Biochemical Systematics and Ecology 122 (2025) 105028

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Chemical composition, α-amylase inhibition, and antioxidant activities of *Scabiosa hololeuca* Bornm.

Kübra Öğüt [a,*], Gülmira Özek [a], Nilgün Öztürk [a], Ömer Koray Yaylacı [b], Temel Özek [a]

[a] Anadolu University, Department of Pharmacognosy, Faculty of Pharmacy, Eskişehir, 26470, Türkiye
[b] Anadolu University, Department of Pharmaceutical Botany, Faculty of Pharmacy, Eskişehir, 26470, Türkiye

ARTICLE INFO

Keywords:
Scabiosa hololeuca
Lomelosia hololeuca
Caprifoliaceae
Essential oil
Phenolic acids
Derivatization
Antioxidant activity

ABSTRACT

Scabiosa hololeuca Bornm., a plant from the Caprifoliaceae family, grows naturally in the Kütahya-Eskişehir region of Türkiye. This study investigates the chemical composition and biological activities of *S. hololeuca* extracts. The plant was subjected to various extraction methods, including essential oil, fatty acids, *n*-hexane, methanol, and water extracts. The GC-FID/MS technique was employed to analyze the chemical composition of essential oil and fatty acids. In the hydrodistilled essential oil, dodecanoic acid (11.000 ± 0.0015 %) and tetradecanoic acid (10.592 ± 0.033 %) were identified as the major volatile compounds. Volatile compounds in the leaves and flowers were extracted via the MSD-SPME technique, with alloaromadendrene (24.908 ± 0.033 %) as the predominant volatile in the leaves and naphthalene (14.106 ± 0.045 %) in the flowers. Lipophilic compounds, such as methyl nonadecanoate (44.903 ± 0.001 %) and methyl hexadecanoate (41.299 ± 0.011 %), were the main fatty acids in the leaves and flowers, respectively. The antioxidant potential of the extracts was evaluated through various assays, including DPPH• and ABTS+• free radical scavenging, lipid peroxidation inhibition (β-carotene bleaching), and ORAC. The methanol extract exhibited the highest antioxidant activity, with the strongest DPPH• scavenging activity (IC_{50} 0.24 ± 0.01 mg/mL) and significant β-carotene inhibition (IC_{50} 1.3 ± 0.02 mg/mL). The water extract showed the highest ORAC values. However, S. hololeuca extracts did not show effective α-amylase inhibition. The RP-HPLC analysis revealed *o*-coumaric and caffeic acids as major phenolic components in the methanol extract. These findings suggest potential industrial applications of *S. hololeuca* due to its rich chemical profile and antioxidant properties.

1. Introduction

The genus *Scabiosa* L. belongs to Caprifoliaceae family and encompasses approximately 100 species. This genus with its numerous species found throughout the Mediterranean Basin, Asia, and southern Africa, is considered a vast and taxonomically complicated genus (Carlson et al., 2012; Mostafa and Siahkola, 2016).

Scabiosa species are used traditionally to cure a variety of ailments, including ulcers and dermatitis, to control menstruation, and have diuretic effects (Hlila et al., 2018; Mouffouk et al., 2018). Also, current literatures shows that *Scabiosa* genus has anti-diabetic, hepatoprotective, analgesic, anti-inflammatory, antioxidant, antibacterial, anti-melanogenesis, anti-tyrosinase, and anti-parasitic effects, depending on its phenolic content. Previous phytochemical investigations of *Scabiosa* species have resulted with coumarins, flavonoids, iridoids, triterpenes, iridoid glucosides, and monoterpenoid glucoindole alkaloids (Lehbili et al., 2018; Otang-Mbeng and Sagbo, 2020).

Scientists have studied the *Scabiosa* genus, which comprises *Scabiosa* L., *Sixalix Rafinesque*, and *Lomelosia Rafinesque*, with a particular focus on differences in their involucel structures (Albayrak et al., 2024). Moreover, literature indicates that *Scabiosa hololeuca* Bornm., also referred to as *Lomelosia hololeuca* Bornm., is classified within the *Scabiosa* genus (Govaerts et al., 2021).

Although no previous studies have been conducted on *Scabiosa hololeuca* (*Lomelosia hololeuca*) Bornm., the presence of biologically active compounds in other *Scabiosa* species highlights the need for further investigation of this species. This study provides the first comprehensive data on the silyl derivatives, phenolic acids, fatty acids, volatile compounds, antioxidant properties, and α-amylase inhibition activities of *Scabiosa hololeuca*. The aim of this study is to determine the volatile

Abbreviations: Abs, absorbance; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GC-FID, gas chromatography-flame ionization detector; MS, mass spectrometry.

