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Original Article

Comprehensive phenolic profiling and *in vitro* bioactivities of *Salvia yangii* (B.T. Drew) extracts using gas chromatography–flame ionization detection/mass spectrometry and ultra-high-performance liquid chromatography–high-resolution mass spectrometry

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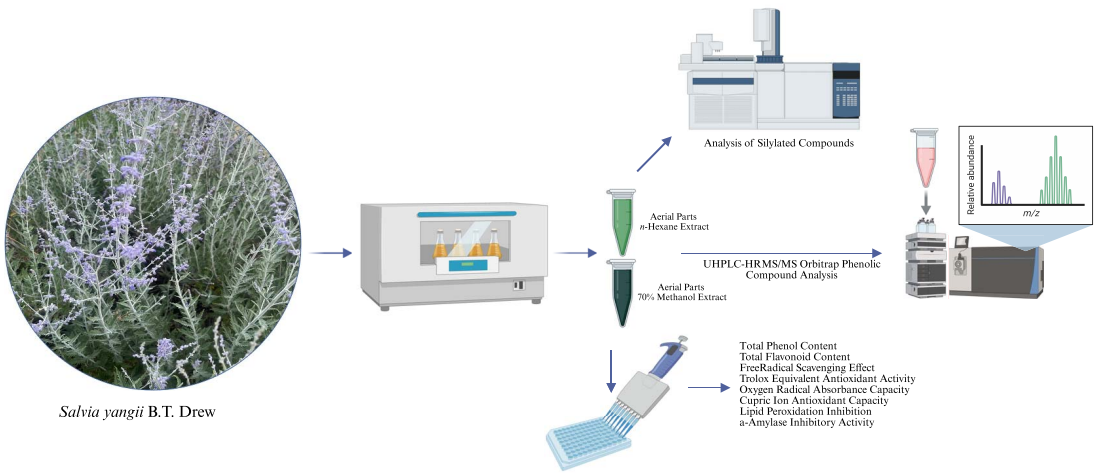
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Abstract

Salvia yangii (B.T. Drew) has attracted attention for its chemical diversity and biological potential. In this study, the aerial parts were extracted using *n*-hexane and aqueous methanol (70%) extract to obtain apolar and polar extracts, respectively. A silylation-based GC–MS/flame ionization detection analysis identified carnosic acid and rosmarinic acid as the major constituents of the *n*-hexane and aqueous methanol (70%) extracts. Ultra-HPLC–high-resolution MS/MS revealed additional phenolic compounds, including vanillin, caffeic acid, hesperidin, and isosakuranetin. Remarkably, 3-hydroxytyrosol and isosakuranetin were reported for the first time in this species. The *n*-hexane extract demonstrated a higher antioxidant capacity in 2,2-diphenyl-1-picrylhydrazyl, β-carotene bleaching, and α-amylase inhibition assays, whereas the methanolic extract exhibited superior results in the oxygen radical absorbance capacity, Trolox equivalent antioxidant capacity, and cupric-reducing antioxidant capacity tests. These findings highlight the distinct phytochemical profiles and biological activities of *S. yangii* extracts, suggesting their potential application as natural antioxidants and functional food ingredients.

Keywords *Salvia yangii*, *Perovskia* atriplicifolia, 3-hydroxytyrosol, isosakuranetin, UHPLC–HRMS/MS, GC–MS/FID, silylation

Graphical abstract



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Introduction

Salvia yangii B.T. Drew, formerly known as *Perovskia atriplicifolia* Benth, is an aromatic perennial plant from the Lamiaceae family that grows in the mountains of Central and Southwest Asia (Drew et al., 2017). Locals often call it Russian sage (Moallem & Niapour, 2008) due to its bright bluish-purple flowers, nice smell, and can grow in a variety of conditions (Öğüt & Özek, 2025). The plant has become more popular because of its chemical richness and possible health benefits, in addition to its value as a garden plant (Gostin & Popescu, 2025). *Salvia* and *Perovskia* species have been used in traditional medicine to treat a variety of conditions, including rheumatism, fever, and skin inflammation, as well as to improve overall health. These ethnobotanical uses point to the existence of compounds that could be useful for medicine (Ahmad et al., 2015). Prior phytochemical investigations have validated that species within this group encompass various classes of bioactive compounds, notably phenolic acids, flavonoids, and terpenoids (Afshari et al., 2024). These metabolites are well-known for their antioxidant, antimicrobial, and anti-inflammatory properties, and they have recently garnered interest as natural alternatives for food preservation and functional food development (Miroliaei et al., 2017). In the literature, *S. yangii* has been reported to contain compounds including rosmarinic acid and carnosic acid (Kozłowska et al., 2022). Still, there is not plenty of data available right now about its detailed phenolic composition and the biological activities that go along with it. To fully understand its chemical diversity and possible uses, we need thorough studies that use both qualitative and quantitative methods. It is crucial to investigate these plants, as phenolic compounds and enzyme inhibitors derived from natural sources are gaining significance in food science and technology for their contributions to reducing oxidative stress and controlling carbohydrate metabolism.

This study was conducted to characterise, for the first time, the chemical composition and biological potential of *S. yangii* extracts derived from plants cultivated in Türkiye. Phenolic compounds were qualitatively and quantitatively analysed using a UHPLC–MS/MS method, and the chemical profiles of the silylated derivatives were elucidated by GC–MS/flame ionization detection (FID) analysis. Considering that phenolic compounds may be associated with antioxidant and α-amylase inhibitory activities, the antioxidant potential was assessed using cupric-reducing antioxidant capacity (CUPRAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), and β-carotene bleaching assays, together with the α-amylase inhibition test for *in vitro* antidiabetic evalution. The results aim to enhance the phytochemical knowledge of *S. yangii* and underscore its potential as a valuable natural source of bioactive compounds for use in nutraceutical or food formulations.

Materials and methods

Chemicals

The following chemicals were purchased: glacial acetic acid, formic acid, *n*-hexane, methanol, ethanol, dimethyl sulfoxide (DMSO), N,O-bis (trimethylsilyl) trifluoroacetamide (BTSFA, with 1% trimethylchlorosilane (TMCS)), and hydrochloric acid from Sigma-Aldrich (USA); anisaldehyde, 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), butylated hydroxyanisole (BHA), DPPH, Folin & Ciocalteu's phenol reagent (FCR), aluminium chloride (reagent grade, 98%), fluorescein, linoleic acid, gallic acid, and quercetin from Merck (Germany); natural product reagent, polyethylene glycol 400, potassium iodide, potassium dihydrogen phosphate, potassium hydroxide, potassium carbonate (≥99.5%, ACS reagent), boron trifluoride reagent (BF_3), and Tween 20 from Merck (Germany). Additionally, β-carotene (≥97.0%, UV) was obtained from Sigma-Aldrich (USA), and the C_8–C_{40} *n*-alkane standard solution was obtained from Fluka (Buchs, Switzerland).

