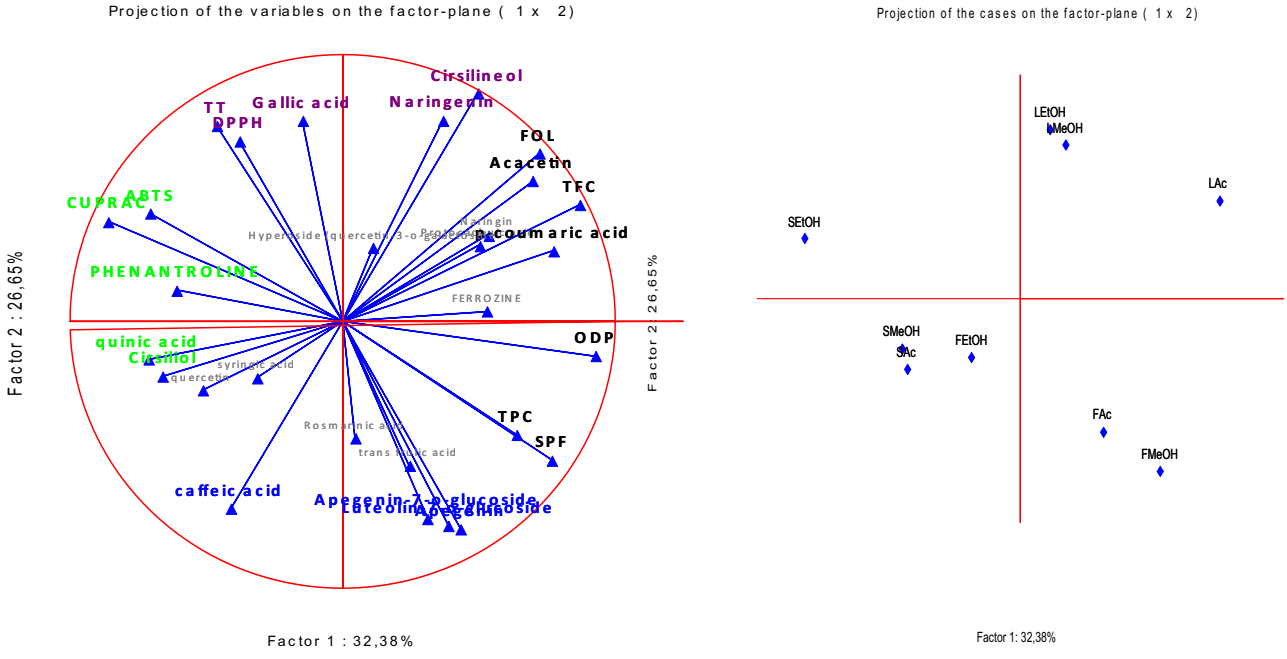

Figure 5: The results of a chemometric analysis using the PCA approach.