* Corresponding author.
E-mail address: kubraogut@anadolu.edu.tr (K. Öğüt).

https://doi.org/10.1016/j.bse.2025.105028
Received 30 September 2024; Received in revised form 10 April 2025; Accepted 15 April 2025
Available online 20 April 2025
0305-1978/© 2025 Elsevier Ltd. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

composition and fatty and phenolic acids, as well as the antioxidant and α-amylase inhibition activities of *n*-hexan, methanol, and water extracts obtained from the aerial parts of *S. hololeuca*. Given the chemical diversity of *S. hololeuca*, a multifaceted analytical approach was employed to characterize its phytochemical profile comprehensively. The volatile components were investigated through hydrodistillation and SPME techniques, while fatty acid composition was analyzed using GC-MS. Additionally, silylation derivatization was applied to enhance the detectability of non-volatile and semi-volatile compounds, particularly phenolic acids and triterpenoids, allowing for a more in-depth exploration of the plant's bioactive constituents.

Researchers have proven that the SPME analysis represents a green chemical method (Spietelun et al., 2013; Zhou and Pawliszyn, 2024). This method is prompt, easy to understand, and reproducible; it necessitates no solvent usage and is appropriate for the extraction and concentration of various volatile and semi-volatile chemicals from aqueous solutions (Harmon, 2020; Yu et al., 2012). Moreover, SPME techniques require minimal sample quantities, and their integration with gas chromatography and mass spectrometry (GC/MS) enhances their sensitivity. This method has been employed to prevent further plant destruction, as the leaves and flowers of *S. hololeuca*, an endemic species in Türkiye, are deficient in essential oils. In the literature, there are also articles that include both SPME and hydrodistillation studies (Andreica et al., 2024; Benyelles et al., 2014; Mutavski et al., 2024; Özek et al., 2005, 2006, 2008, 2018). Additionally, the silylation method was employed to enhance the volatility and stability of non-volatile and semi-volatile compounds, particularly phenolic acids and triterpenoids, enabling their effective analysis by GC-MS (Rohloff, 2015). This derivatization technique facilitates the identification of thermolabile and hydroxyl-containing compounds by converting them into their more stable trimethylsilyl (TMS) derivatives (Halket et al., 2005; Jorge et al., 2016; Kumar et al., 2024). The combination of silylation and GC-MS has been extensively applied in phytochemical studies to elucidate the structures of bioactive compounds (Kumar et al., 2024; Liang et al., 2024; Zarate et al., 2016).

2. Materials and methods

2.1. Chemicals

The chemicals utilized in this study; *n*-hexane, dimethyl sulfoxide, methanol, ethanol, formic acid, hydrochloric acid, glacial acetic acid, Folin-Ciocalteu reagent (FCR), boron trifluoride (BF_3) reagent, and butylated hydroxyanisole (BHA) were purchased from Sigma-Aldrich (USA). The lipid extraction kit, α-amylase produced from porcine pancreas (Type VI-B, ≥10 units/mg solid), and acarbose were purchased from Sigma (USA). However, the rest of the chemicals utilized in this study were obtained from Merck (Germany). The standard *n*-alkanes C_8–C_{40} were purchased from Fluka in Buchs. The phenolic acids utilized in this investigation, namely caffeic acid, chlorogenic acid, ferulic acid, gallic acid (GA), protocatechuic acid, *p*-hydroxybenzoic acid, syringic acid, and vanillic acid were acquired from Sigma-Aldrich (St. Louis, MO, USA) or Merck (GmbH, Darmstadt, Germany).