Instruments

An Agilent 5975 GC–mass selective detector (MSD) system (Agilent Technologies, Santa Clara, CA, USA) was used for the GC–MS analysis. Absorbance values were recorded using a Microplate Reader ELISA system (Biotek Powerwave XS, USA). Samples were pipetted into microplate wells using an Eppendorf® Xplorer® (10–300 μl) 12-channel pipettor. A 96-deep well round-bottom polypropylene plate (2.2 ml volume) and a 96-well flat-bottom white polystyrene microplate (nonsterile; Greiner, Germany) were purchased from Sigma-Aldrich. For the SPME procedure of the volatiles, a manual SPME holder (57330-U, SUPELCO, Bellefonte, PA, USA) and a blue-type PDMS-DVB fibre (65 μm) were utilised. UHPLC–HRMS/MS analyses were performed using a Dionex Ultimate 3000 RS UHPLC system coupled to a quadrupole-Orbitrap QExactive hybrid mass spectrometer (Thermo Fisher Scientific, USA).

Plant material

Aerial parts of *S. yangii* were collected from the village of Günalan, Ankara. After collection, the plant material was air-dried in the shade at room temperature. The identification of the plant was performed by Dr Koray Yaylacı (Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Botany). Voucher specimens of *S. yangii* were prepared, and the herbarium specimen (ESSE 16364) was registered at the Herbarium of the Faculty of Pharmacy at Anadolu University.

Extraction of plant material

The powdered aerial parts of *S. yangii* (80 g) were first extracted with *n*-hexane by maceration under continuous shaking for 24 hr at room temperature. This process was repeated seven times until the colour of the solvent had significantly faded. The combined supernatants were filtered using Whatman filter paper, and the organic solvent was evaporated under reduced pressure to obtain the *n*-hexane extract. Subsequently, the plant material was extracted with aqueous methanol (70%) (24 hr × 2), followed by microwave-assisted extraction to enhance the extraction efficiency. Under fixed-power conditions, the extraction was carried out using 1,200 ml of solvent, with irradiation at 500 W for 3 min followed by 200 W for 30 min. Methanol was evaporated from the filtrates under reduced pressure to obtain the dry extracts, and the aqueous phase from the aqueous methanol (70%) extract was dried by lyophilisation. The dried extracts were kept in amber vials at 4 °C until further analysis.

Preparation of silyl derivatives of extracts

The dry extracts obtained from the aerial parts of *S. yangii* were initially subjected to silylation for chemical composition analysis, followed by GC–MS/FID examination. For the derivatisation process,

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2.0 mg of each extract was treated with 80 μl of pyridine and 80 μl of BTSFA reagent containing 1% TMCS. The mixture was then heated at 100 °C in a sand bath for 1 hr. Subsequently, 1.0 μl of the silylated sample was directly injected into the GC–FID and GC–MS systems for analysis (Isidorov, 2020).

GC–MS and GC–flame ionisation detection analysis

The *n*-hexane and aqueous methanol (70%) extracts of *S. yangii* were silylated for GC-FID/MS chemical composition analysis. To conduct the experiment, the dry extract was dissolved in pyridine, and 80 μl of BSTFA containing 1% TMCS was added. An hour was spent vortexing and heating the mixture in a 100 °C sand bath. After cooling, 1.0 μl of the silylated solution was loaded into the GC−FID/MS. An Agilent 7890A gas chromatograph and a 5975C inert MSD with a triple-axis detector were used to analyse *S. yangii* aerial part silylated derivatives extracted in *n*-hexane and aqueous methanol (70%). Chromatographic separation was carried out on an HP-5 FSC column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, Wilmington, DE, USA) using helium as the carrier gas at 0.8 ml/min. After increasing from 100 to 320 °C at 3 °C per min, the oven was held at 320 °C for 16.67 min. Splitless injections were used. The injector and FID were heated to 250 and 300 °C. Mass spectra were taken at 70 eV using electron impact mode for *m/z* 35–1,050. Retention durations and mass spectral data were compared to chemical identification standards and the literature. Identification was done using the Wiley GC/MS Library (Wiley, NY, USA) and the "Special Özek Silyl Derivative Library" for silylated substances. Quantifying chemicals using FID chromatogram relative peak areas (Çetin et al., 2025; Isidorov, 2020).

UHPLC–HRMS/MS orbitrap phenolic compounds analysis

Phenolic compounds were examined with a Thermo Scientific LC system, comprising a binary UHPLC Dionex Ultimate 3000 RS, linked to a quadrupole-Orbitrap QExactive hybrid MS (ThermoFisher Scientific, USA) equipped with a HESI ionisation probe. The Xcalibur software was carried out instrument operation and data collection. Separation was performed utilising an Acquity BEH C18 column (1.7 μm particle size, 100 × 2.1 mm) (waters) at 40 °C with a flow rate of 0.5 ml/min. A binary gradient comprising (A) water and (B) methanol, each containing 0.1% formic acid, was employed with the subsequent elution profile: 5% B (1 min), a linear gradient to 100% B (9 min), 100% B (2 min), and concluding with 5% B (3 min). The injection volume was 5 μl. A data-dependent acquisition procedure (Top5) was employed in negative mode with a resolution of 70,000 and 17,500 at *m/z* 200 full width at half maximum (FWHM) for the full scan and product ion scan, respectively. The parameters for the HESI source were as follows: spray voltage, −3.0 kV; S-lens RF level, 50; capillary temperature, 320 °C; sheath and auxiliary gas flow, 60 and 25 (arbitrary units); and probe heater temperature, 400 °C. The Trace Finder 5.1 programme was utilised for data processing. Identification was achieved by comparing retention times, exact masses of pseudomolecular ions ($[M-H]^-$), and characteristic fragment ions with those of reference standards and literature data. A maximum mass error of ±5 ppm was accepted, and isotopic pattern matching scores higher than 80% were considered as confirmation criteria. Additionally, a phenolic compound database containing 87 reference compounds, implemented as a commercially available library within the TraceFinder software (Thermo Fisher Scientific), was used to support compound annotation. For quantitative analysis, commercially available phenolic standards were used. A mixed standard solution was prepared and serially diluted to obtain 12 concentration levels. Calibration curves were constructed for each compound based on peak areas, and linearity was evaluated. Quantification of phenolic compounds in the extracts was performed using external calibration. The results were expressed as mg/g of dry extract. All analyses were performed in duplicate (Álvarez-Fernández et al., 2015; Öğüt et al., 2026a).