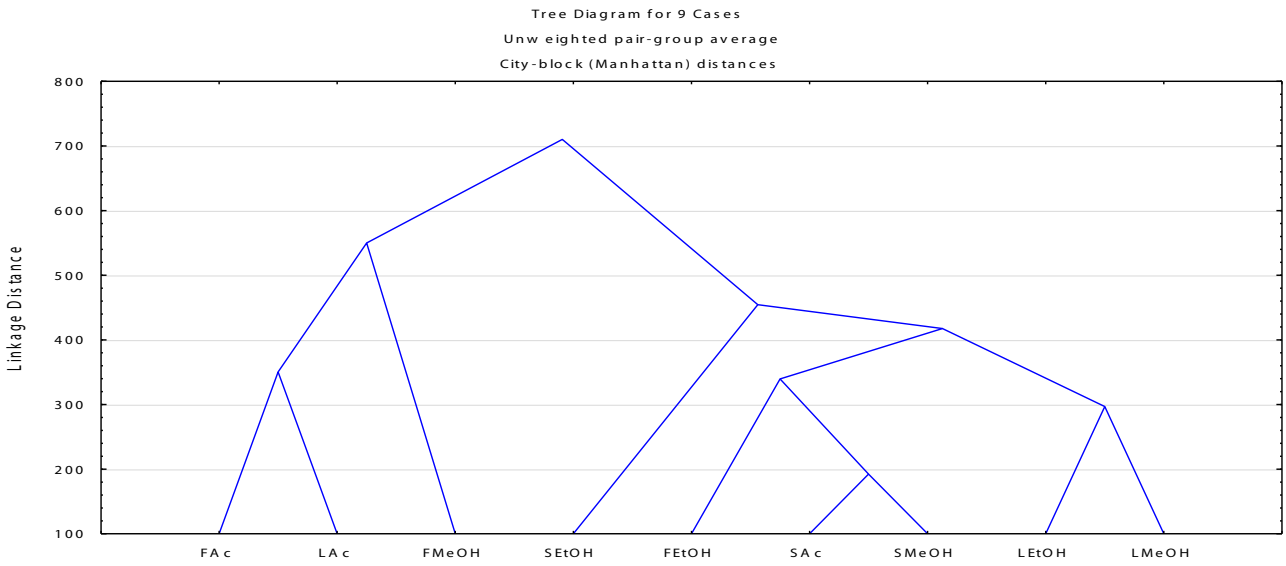

Figure 6: Dendrogram of SBCEs, based on Manhattan Similarity distance.

the extracts with less antioxidant activity (lower values of IC50%) are in the second cluster (Figure 6).

It can be concluded from all these that in the distribution of examined extracts, the most dominant influence is biological activities and phytochemical contents, which both have a very similar influence regarding the positioning of these extracts on the score plot.

Pearson's correlation matrix (Table S1) clearly shows that ODP was negatively and significantly correlated with ABTS (r = −0.748, P = 0.02), CUPRAC (r = −0.876, P = 0.002), and phenanthroline assays (r = −0.718, P = 0.029). Meanwhile, it correlated positively with TPC (r = 0.764, P = 0.017) and TFC (r = 0.761, P = 0.017). TPC was negatively and significantly correlated with ABTS (r = −0.718, P = 0.029), CUPRAC (r = −0.83, P = 0.006), and DPPH assays (r = −0.808, P = 0.008). The DPPH assay was negatively and significantly correlated with rosmarinic acid (r = −0.796, P = 0.01). The SPF assay correlated positively and significantly with apegenin (r = 0.74, P = 0.014) and ODP (r = 0.78, P = 0.013), whereas it correlated negatively with CUPRAC (r = −0.82, P = 0.07) and ABTS (r = −0.78, P = 0.013). The negative correlation is mainly related to the inverse relationship between $IC_{50}/A_{0.5}$ values and antioxidant capacity, suggesting that among the various antioxidant components present in different extracts of *Salvia balansae*, TPC and ODP appear to have the most significant impact on the observed antioxidant capacity. These results align with previous studies conducted on different plant species [54,70], which have also highlighted the significant influence of TPC on the antioxidant potential of various plant extracts.

4 Conclusions

This study is the first to thoroughly understand the phytochemicals and antioxidant activities of *S. balansae* harvested from the Aures Mountains, which is endemic to this region, using chemo-metric analysis. LC-MS analysis showed the prevalence of quinic acid, luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside, cirsiliol, and rosmarinic acid in *S. balansae* extracts, and confirmed the distinction of this taxon to those located in the Mostaganem region. The PCA analyses allowed the recognition of three distinct extract types based on this composition: the flower extracts, characterized by high concentrations of luteolin-7-*o*-glucoside and apigenin-7-*o*-glucoside; the stem extracts, which are characterized by a high content of quinic acid and cirsiliol, and finally, the leaf extracts, characterized by the presence of cirsilineol, naringenin, protocatechuic acid, p-coumaric acid, and acacetin. The antioxidant and photo-protective activities were investigated using *in vitro* assays, and the polar extracts showed significant antioxidant activity in direct proportion to the higher concentrations of total phenolics and ODP content. The relationship between the phytochemicals and biological activities of the extracts was determined by PCA, UPGMA, and correlation analysis. Ultimately, this work provides a thorough examination that reveals the importance of *S. balansae* as a reservoir of natural phytochemicals for the development of phytopharmaceuticals, specifically targeting diseases associated with oxidative stress.

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Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: All data generated or analyzed during this study are included in this published article and its supplementary information files.

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