2.2. Instruments

An Agilent 5975 GC-MSD system manufactured by Agilent Technologies (Santa Clara, CA, USA) was used to perform GC-MS analyses. The SPME technique for volatiles was conducted using a manual SPME holder (57330-U, SUPELCO, Bellefonte, PA) and the PDMS-DVB (polydimethylsiloxanedivinylbenzene) 65 μm fiber "blue type". In the microtiter assays, the sample solutions were pipetted into microplate wells using an Eppendorf® Xplorer® 12-channel pipettor with a volume range of 10–300 μL. Two types of microplates were acquired from Sigma-Aldrich: a 96-well flat-bottom white polystyrene microplate, which was non-sterile (Grainer), and a 96-deep well round-bottom polypropylene plate with a volume of 2.2 mL. The absorbance readings were recorded using a Microtek Powerwave XS microplate reader ELISA device. Agilent 1100 series autosampler system from Agilent, GL Sciences Inc. (Waldbronn, Germany). The equipment was outfitted with a system controller, a DAD detector (G 1315B, 280 nm), and a quaternary LC pump (G1311A). The separation method was performed using a Zorbax Eclipse XDB-C_{18} column (150 mm, 4.6 mm, and 5 μm particle size) manufactured by Agilent in Waldbronn, Germany. The Human UP 9000 System's (18 mW) water purification system provided ultrapure water. The liquid chromatographic system (Shimadzu LC 10Avp, Kyoto, Japan) had an in-line degasser, pump, and controller connected to an SPD-M10Avp photodiode array detector with an automatic injector and Class VP chromatography manager software. A reverse-phase C_{18} Ultrasphere column (INERTSIL, Waldbronn, Germany) (100 × 4.6 mm id. 3 μ) was used to analyze phenolic acids.

2.3. Plant material

S. hololeuca was collected from the west of Eskişehir and around Kütahya provinces of Türkiye. The identification of the plant was performed by Dr. Koray Yaylacı (Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Botany). The herbarium sample was registered (ESSE 15843) in Anadolu University Faculty of Pharmacy Herbarium.

2.4. Hydrodistillation of essential oil

The essential oil from the air-dried aerial parts of the plant was hydrodistilled (3 hous) using Clevenger-type equipment, following the guidelines outlined in the European Pharmacopoeia Commission (2023). The essential oil's yield was calculated on the basis of moisture free amount of the plant material. Anhydrous sodium sulphate was used to dry the oil, and it was then kept at 4 °C in an amber vial until it was used for gas chromatography and biological activity studies.

2.5. Microsteam distillation - solid phase microextraction (MSD-SPME)

The plant material (leaves and flowers, separately) weighing 1.0 g, was placed into a flask with a volume of 25 mL (Göger et al., 2021). Additionally, 3.0 mL of water was added to the flask. The mixture was then heated. The flask was equipped with a Claisen distillation head and a reflux condenser, rather than a distillation setup. The electric heater was utilized for heating, while the SPME fiber assembly was secured using a threaded plug. The SPME process for volatiles was conducted using a manual SPME holder and a PDMS-DVB 65 μm fiber known as "blue-type." Before the experiment, the fiber underwent a conditioning process at a temperature of 250 °C for 15 min. The SPME needle penetrated the plug, pushed out the fiber, and brought it into contact with the air space above a plant sample. The MSD-SPME method was performed at the boiling point of water, which was used as the solvent. The time of equilibrium refers to the period between the loading of the SPME fibre into the flask and the initiation of the extraction step. A 3.0-min extraction time was determined to be the appropriate duration after reaching equilibrium. Following the extraction of the volatile substances, the SPME fiber containing the extracted substances was retracted into the needle. The needle was then detached from the plug and employed for thermal desorption at the injection port of GC-FID/MS systems.

2.6. Lipid extraction and fatty acid derivatization

Fatty acid research involved a series of consecutive steps, which include preparing the sample, extracting total lipids, methylating fatty acids, and then analyzing the fatty acid methyl esters using GC-MS/FID (Möller et al., 2019). The Lipid Extraction Kit was employed to extract the total lipids from the aerial parts of the plant material. The extraction

of lipids requires a dual solvent partition mechanism, which comprises an aqueous and a lipophilic solvent (for example, chloroform). The lipids were retained in the lower layer of chloroform, whereas the aqueous-soluble chemicals were retained in the upper layer of methanol-water. In experiment, the mill-ground plant material (0.15 g) was homogenized in extraction solvent (3.0 mL) of the kit. Following the homogenization and vortexing, 0.5 mL of the buffer solution supplied in the kit was added into the mixture and vortexed again. Following that, the mixture was filtered through special filter cartridge of the kit. A 200 μL of the extract was dried under nitrogen gas and then subjected to transesterification with BF_3-methanol reagent. The mixture was subjected to reflux for 1 h at 95 °C. After that, *n*-hexane (1.0 mL) and distilled water (1.0 mL) were added to the reaction vessel. The mixture was vortexed and centrifugated at 500 rpm for 5 min. The uppermost layer, hexane was transported in a vial, concentrated under nitrogen gas, and thereafter injected into the GC-MS/FID system.