Determination of total phenolic and flavonoid contents

The extracts' total phenolic content was evaluated as gallic acid equivalent (GAE) using Folin–Ciocalteu reagent (FCR) per singleton (Vl, 1999). Extract and gallic acid stock solutions were made with methanol. The experiment involved mixing 20 μl of sample solution, 1,560 μl of ultrapure water, and 100 μl of FCR in 96 deep wells using a 12-channel micropipette. After 1–8 min of incubation, add 300 μl of 20% sodium carbonate solution and mix again. The mixture was then incubated for 2 hr at 25 °C in the dark. After adding 300 μl of the mixture into a 96-well microplate, absorbance values at 760 nm were compared to a 5-point gallic acid calibration curve (0.01–1.0 mg/ml). The experiment was performed in triplicate. The calibration curve was obtained for gallic acid ($y = 0.8421x + 0.077$, $r^2 = 0.9993$). We calculated the mean content value with +/−*SE*. The results were calculated as mg GAE/$g_{extract}$.

In the previous method (Öğüt et al., 2026a), the extracts' total flavonoid concentration was evaluated as quercetin equivalent (QE) using $AlCl_3$ reagent. A 12-channel Eppendorf Xplorer micropipettor was used to mix 80 μl of the extract or quercetin, 80 μl of $AlCl_3$, and 1840 μl of pure ethanol in 96 deep wells. For the blank samples, 10 μl of 99.8% acetic acid substituted aluminium chloride reagent. Post-40 min dark incubation, 300 μl of the mixture was transferred to a 96-well microplate, and the absorbance was read at 415 nm with a microplate reader. The total flavonoids were calculated bu using the quercetin calibration curve. The five-point calibration curve ranged from 0.01 to 1.0 mg/ml. The r^2 of quercetin's calibration curve for quercetin was obtained ($y = 1.857x + 0.0088$) and r^2 was 0.9996. Average content had a *SE*. The reported value was calculated as mg QE/$g_{extract}$.

Free radical scavenging effect (DPPH)

The free radical scavenging activity of the extracts was evaluated using a modified DPPH assay in accordance with the approach of Brand-Williams et al. (1999). Extract and reference samples were dissolved in methanol containing 10% DMSO. The samples were combined with a DPPH solution in a 96-well microplate and incubated in the dark for 30 min. Absorbance was measured at 517 nm, with gallic acid used as the reference antioxidant. The experiments were performed in triplicate. The percentages of radical scavenging and IC_{50} values were derived from the concentration–response curves. *SigmaPlot* was utilised to analyse the data (Version 15.0).

Trolox equivalent antioxidant capacity (TEAC) assay

The antioxidant capacity of the extracts was determined using the $ABTS^{+\bullet}$ radical scavenging assay as previously described (28). The

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$ABTS^{+\bullet}$ working solution was prepared from ABTS and potassium persulfate and adjusted to an absorbance of 0.700–0.800 at 734 nm. Briefly, 10 μL of sample or Trolox standard solution was mixed with 990 μL of $ABTS^{+\bullet}$ solution and incubated in the dark at room temperature for 30 min. Absorbance was measured at 734 nm using a microplate reader. Antioxidant activity was expressed as Trolox equivalent antioxidant capacity (TEAC, μmol TE/gextract) based on the Trolox calibration curve. All analyses were performed in quadruplicate.

Oxygen radical absorbance capacity (ORAC) test

The procedure, with few modifications, was based on a previously published method (Gillespie et al., 2007). Each reaction mixture consisted of 50 μl of sample or Trolox standard, 100 μl of fluorescein solution (45 nM), and 50 μl of AAPH solution (15 mM). Fluorescence was recorded every minute for 60 min using an excitation wavelength of 485 nm and an emission wavelength of 528 nm. Measurements were performed in quadruplicate using a multi-mode microplate reader (Synergy HT, Biotek, USA). Trolox was used as a calibration standard within the concentration range of 0.5–9.5 μM. ORAC values were determined by calculating the difference between the areas under the fluorescein decay curves of the blank and the sample. The results were expressed as mmol Trolox equivalents (TE) per gram of extract.

Cupric-reducing antioxidant capacity (CUPRAC) test

The cupric ion-reducing capacity of the extracts was assessed using the CUPRAC method, with slight modidfications (Apak et al. (2008) with slight modifications. Extract solutions were prepared in a 10% DMSO–methanol mixture. Solutions of $CuCl_2$ (1.0×10^{-2} M) and ammonium acetate buffer (1.0 M, pH 7.0) were prepared in ultrapure water, and a neocuproine (Nc) solution (7.5×10^{-3} M) was prepared in pure ethanol. Each well contained 55 μl of sample solution (extract or standard), 50 μl of $CuCl_2$ solution, 50 μl of neocuproine solution, and 50 μl of ammonium acetate buffer. For the control wells, the 10% DMSO–methanol mixture was used instead of the sample solution. After incubation for 30 min at 25 °C, the absorbance was measured at 450 nm using a Biotek Powerwave XS ELISA microplate reader. Trolox equivalents were calculated using the standard calibration curve: $y = 1.069x + 0.1341$ ($r^2 = 0.9986$), where y represents absorbance and x indicates the cupric reducing capacity in TE. The results were expressed as mM presented as $M \pm SD$.

Lipid peroxidation inhibition (β-carotene bleaching) test

The antioxidant capacity of the extracts was assessed using a modified β-carotene bleaching test (Baderschneider et al., 1999). The emulsion was prepared using β-carotene, linoleic acid, and Tween-20 following the evaporation of chloroform under reduced pressure. Oxygenated water was subsequently incorporated to achieve a stable emulsion, which was freshly prepared before each analysis. Butylated hydroxyanisole was used as the benchmark antioxidant. In a 96-well plate, the sample or reference solutions were combined with the emulsion, and the absorbance was measured at 492 nm for 105 min at 50 °C. The antioxidant activity was quantified as the percentage of β-carotene discoloration inhibition compared to the control.

Table 1 Yields of *S. yangii* extracts.