2.7. Extraction of plant material

The aerial parts of *S. hololeuca* were extracted using a maceration technique with *n*-hexane, methanol, and water. The extraction process involved continuous shaking at room temperature for 24 × 2 h to obtain the various extracts. The supernatants obtained were passed through a Whatman filter paper for filtration and, the organic solvents were evaporated from the filtrates using reduced pressure to get dry extracts. The aqueous extracts were dehydrated using the lyophilization process. The dried extracts were kept in amber vials at a temperature of 4 °C until further analysis.

2.8. Gas chromatography-mass spectrometry (GC/MS) analysis

GC-MS analysis was performed using the previously published conditions (Göger et al., 2021). A 60-m Agilent Innowax FSC column with a 0.25 mm ID and 0.25 μm film thickness was utilized, in addition to a carrier gas flow rate of 0.8 mL/min. The GC oven was initially set to a temperature of 60 °C for 10 min. Then temperature was gradually raised to 220 °C, increasing at a rate of 4 °C/min, and then maintained at that temperature for 10 min. Ultimately, the temperature was elevated to 240 °C steadily, with an increment of 1 °C/min. The split ratio was set to 40:1, while the injector temperature was determined to be 250 °C. The mass spectra were recorded at 70 eV, encompassing a mass range from 35 to 450 *m/z*.

2.9. Gas chromatography (GC-FID) analysis

The gas chromatography (GC) assay was conducted with an Agilent 6890 N GC equipment. In order to achieve an equivalent elution sequence as observed in GC-MS, the elution line was divided between MS and FID detectors, and a solitary injection was conducted utilizing the identical column and suitable operational parameters. The temperature of the flame ionization detector (FID) was set at 300 °C. The contents of the essential oil, and fatty acid methyl esters were achieved by co-injecting them with standards procured commercially or obtained from pure organic sources whenever feasible. Furthermore, the confirmation of compound identities was achieved through the comparison of their mass spectra with records available in the Wiley-NIST GC/MS Library (Wiley, NY, USA), MassFinder software 4.0 (Dr. Hochmuth Scientific Consulting, Hamburg, Germany and Adams Library. Confirmation was additionally accomplished by utilizing the exclusive "Başer Library of Essential Oil Constituents" database, acquired through chromatographic investigations conducted on pure compounds under identical equipment and conditions. For confirmation of identified compounds, each of the compound's relative retention indices (RRI) was calculated with a C_8–C_{40} *n*-alkane standard mixture (Fluka, Buchs, Switzerland). The FID chromatograms were used to calculate the relative percentage of the separated individual compounds without normalization.

2.10. Preparation of silyl derivatives of extracts

The extracts obtained from the aerial parts of *S. hololeuca* were first subjected to silylation to determine their chemical composition and then analyzed using GC-MS/FID techniques. For this purpose, after adding 80 μL of pyridine and 80 μL of BTSFA (containing 1 % TMCS) reagent to the extracts (2.0 mg), the silylation reaction was carried out by heating the mixture in a sand bath at 100 °C for 1 h. The derivatized samples were directly injected (1.0 μL) into the GC-FID and GC-MS systems (Isidorov, 2020).

2.11. Analysis of silylated compounds

For the analysis of silyl derivative forms prepared from *n*-hexane, methanol, and water extracts, a chromatographic system consisting of an Agilent 7890A GC system coupled with a 5975C Inert MSD with a Triple-Axis detector was used. Chromatographic separation was performed using an HP-5 FSC column (30 m × 0.25 mm, 0.25 μm film thickness, Agilent, Walt & Jennings Scientific, Wilmington, DE, USA). Helium was used as the carrier gas at a flow rate of 0.8 mL/min. The oven temperature was programmed to increase from 100 °C to 320 °C at a rate of 3 °C per minute, and then held at 320 °C for 16.67 min. The sample injection was performed in splitless mode. The injection chamber temperature was maintained at 250 °C, while the FID detector temperature was set at 300 °C. Mass spectra of each compound separated in the column were obtained at 70 eV within the mass range of 35–1050 *m/z*. For compound identification, retention times and mass spectra were evaluated based on reference standards and literature data. The Wiley GC/MS Library (Wiley, New York, NY, USA) and the "Special Özek Silyl Derivative Library," created from the silyl derivatives of standard compounds, were used as reference sources. Quantitative analysis was conducted by calculating the percentage areas of the peaks obtained from the flame ionization detector (FID) (Isidorov, 2020).