Extract type	Amounts of extract (g)	Yield[a] (%)
n-Hexane extract	5.42	6.78
Aqueous methanol (70%) extract	13.17	16.47

[a]Yield was calculated based on air dried plant weight.

α-Amylase inhibition test

The α-amylase inhibitory activity was assessed with a colorimetric I_2/KI technique with slight adjustments (Zengin et al., 2014). Acarbose was employed as the reference inhibitor. Extracts were produced in methanol containing 10% DMSO. In a 96-well plate, equal amounts of the sample and enzyme solutions were incubated at 37 °C, after which the starch substrate was added. Following a subsequent incubation, the reaction was stopped with HCl and subjected to I_2/KI reagent processing. The absorbance was measured at 630 nm, and the inhibition was determined in relation to the control wells.

Results and discussion

Yields obtained

Within the scope of this investigation, the bioactivity and profile analyses of *S. yangii* helped to fill existing gaps and provided comparative insights into the literature. The aerial parts of *S. yangii* were extracted using *n*-hexane and aqueous methanol (70%). The yields of the prepared extracts are presented in Table 1.

The extraction yields observed in this study were likely influenced by multiple factors, including solvent polarity, extraction technique, plant organ, and species-specific phytochemical composition (Ng et al., 2020; Tourabi et al., 2025; Wakeel et al., 2019). In the genus *Salvia*, extract yields have been reported over a broad range depending on the solvent system and methodology employed. For instance, values of ~25% for *S. officinalis* (Fatma Ebru et al., 2017), 12%–14% for *S. amplexicaulis* flowers (Alimpić et al., 2014), and 3%–15.7% for *S. nemorosa* have been described (Ivanov et al., 2022). In contrast, the lower yield of the *n*-hexane extract is in line with the more limited recovery of predominantly lipophilic compounds (Kartika et al., 2018).

The aqueous methanol (70%) extract was additionally subjected to microwave-assisted extraction, whereas the *n*-hexane extract was obtained solely by repeated maceration. Considering that nonpolar solvents such as *n*-hexane exhibit a low microwave absorption capacity, this approach was considered technically appropriate (Bagade & Patil, 2019; Lefebvre et al., 2020). Nevertheless, the combined influence of solvent polarity and the extraction procedure should be taken into account when interpreting the differences in extraction yield and composition. Accordingly, the relatively higher yield obtained for the aqueous methanol extract of *S. yangii* may reflect not only the ability of the hydroalcoholic solvent to recover polar constituents more efficiently, but also the enhanced extraction efficiency associated with microwave-assisted extraction when applied to polar solvents (Amarni and Kadi, 2010; Irakli et al., 2023; Kaderides et al., 2019; Turan et al., 2024). Overall, these findings indicate that the extraction yield in *Salvia* species is governed by a combination of solvent polarity and extraction conditions, which

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Table 2 GC-FID/MS analysis results of the silylated extracts obtained from the aerial parts of *S. yangii.*

No.	RRI	Compound	*n*-Hexane extract (%)	Aqueous methanol (70%) extract (%)
1	1506	Malic acid – 3TMS	–	1.27 ± 0.07
2	1843	α-Fructofuranose – 5TMS	–	1.19 ± 0.04
3	1852	β-D-Fructofuranose – 5TMS (*isomer-2*)	–	1.86 ± 0.02
4	1898	Quinic acid – 5TMS	–	3.38 ± 0.05
5	1929	α-Glucopyranose – 5TMS	–	0.99 ± 0.01
6	2023	β-Glucopyranose – 5TMS	–	2.61 ± 0.11
7	2046	Gluconic acid – 6TMS	–	1.12 ± 0.03
8	2093	Catechollactate – 3TMS	–	3.98 ± 0.06
9	2510	Rosmaricin – 2TMS	0.73 ± 0.00	–
10	2151	(*E*)-Caffeic acid – 3TMS	–	4.42 ± 0.03
11	2591	Sucrose derivative	–	2.49 ± 0.05
12	2595	Carnosic acid – 3TMS	26.31 ± 0.17	-
13	2654	Sucrose derivative	–	3.21 ± 0.07
14	2666	Carnosol – 2TMS	4.44 ± 0.03	0.65 ± 0.02
15	2713	Sucrose – 8TMS	–	4.41 ± 0.03
16	2717	Sucrose derivative	–	3.19 ± 0.12
17	2752	Rosmanol – 3TMS	1.24 ± 0.01	–
18	2900	Nonacosane	0.71 ± 0.05	–
19	3100	Hentriacontane	2.88 ± 0.11	–
20	3159	α-Tocopherol – TMS	0.59 ± 0.01	–
21	3200	Dotriacontane	0.60 ± 0.02	–
22	3300	Tritriacontane	3.47 ± 0.02	–
23	3368	β-Sitosterol – TMS	1.78 ± 0.01	–
24	3469	Rosmarinic acid – 5TMS	–	22.12 ± 0.04
25	3602	Oleanolic acid – 2TMS	9.75 ± 0.08	4.50 ± 0.05
26	3613	Betulinic acid – 2TMS	6.00 ± 0.13	–
27	3647	Ursolic acid – 2TMS	6.27 ± 0.02	8.76 ± 0.03
28	3680	Betulonic acid – 2TMS	2.06 ± 0.12	3.18 ± 0.04

Note. RRI = relative retention indices calculated against *n*-alkanes for apolar column.

supports the observed differences between the *n*-hexane and aqueous methanol (70%) extracts in this study.

Analysis of silylated compounds

The compounds identified in the silylated derivatives of *S. yangii* extracts were characterised using GC–MS/FID techniques. The identified compounds are listed in Table 2, and the results are expressed as mean values ± *SD* from three independent replicates.