2.12. Liquid chromatography (RP-HPLC) analysis

The extracts of *S. hololeuca* were subjected to reverse-phase high-performance liquid chromatography (RP-HPLC) to obtain the phenolic acid profiles. The analytical procedure was conducted in accordance with the protocol established by Öztürk et al. (2007), which had previously undergone comprehensive validation to ensure accuracy, precision, and reproducibility. Chromatographic separation was achieved using two solvent systems: (A) methanol, water, and formic acid (10:88:2, v/v/v) and (B) methanol, water, and formic acid (90:8:2, v/v/v). The gradient elution program was as follows: 15–20 min, 85 % A; 20–30 min, 50 % A; 30–35 min, 0 % A; and 36–42 min, re-equilibration to 100 % A. The flow rate was maintained at 1.0 mL/min, and the injection volume was set at 10 μL. Detection was performed at 280 nm.

Prior to injection, the extracts were dissolved in a methanol-water mixture (1:1, v/v). Peak identification and quantification were based on the retention times and calibration curves previously validated and reported by Öztürk et al. (2007), without any modification to the original methodology (Öztürk et al., 2007).

2.13. Total phenol content

The extracts of *S. hololeuca* were evaluated for the total phenolic content measured as gallic acid equivalent (GAE) using an FC reagent, according to previous procedure (Vl, 1999). Methanol was used to prepare the stock solutions of the extracts and gallic acid. The experimental procedure involved the combination of 20 μL of the sample solution (extract/gallic acid), 1560 μL of ultrapure water, and 100 μL of FC reagent in 96-deep wells. Following an incubation period of 1–8 min,

300 μL of a sodium carbonate solution (20 %) was added to the mixture. The mixture was subjected to a 2-h incubation period (at 25 °C, in dark). Subsequently, a 300 mL of the mixture was put into a 96-well microplate. The absorbance readings at a wavelength of 760 nm were subsequently compared to a gallic acid calibration curve, which was established using a 5-point calibration range spanning from 0.01 to 1.0 mg/mL. The experiment was repeated three times. The calibration curve for gallic acid had a regression coefficient r^2 of 0.9992, calculated as y = 0.7489x + 0.0551. The average content value was computed using a standard error of ±. The findings were quantified as mgGAE/$g_{extract}$.

2.14. Total flavonoid content

The total flavonoid contents of the extracts were quantified using the $AlCl_3$ reagent as quercetin equivalent (QE) following a previously established method (Yur et al., 2017). In the experiment, 80 μL of the sample solution (extract/quercetin), 80 μL of $AlCl_3$, and 1840 μL of absolute ethanol were added into 96-deep wells. In the blank samples, 10 μL of acetic acid (15 %) was added instead of aluminum chloride. Following an incubation period of 40 min a 300 μL of the mixture was transferred into the 96-well microplate. The absorbance values were measured at a wavelength of 415 nm using a microplate reader. The quantification of total flavonoid content was performed using a calibration curve based on quercetin. A 5-point calibration was used to plot the calibration curve within the concentration range of 0.01–1.0 mg/mL. The calibration curve for quercetin had a regression coefficient r^2 of 0.9996, calculated as y = 1.857x + 0.0088. The average content value was computed using a standard error of ±. The results were quantitatively represented as mgQE/$g_{extract}$.

2.15. Trolox equivalent antioxidant activity (TEAC)

The antioxidant potential of the extracts and essential oil of *S. hololeuca* was assessed using $ABTS^{+\bullet}$ and calculated as Trolox equivalent (Re et al., 1999). In the beginning, a 7 mM solution of ABTS was prepated by combining it with 6.6 mg of potassium persulfate in 10 mL of distilled water. After being incubated for 16 h in the absence of light, the solution was diluted with absolute ethanol until it attained an absorbance ranging from 0.700 to 0.800 at a wavelength of 734 nm. The extracts, essential oil (2 mg/mL), and Trolox (5 dilutions ranging from 3.0 to 0.125 mM) were prepared as stock solutions in methanol (with 10 % DMSO). The experiment entailed mixing a 10 μL sample of essential oil/extract/trolox with a 990 μL $ABTS^{+\bullet}$ solution on a 96-deep well plate. After incubating for 30 min in the dark, there was a reduction in absorbance values at a wavelength of 734 nm, as measured by a microplate reader. The ABTS•+ scavenging activity of the samples was measured in terms of their Trolox equivalent antioxidant capacity (TEAC). The linear equation for Trolox was determined as $y = 29.997x - 0.6918$. The regression coefficient (r^2) for the calibration curve was found to be 0.9989. All experiments were performed in triplicate.