Silylation is applied to render nonvolatile or polar and thermally unstable compounds volatile and thermally stable, thereby enabling their analysis by the GC technique. Through this process, the identification of the compounds was made possible (Öğüt et al., 2026b). Although silylation procedures have been widely used for various plant species, studies investigating the silylated derivatives of *Salvia* or *Perovskia* species are quite limited. In the silylated *n*-hexane extract, carnosic acid – 3TMS was identified as the major compound (26.31 ± 0.17%). This observation is consistent with the lipophilic nature of carnosic acid, which is a well-known phenolic abietane-type diterpenoid commonly reported in *Salvia* species (*S. officinalis*, *S. fruticosa*, *S. rosmarinus*, *S. canariensis*) and is preferentially extracted using nonpolar solvents (Castañeta et al., 2022; Jakovljević et al., 2021; Kallimanis et al., 2024; López-Cabeza et al., 2024; Šulniūtė et al., 2017). The relatively high abundance of carnosic acid in this extract suggests that the *n*-hexane extract may represent a potential source of bioactive compounds with antioxidant and enzyme inhibitory properties (Bhadouria et al., 2024; Iorio et al., 2022). In addition to these activities, carnosic acid has been reported to exhibit various pharmacological effects, including cytoprotective, anti-inflammatory, anticancer, and antidiabetic activities (Banerjee et al., 2025; Singh et al., 2025; Mirza et al., 2023; Wu et al., 2025; Yana et al., 2025). Therefore, the observed biological activities of the *n*-hexane extract may be at least partially attributed to the presence of this compound. In contrast, the aqueous methanol (70%) extract was dominated by rosmarinic acid – 5TMS (22.12 ± 0.04%), which can be attributed to its polar structure and high solubility in hydroalcoholic solvents (Caleja et al., 2017; Li et al., 2017). Rosmarinic acid is a well-known phenolic compound widely reported for its strong antioxidant activity, which is considered a key contributor to its biological effects (Guan et al., 2022; Leong et al., 2025). In addition, it has been associated with anti-inflammatory, antimicrobial, and enzyme inhibitory properties, including α-amylase inhibition (Konstantinou et al., 2023; McCue & Shetty, 2004; Noor et al., 2022). The predominance of rosmarinic acid in the aqueous methanol (70%) extract suggests that this extract may represent a rich source of bioactive compounds contributing to the observed antioxidant and enzyme inhibitory activities. Therefore, the biological activity profile of the aqueous methanol extract may be at least partially attributed to the presence of rosmarinic acid (Moreno et al., 2006; Nie et al., 2019).

It should be noted that rosmarinic acid is widely recognised as a characteristic phenolic compound of the *Salvia* genus and has been predominantly identified and quantified using HPLC-based analytical techniques (Bardakçi et al., 2011; Ghanadian et al., 2017). In contrast, the present study demonstrates that rosmarinic acid can also be

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successfully detected in its silylated form using GC–FID/MS analysis, highlighting the applicability of silylation-based GC techniques as a complementary approach for the analysis of polar phenolic compounds. These findings are consistent with previous studies on *Salvia* species, where carnosic acid derivatives are typically associated with nonpolar extracts, whereas rosmarinic acid is predominantly detected in polar extracts. This distinct distribution of compounds between the extracts highlights the strong influence of solvent polarity on phytochemical composition and, consequently, on biological activity profiles.

UHPLC–HRMS/MS orbitrap phenolic compounds analysis

Initially, qualitative UHPLC–HRMS/MS analysis was performed to identify the phenolic compounds present in each extract. A total of 13 phenolic compounds were detected in the *n*-hexane extract, whereas 27 compounds were identified in the aqueous methanol (70%) extract. Quantitative analysis revealed differences in the concentrations of these compounds between the extracts, as presented in Tables 3 and 4.

In the aqueous methanol (70%) extract, qualitative analysis identified a broader range of phenolic compounds. Quantitative analysis revealed that hesperidin (211.54 ± 10.05 mg/$g_{extract}$) was the major constituent, followed by caffeic acid (114.98 ± 3.37 mg/$g_{extract}$) and isosakuranetin (77.47 ± 0.17 mg/$g_{extract}$). When expressed on a plant basis, taking into account the extraction yield of the aqueous methanol (70%) extract (16.47%), the hesperidin content corresponds to approximately 34.84 mg/$g_{dried\ plant\ material}$. In contrast, qualitative analysis of the *n*-hexane extract revealed the presence of several phenolic compounds. Subsequently, phenolic compounds were quantified, with vanillin (0.866 ± 0.024 mg/$g_{extract}$) being the most abundant, followed by isosakuranetin (0.827 ± 0.013 mg/$g_{extract}$) and luteolin (0.181 ± 0.003 mg/$g_{extract}$). Although phenolic compounds are generally associated with polar extracts, their presence in the *n*-hexane extract may be attributed to several factors (Alara et al., 2021; Alu'datt et al., 2018; Benito-Román et al., 2020; Gil-Martín et al., 2021; Keddar et al., 2020; Mojzer et al., 2016; Sánchez et al., 2025; Sun & Shahrajabian, 2023). Less polar phenolics or flavonoid aglycones may exhibit partial affinity toward nonpolar solvents (Akomeng & Adusei, 2021; Wijaya et al., 2017). In addition, coextraction with lipophilic matrix components such as waxes, lipids, and terpenoids may facilitate their transfer into the *n*-hexane phase (Ng et al., 2020; Syukur et al., 2023). Sequential extraction processes may also contribute to the carry-over of certain phenolic compounds into the nonpolar fraction (Kotecha & Ram, 2023). These factors, together with the high sensitivity of UHPLC–HRMS/MS, may account for the detection of low-abundance phenolic compounds in the *n*-hexane extract.

In previous studies, caffeic acid derivatives are the predominant phenolic acids in *Salvia* species. Caffeic acid is integral to the biochemistry of the Lamiaceae family (Kamatou et al., 2010). Gallic acid (26.2 ± 0.04 mg/100 g), vanillic acid (14.0 ± 0.01 mg/100 g), caffeic acid (15.4 ± 0.1 mg/100 g), syringic acid (17.9 ± 0.1 mg/100 g), *p*-coumaric acid (72.5 ± 0.3 mg/100 g), ferulic acid (17.0 ± 0.3 mg/100 g), rosmarinic acid (341.1 ± 7.2 mg/100 g), and cinnamic acid (65.4 ± 0.3 mg/100 g) were detected in the methanol extract of *S. abrotanoides* (Ghaderi et al., 2019). In other studies, the methanol/ethanol extracts of *Salvia* species were reported to contain gallic acid (1.294 g/100 g d.m), rosmarinic acid (1.085 g/100 g d.m), luteolin (0.277 g/100 g d.m), rutoside (0.561 g/100 g d.m) (Afshari et al., 2024; Kostić et al., 2015; Grzegorczyk-Karolak & Kiss, 2018; Quradha et al., 2024; Stanković et al., 2020; Uysal et al., 2021). An HPLC study on *S. abrotanoides* and *S. yangii* identified rosmarinic acid and rutin as the major components. In addition, gallic acid, chlorogenic acid, caffeic acid, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid, rosmarinic acid 3-*O*-glucoside, and quercetin were also detected in both plants (Afshari et al., 2024). In another report, a significant concentration of caffeic acid was detected in the *S. yangii* plant (Khodadadi et al., 2023). Moreover, different HPLC analyses showed that there were flavonoids, phenolic diterpenes, various phenolic acids, particularly caffeic acid derivatives (Farhat et al., 2013; Llorent-Martínez et al., 2025).