2.16. Free radical scavenging effect (DPPH)

The samples' ability to scavenge DPPH radicals was assessed using a modified version of the Brand-Williams method (Brand-Williams et al., 1995). The sample (extracts/essential oil) and reference inhibitor (gallic acid) stock solutions were prepared in methanol (with 10 % DMSO). In the experiment, 100 μL of the sample solution was combined with 100 μL of DPPH solution (0.08 mg/mL in MeOH) in 96-flat bottom well plate cells. The mixtures were incubated in the dark for 30 min. The reduction in absorbance at a wavelength of 517 nm was recorded with the microplate reader. Gallic acid solution was employed in this test as the positive control. The experiments were repeated three times. The samples' free radical scavenging activity was quantified as a percentage of inhibition, which was determined using the following equation:

$$\%\ Inh = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right) \times 100$$

The absorbance of the control, which includes all reagents except the test substance, is indicated as $Abs_{control}$. On the other hand, Abs_{sample} represents the absorbance of the sample after the addition of DPPH (Version 12.0).

2.17. Lipid peroxidation inhibition (β-carotene bleaching) test

The present study aimed to assess the inhibitory impact of extracts and essential oils on lipid peroxidation through the use of the β-carotene bleaching test, utilizing certain modifications (Baderschneider et al., 1999). A chloroform solution containing 1 mg/mL of β-carotene was prepared and subsequently combined with 200 mg of Tween-20 and 25 μL of linoleic acid to generate an emulsion. Chloroform was completely evaporated using a vacuum and nitrogen gas flow. Following that, oxygenated ultrapure water (50 mL) was added and the mixture was vortexed. Before each experiment, the emulsion solution was freshly produced and stored in the dark. The standard antioxidant employed in the study was BHA. The stock solutions of the samples were prepared in methanol (containing 10 % DMSO) at a concentration of 5 mg/mL. In the experiment, 60 μL of a sample (extract/essential oil/BHA) was combined with 250 μL of an emulsion solution within a 96-well plate. The absorbance values were measured at 50 °C every 15 min for a total of 105 min at a wavelength of 492 nm. The experiments were conducted three times. The results of the experiment were calculated using the following formula:

$$\%\ AA = \left[1 - \left(\frac{Abs_{0\ sample} - Abs_{120sample}}{Abs_{0control} - Abs_{120control}}\right)\right] \times 100$$

Where; AA is the antioxidant activity, $Abs_{0sample}$ and $Abs_{120sample}$ are the absorbance values of the sample at 0 min and 120 min, $Abs_{0control}$ and $Abs_{120control}$ are the absorbance values of the control at 0 min and 120 min.

2.18. Oxygen radical absorbance capacity (ORAC) test

The oxygen radical absorption capacity of the extracts and essential oil of *S. hololeuca* was assessed using a microtiter assay (Gillespie et al., 2007) with certain modifications. Fluorescence was employed as a fluorescent probe in the assay, while 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) was utilized as a free radical generator. The standard antioxidant employed in the study was Trolox. The stock solution was prepared by dissolving 15 mg of fluorescein in 10 mL of phosphate buffer solution (0.075 M, pH 7.4). The resulting solution was stored in the dark at 4 °C. In a 96-well plate, 25 μL of a sample (extract/essential oil/trolox) and 150 μL of fluorescein solutions were combined and incubated for 30 min at 37 °C in the dark. The plate was shaked for 10 s after the addition of 25 μL of AAPH reagent following incubation. The fluorescence values were measured at 37 °C each 60-s intervals during a duration of 180 min. The excitation wavelength was 485 nm, and the emission wavelength was 535 nm. The AUC values were determined using the SigmaPlot program. The net AUC values were obtained using the following formula:

$$\text{NET AUC} = AUC_{sample} - AUC_{blank}$$

The standard curve was generated by plotting the net area under the curve (AUC) against the linear concentration of Trolox. The ORAC values were calculated using Trolox equivalents. The experiments were performed on three separate times.