In this species, 3-hydroxytyrosol and isosakuranetin were reported for the first time in the present study. 3-Hydroxytyrosol is a dopamine metabolite and a natural antioxidant found in olive oil and wine, and it is also endogenously produced in humans (Gallardo-Fernández et al., 2019). Isosakuranetin is a flavonoid known for its diverse biological properties, including antioxidant, anti-inflammatory, antimicrobial, cardioprotective, and potential anticancer activities (Khan & Dangles, 2014). The detection of both compounds in the present study provides new insight into the phytochemical and pharmacological potential of *S. yangii*, suggesting that they may collectively contribute to the plant's bioactivity profile. The presence of and these compounds in *S. yangii* highlights the potential contribution of these bioactive compounds to the plant's pharmacological profile. Given their well-documented antioxidant and protective effects, we further evaluated these metabolites for their potential health benefits, revealing promising implications for enhancing overall wellness and mitigating the risk of chronic diseases. Nevertheless, further studies are warranted to elucidate their underlying mechanisms, explore possible synergistic interactions, and define optimal concentrations for applications in preventive health strategies, including dietary supplementation and functional food development.

Total phenolic, flavonoid contents, and biological activity results

Phenolic compounds significantly contribute to the total antioxidant capacity of natural products. The total phenolic content was determined using the FCR, and the results were expressed as GAE. The total flavonoid content was determined spectrophotometrically using the $AlCl_3$ reagent. Table 5 presents the flavonoid contents of the extracts expressed as QE.

In this study, the aqueous methanol (70%) extract exhibited the highest total phenolic content 874.35 ± 15.66 mg GAE/$g_{extract}$), and the highest flavonoid content was found (150.17 ± 1.76 mg QE/$g_{extract}$). Three *Salvia* species were reported to have high flavonoid contents: *Salvia heirosolymitana* Boiss. (770.85 ± 5.26 mg QE/$g_{dry\ extract}$), *Salvia eigii* Zohary. (520.60 ± 6.24 mg QE/g), and *Salvia viridis* L. (311.36 ± 4.41 mg QE/g) (Al-Jaber et al., 2020). The methanolic extracts of *Salvia cerino-pruinosa* Rech. f. var. *cerino-pruinosa*, *Sarga trichoclada* Benth., *Sitilias multicaulis* Vahl., *Salvia palestina* Benth., *Salvia virgata* Jacq., *Salvia divaricata* Montbret & Aucher ex Benth., *Salvia candidissima* Vahl, and *Salvia aucheri* Benth. exhibited phenolic contents ranging from 53.18 to 124.15 mg GAE/g. Among them, *S. cerino-pruinosa* (37.00 mg RE/g), *S. divaricata* (35.97 mg RE/g), and *S. palestina* (35.93 mg RE/g) showed the highest total flavonoid contents (Llorent-Martínez et al., 2025).

The total phenolic content obtained in this study was relatively higher than many values reported for *Salvia* species in the literature, whereas the flavonoid content was comparatively lower. This

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Table 3 Identified and quantified phenolic compounds in the *n*-hexane extract of *S. yangii*.

Compound	RT	Mol. formula	Exact mass $(M-H)^-$	*m/z* (Expected)	*m/z* (Apex)	Δmass (ppm)	MS/MS fragments	Ref.	$mg/g_{extract}$
Protocatechuic acid	2.21	$C_7H_6O_4$	153.0193	153.01933	153.01926	0.2803	108.02177; 109.02956	[a]	0.095 ± 0.007
4-Hydroxybenzoic acid	3.14	$C_7H_6O_3$	137.0244	137.02442	137.02432	0.71148	93.03477; 137.02455	[a]	N.d.
4-O-Caffeoylquinic acid	3.87	$C_{16}H_{18}O_9$	353.0878	353.08781	353.0878	0.03095	89.03974; 107.05046; 134.03754; 135.04532; 179.03529	[a]	N.d.
Caffeic acid	4.03	$C_9H_8O_4$	179.0350	179.03498	179.03494	0.20875	107.05046; 134.03754; 135.04532; 179.03529	[a]	0.025 ± 0.003
Vanillic acid	4.05	$C_8H_8O_4$	168.0422	167.03498	167.03488	0.58915	91.01913; 108.02185; 123.04529; 152.01169; 167.03526	(Grieman et al., 2015)	N.d.
Abscisic acid	6.58	$C_{15}H_{20}O_4$	263.1288	263.12888	263.12881	0.24818	122.03745; 153.09235; 203.10788; 204.11575; 219.13942	(The Scripps Research Institute, 2026)	0.252 ± 0.008
Vanillin	4.48	$C_8H_8O_3$	151.040	151.04007	151.03996	0.60893	92.02692; 108.02183; 136.01672; 151.04018	[a]	0.866 ± 0.024
p-Coumaric acid	4.84	$C_9H_8O_3$	163.0400	163.04007	163.04002	0.18975	91.05575; 93.03465; 119.05035	[a]	N.d.
Taxifoline	5.01	$C_{15}H_{12}O_7$	304.05830	303.05103	303.05087	0.51872	57.03462; 125.02453; 151.00378; 175.04005; 285.04086	(The Scripps Research Institute, 2026)	N.d.
Aromadendrin	5.64	$C_{15}H_{12}O_6$	288.0633	287.05611	287.05609	0.06511	65.00342; 83.01392; 125.02453; 177.05547; 259.06116	(Tsugawa et al., 2016)	N.d.
Hyperoside	5.64	$C_{21}H_{20}O_{12}$	464.0955	463.0882	463.08813	0.14087	227.03555; 243.02991; 255.03001; 271.02509; 300.02774	(Lantz et al., 2021)	N.d.
Luteolin	6.87	$C_{15}H_{10}O_6$	286.0404	285.04046	285.04034	0.40612	65.00339; 107.01401; 133.02968; 151.00371; 175.04092	[a]	0.181 ± 0.003

Note. Δmass is the deviation of the observed ion mass from the corresponding calculated monoisotopic mass. Ref. = previous reports of compounds or spectral library matches; N.d. = no determined. [a]Analytes were confirmed by comparison with pure standards. SDs of 0.00 indicate values below the reporting precision (two decimal places).