2.19. α-Amylase inhibition test

The extracts and essential oil have been tested for their capacity to

inhibit the α-amylase enzyme following the previously reported assay (Zengin et al., 2014). Acarbose was the reference inhibitor of the α-amylase enzyme used in this study. The extracts and essential oil solutions were prepared in methanol (with added 10 % DMSO). In the test, 50 μL of the sample solution (extract/essential oil/acarbose) was mixed with 50 μL of an enzyme solution (0.8 U/mL in a 20 mM sodium phosphate buffer at pH 6.9) in a 96-well plate. Subsequently, the mixture was subjected to incubation for 10 min in the dark at 37 °C. After the period of incubation, 50 μL of starch solution (0.05 %) was added into wells. The resulting mixture was then incubated for an additional 10 min in the dark at 37 °C. The reaction was stopped by adding 25 μL of HCl (1 M) solution. At the final step, 100 μL of I_2/KI reagent solution was added to the wells. In the blank samples, a buffer solution was added to the wells instead of an enzyme. In the control wells, all reagents were included except for the sample. Absorbance measurements at 630 nm were recorded using a plate reader. The % inhibition was calculated using the following formula:

$$\%\ \mathrm{Inh} = \left(\frac{(Abs_{0\ control} - Abs_{control\ blank}) - (Abs_{sample} - Abs_{sample\ blank})}{(Abs_{0\ control} - A(Abs_{control\ blank})} \right) \times 100$$

Phenolic compounds significantly contribute to the total antioxidant capacity of natural products. Therefore, the phenolic content of the extracts was quantified, and their characterization was determined. The Folin-Ciocalteu reagent was used to assess the total phenolic content of *S. hololeuca* extracts, and the results were expressed in gallic acid equivalents (GAE, mg/g extract) (Table 4). Similarly, the total flavonoid content was evaluated using an aluminum chloride colorimetric assay, with results expressed in quercetin equivalents (QE, mg/$g_{extract}$) (Table 4).

To further evaluate the biological activity of the extracts, various antioxidant assays were performed. The DPPH free radical scavenging assay was conducted to determine the ability of the extracts to neutralize free radicals, with results expressed as IC_{50} values (mg/mL), representing the concentration required to inhibit 50 % of DPPH radicals. The Trolox equivalent antioxidant capacity (TEAC) assay was used to quantify the antioxidant potential in mM Trolox equivalents, while the oxygen radical absorbance capacity (ORAC) assay measured the ability of the extracts to scavenge peroxyl radicals, with values expressed as Trolox equivalents per micromole (TEμmol). Additionally, the β-carotene peroxidation inhibition assay was performed to evaluate lipid peroxidation inhibition, and results were expressed as IC_{50} values (mg/mL). The potential inhibitory effect of the extracts on α-amylase enzyme activity was assessed, and inhibition percentages were reported at a concentration of 10 mg/mL.

3. Results and discussion

In the present work, the phytochemical profile and bioactivity of *S. hololeuca* have been evaluated for the first time. These findings contributed to the lack of information in the existing literature. The aerial parts of *S. hololeuca* were extracted using *n*-hexane, methanol, and water. The hydrodistillation of *S. hololeuca* resulted with colorless and odorless essential oil. Table 1 shows the quantities obtained from the extraction and essential oil process.

Table 1
Extracts yields.

Extract Type	Amounts of Extract, g	Yield[a], %
n-Hexane extract	0.16	0.45
Methanol extract	4.33	12.29
Water extract	4.67	13.26
Essential oil		0.01

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

3.1. Chemical composition of essential oil and volatile constituents

The constituents making up the essential oil from *S. hololeuca* aerial parts have been analyzed using GC-FID/MS techniques. In addition to this, the combined MSD-SPME technique was utilized to investigate the volatiles from the leaves and flowers, separately. The reported values, including the mean of three independent measurements and their standard deviations, are presented in Table 2, which lists the compounds in the order of their elution on the HP-Innowax FSC column, along with their relative percentages, retention indices, and method of identification.

Gas chromatographic analysis of *S. hololeuca* essential oil resulted with identification of 111 compounds representing 90.873 % of the oil. Dodecanoic acid (11.000 ± 0.015 %), tetradecanoic acid (10.592 ± 0.033 %), and alloaromadendrene (9.023 ± 0.036 %) were the main compounds of the essential oil obtained with the hydrodistillation of *S. hololeuca* aerial parts. Analysis of the leaf and flower volatiles obtained with MSD-SPME technique resulted with 104 volatile components, accounting for 96.605 % of all the components identified. In the leaves, alloaromadendrene accounted for 24.908 ± 0.033 % of the major volatile components, followed by naphtalene (9.407 ± 0.040 %) and *(Z)*-2-hexenal (8.181 ± 0.169 %). In the flowers, a total of 103 volatile compounds were identified, accounting for 95.814 % of all detected components. The main volatile compound was naphthalene (14.106 ± 0.045 %), followed by 4-vinylpyridine (7.603 ± 0.032 %) and γ-muurolene (5.604 ± 0.048 %). Table 2 presents these findings.