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Table 4 Qualitatively identified phenolic compounds in the aqueous methanol (70%) extract of *S. yangii*.

Compound	RT	Mol. formula	Exact mass $(M-H)^-$	*m/z* (Expected)	*m/z* (Apex)	Δmass (ppm)	MS/MS fragments	Ref.	mg/g$_{extract}$
Quinic acid	0.52	$C_7H_{12}O_6$	191.05611	191.05611	191.05608	0.1777	85.02957; 93.03463; 109.02955; 127.04015; 173.04565	(Moyo & Madala, 2025)	N.d.
Gallic acid	1.12	$C_7H_6O_5$	169.01425	169.01425	169.01413	0.71214	69.03468; 79.01904; 81.03468; 97.02958; 125.02451	(HighChem LLC, 2026)	N.d.
Protocatechuic acid	2.21	$C_7H_6O_4$	153.0193	153.01933	153.01927	0.38001	65.00338; 81.03468; 91.01903; 108.02185; 109.02962	[a]	34.90 ± 0.31
3-Hydroxytyrosol	2.67	$C_8H_{10}O_3$	93.0704	153.05572	153.05568	0.26578	93.03471; 95.05044; 108.02178; 123.04531	[a]	1.00 ± 0.03
4-Hydroxybenzoic acid	3.16	$C_7H_6O_3$	137.02387	137.02442	137.02431	0.82284	65.0396; 93.03473; 137.02458	[a]	22.00 ± 0.47
Gentisic acid	3.24	$C_7H_6O_4$	154.0266	153.01933	153.01924	0.57945	153.0193; 109.0295; 91.0189; 135.0087; 108.02113; 93.03404; 65.03913	[a]	3.79 ± 0.09
Caffeic acid	4.03	$C_9H_8O_4$	179.03498	179.03498	179.03491	0.3792	89.03984; 107.05035; 134.03749; 135.04535; 179.03522	[a]	114.98 ± 3.37
4-*O*-Caffeoylquinic acid	4.04	$C_{16}H_{18}O_9$	353.08781	353.08781	353.08777	0.11738	93.03468; 135.04538; 173.04575; 179.0352; 191.05644	[a]	72.17 ± 1.88
Chlorogenic acid	4.04	$C_{16}H_{18}O_9$	353.08781	353.08781	353.08777	0.11738	59.01397; 85.02962; 93.03468; 127.0401; 191.05644	[a]	5.90 ± 0.003
p-Coumaric acid	4.81	$C_9H_8O_3$	163.0395	163.04007	163.04007	0.00258	65.03975; 91.05544; 93.03465; 104.02671; 119.05033	[a]	4.10 ± 0.11
Abscisic acid	6.57	$C_{15}H_{20}O_4$	263.12888	263.12888	263.12878	0.36416	122.03738; 153.09239; 203.10831; 204.1154; 219.13914	(The Scripps Research Institute, 2026)	N.d.
Taxifoline	5.02	$C_{15}H_{12}O_7$	303.05830	303.05103	303.05093	0.31731	57.03462; 125.02453; 151.00378; 175.04005; 285.04086	(Yang et al., 2016)	N.d.
Aromadendrin	5.61	$C_{15}H_{12}O_6$	287.05611	287.05611	287.05615	0.14751	65.00339; 83.01398; 125.02453; 177.05595; 259.06143	(Zhao et al., 2020)	N.d.
Hyperoside	5.64	$C_{21}H_{20}O_{12}$	463.08820	463.0882	463.08865	0.97943	227.03516; 243.02997; 255.03017; 271.02512; 300.02777	(Dugay et al., 2025)	N.d.
Naringin	5.69	$C_{27}H_{32}O_{14}$	579.1719	579.17193	579.17139	0.93803	65.00337; 107.01395; 119.05031; 151.00381; 271.06158	[a]	2.06 ± 0.01
Hesperidin	5.93	$C_{28}H_{34}O_{15}$	609.18249	609.18249	609.18329	1.31089	108.02178; 134.03749; 151.00378; 164.01172; 301.07211	[a]	211.54 ± 10.05
Neohesperidin	5.93	$C_{28}H_{34}O_{15}$	609.1824	609.18249	609.18329	1.31089	107.01395; 134.03749; 151.00378; 164.01172; 301.07211	(Tong et al., 2019)	N.d.
Kaempferol-3-*O*-glucoside	6.08	$C_{21}H_{20}O_{11}$	447.09328	447.09328	447.09329	0.02737	183.04532; 211.04048; 227.03529; 255.0302; 284.03287	(March et al., 2006)	N.d.
Quercitrin	6.08	$C_{21}H_{20}O_{11}$	447.0930	447.09328	447.09329	0.02737	151.00371; 255.0302	(Adeniyi et al., 2022)	N.d.
Luteolin-4'-*O*-glucoside	6.08	$C_{21}H_{20}O_{11}$	447.0933	447.09328	447.09329	0.02737	65.00333; 107.01392; 133.02946; 151.00371; 285.04077	(Śliwka-Kaszyńska et al., 2022)	N.d.
Quercetin	6.66	$C_{15}H_{10}O_7$	301.03438	301.03538	301.03525	0.43914	65.0034; 107.01403; 121.02964; 151.00386; 178.99893	(Yu-hang et al., 2009)	N.d.
Naringenin	6.82	$C_{15}H_{12}O_5$	271.06065	271.0612	271.06122	0.06737	65.00339; 83.01398; 107.01394; 119.05033; 151.00385	(Corinti et al., 2023)	N.d.
Luteolin	6.85	$C_{15}H_{10}O_6$	286.0477	285.04046	285.04034	0.29906	65.00338; 107.01396; 133.02968; 151.00377; 175.04025	[a]	2.102 ± 0.004
Kaempferol	7.22	$C_{15}H_{10}O_6$	302.0427	285.04046	285.04044	0.08493	65.0034; 93.03467; 117.03473; 161.04552; 187.04037	(Pavlovic et al., 2013)	N.d.
Apigenin	7.33	$C_{15}H_{10}O_5$	269.04555	269.04555	269.04556	0.04737	65.00336; 107.01398; 117.03473; 149.02452; 151.00377	(Xing & Huan, 2022)	N.d.
Diosmetin	7.37	$C_{16}H_{12}O_6$	299.0561	299.05611	299.05582	0.98092	284.033; 256.038; 151.003; 133.029, 107.014; 65.003	[a]	0.53 ± 0.02
Isosakuranetin	8.04	$C_{16}H_{14}O_5$	285.07685	285.07685	285.07681	0.13069	6.500.337; 108.02185; 136.01672; 151.00392; 164.01178	[a]	77.47 ± 0.17

Note. Δmass is deviation of the observed ion mass from the corresponding calculated monoisotopic mass. Ref. = previous reports of compounds or spectral library matches; N.d. = no determined. [a]Analytes confirmed by comparison with pure standards. SDs of 0.00 indicate values below the reporting precision (two decimal places).

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Table 5 The total phenolic and flavonoid contents in the extracts of *S. yangii*.

Extract type	Total phenol content ($mgGAE/g_{extract}$)	Total flavonoid content ($mgQE/g_{extract}$)
n-Hexane extract	475.10 ± 35.11	30.20 ± 1.25
Aqueous methanol (70%) extract	874.35 ± 15.66	150.17 ± 1.76

difference may be attributed to variations in the chemical composition of the plant, as well as differences in extraction conditions, solvent polarity, and analytical methods (Farhat et al., 2013; Moshari–Nasirkandi et al., 2024; Quradha et al., 2024; Paje et al., 2022).

Antioxidant activity was assessed by a series of *in vitro* assays, including DPPH, CUPRAC, TEAC, ORAC, and β-carotene bleaching. The inhibitory potential of α-amylase was evaluated. Data from CUPRAC and TEAC experiments were presented as TE (mM), whereas ORAC results were expressed as μmol Trolox/$mg_{extract}$ the DPPH, β-carotene bleaching, and α-amylase inhibition data were conveyed as IC_{50} values (mg/ml). All results related to biological activity are displayed in Table 6.

Our results demonstrated that the *n*-hexane extract exhibited the strongest DPPH radical scavenging activity (IC_{50}, 0.04 ± 0.00 mg/ml), β-carotene peroxidation inhibition (IC_{50}, 0.11 ± 0.00 mg/ml), as well as α-amylase enzyme inhibitory effect (IC_{50}, 0.56 ± 0.03 mg/ml). In contrast, the 70% methanol extract exhibited the highest ORAC value (1.62 ± 0.01 μmol Trolox/$mg_{extract}$), TEAC value (2.12 ± 0.01 mM), and CUPRAC value (2.27 ± 0.04 mM).

When examining studies conducted on other species, it has been shown that the methanolic extracts of *S. officinalis* possess the strongest antioxidant capacity, evaluated by DPPH (IC_{50}: 10.08–3.37 μg/ml) and ABTS assays (644.85–766.30 μM TE/mg) (Farhat et al., 2013). *S. heirosolymitana* exhibited the highest DPPH radical scavenging activity (IC_{50}: $0.184 \pm 1.22 \times 10^{-2}$ mg/ml) and demonstrated slightly superior $ABTS^{\bullet+}$ scavenging activity compared to *S. eigii*, with values of $0.176 \pm 1.16 \times 10^{-2}$ mg/ml and 0.183 ± 0.031 mg/ml, respectively (Al-Jaber et al., 2020).

In the literature, there are very few studies investigating the biological activities of *S. yangii*. Additionally, only a limited number of studies have focused on the biological activities of other *Perovskia* species.

Conclusion

This study highlights the chemical distinctiveness of *S. yangii*, integrating findings from both UHPLC–HRMS/MS and GC–MS/FID (silylation) analyses. The GC–MS/FID data revealed carnosic acid − 3TMS as the predominant compound in the *n*-hexane extract, while rosmarinic acid − 5TMS was identified as the major constituent in the aqueous methanol (70%) extract. These metabolites are well-known for their potent antioxidant and protective biological effects, which are consistent with the high antioxidant activity observed in the present study. The identification of isosakuranetin and 3-hydroxytyrosol for the first time in this species further expands its phytochemical profile and emphasises its pharmacological potential.

Overall, the results underscore the chemical diversity and bioactive richness of *S. yangii*, suggesting its potential as a valuable source of natural antioxidants and health-promoting compounds. Further investigations, including *in vivo* and pharmacological assessments, are warranted to confirm the biological relevance and possible applications of these metabolites in functional food and phytomedicine development.

Data availability

Data will be made available on request.

Author contributions

Kübra Ögüt (Conceptualisation [equal], Data curation [equal], Methodology [equal], Resources [equal], Software [equal], Validation [equal], Visualisation [equal], Writing—original draft [equal], Writing—review & editing [equal]), M. C. Garcia-Parrilla (Conceptualisation [equal], Data curation [equal], Formal analysis [equal], Investigation [equal], Methodology [equal]), and Gülmira Özek (Conceptualisation [equal], Methodology [equal], Validation [equal], Writing—review & editing [equal]).

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 6 Biological activity results for *S. yangii*.

	DPPH[a] (IC_{50}, mg/ml)	TEAC[b] (mM)	ORAC[c] (μmol Trolox/$mg_{extract}$)	CUPRAC[c] (mM)	β-Carotene peroxidation inhibition[d] (IC_{50}, mg/ml)	α-Amylase inhibition[d] (IC_{50}, mg/ml)
n-Hexane extract	0.04 ± 0.00	2.01 ± 0.02	1.51 ± 0.01	1.53 ± 0.05	0.11 ± 0.00	0.56 ± 0.03
Aqueous methanol (70%) extract	0.07 ± 0.01	2.12 ± 0.01	1.62 ± 0.01	2.27 ± 0.04	0.76 ± 0.01	0.62 ± 0.00
Standard	0.002 ± 0.000[e]	–	–	–	0.01 ± 0.00[f]	0.019 ± 0.00[g]

[a]%Inh.: % inhibition value determined for extracts at 1 mg/ml. [b]The value calculated for *n*-hexane extract and 70% methanol extract is 0.1 mg/ml. [c]The value was calculated for 1 mg/ml *n*-hexane extract and a 2.5 mg/ml 70% methanol extract. [d]%Inh.: % inhibition value determined for extracts at 1 mg/ml. [e]Standard used for DPPH assay: gallic acid. [f]Standard used for β-carotene peroxidation inhibition assay: butylated hydroxyanisole. [g]Standard used for α-amylase inhibition assay: acarbose.

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Statistical analysis

Data were analyzed using analysis of variance (ANOVA). Results are presented as M ± SD. Statistical significance was determined at $p < .05$. Statistical analyses were performed using Statistica version 15.0.

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