A comparative analysis of the volatile compounds in the leaves and flowers was conducted using the SPME method, revealing that they did not contain the same main compounds. Furthermore, the essential oil chemical composition of *S. hololeuca* has been found to differ from that of other *Scabiosa* species.

Earlier it has been reported, in the aerial parts of *S. flavida* the main compounds of the essential oil were tricosane (15.5 %), rosifoliol (15.3 %), (*E*)-caryophyllene (10.7 %), and α-humulene (7.9 %) (Javidnia et al., 2006). In the flowers of *S. maritima* the main compounds of essential oil were identified as 3-vinyl pyridine (23.5 %) and hexahydrofarnesylacetone (19.4 %).

3.2. Fatty acid chemical composition

In our research, *S. hololeuca's* fatty acid profile has been established for the first time. Using microscale extraction technique, the lipids of *S. hololeuca* have been extracted from the leaves and flowers and then transesterified with the BF_3 reagent for subsequent GS-FID/MS analysis. Figs. 4 and 5, along with Table 3, present the results of chromatographic separation and identification of the methyl ester derivatives. The reported values represent the mean of three independent measurements along with their standard deviations.

A total of 8 fatty acids were identified, comprising 99.982 % of the fatty acids in *S. hololeuca* leaves. The major fatty acids were methyl nonadecanoate (44.903 ± 0.001 %), methyl hexadecanoate (20.103 ± 0.046 %), and *(Z,Z,Z)*-9,12,15-methyl octadecatrienoate. Additionally, a total of 17 fatty acids were detected in *S. hololeuca* flowers, accounting for 91.587 %. The major fatty acids in the flowers were methyl hexadecanoate (41.299 ± 0.011 %) and methyl nonadecanoate (14.502 ± 0.020 %).

According to previous research (Ebadi-Nahari et al., 2018), the leaves of *Scabiosa* species contain a diverse range of 19 fatty acids, spanning from lauric acid (C12:0) to nervonic acid (C24:1ω9). The predominant fatty acids identified were palmitic acid (C16:0; 4.63–23 %), behenic acid (C22:0; 2.40–35.52 %), lignoceric acid (C24:0; 1.91–34.02 %), and linoleic acid (C18:3ω6; 0.73–13.95 %). Arachidonic acid methyl ester (C20:4ω6) was detected in negligible amounts among the analyzed species. The total proportion of saturated fatty acids in the examined species ranged from 48.97 % to 80.11 %, whereas the proportion of unsaturated fatty acids was estimated to be between 13.62 %

Table 2
Essential oil and volatiles analysis results of *S. hololeuca* aerial parts, leaves, and flowers.

No	RRI exp.	RRI $_{lit.}$	Compound	HD	MSD-SPME	MSD-SPME	Ref.
				Ess. Oil %	Leaves %	Flowers %	
1	1060	1050	(*E*)-2-Butenal		1.501 ± 0.021	0.309 ± 0.01	Hashizume et al. (2007)
2	1093	1093	Hexanal		0.396 ± 0.015	0.201 ± 0.011	Schepetkin et al. (2023)
3	1152	–	2,3-Dimethyl-3-isopropyl-cyclopentene[a]		0.199 ± 0.013	0.200 ± 0.019	
4	1202	1198	Limonene		0.308 ± 0.004		Babushok et al. (2011)
5	1211	1215	Isoamyl alcohol		0.195 ± 0.008		Kang et al. (2016)
6	1225	1225	*(Z)*-3-Hexenal		0.196 ± 0.001		Babushok et al. (2011)
7	1228	1244	Amyl furan		0.108 ± 0.002	0.207 ± 0.018	Shukurlu et al. (2023)
8	1234	1231	*(E)*-2-Hexenal		8.181 ± 0.169	0.795 ± 0.016	Koprivnjak et al. (2002)
9	1265	1264	3-Octanone		0.187 ± 0.018		Tamura et al. (1990)
10	1282	1261	*cis*-Hexenyl formate		0.102 ± 0.001		Binder et al. (1989)
11	1288	1287	Octanal			0.505 ± 0.011	Babushok et al. (2011)
12	1295	1282	Terpinolene		0.107 ± 0.001		Babushok et al. (2011)
13	1297	1297	2-Octanone		0.108 ± 0.007		Nielsen et al. (2003)
14	1298	1282	*trans*-2-(2-Pentenyl) furan		0.104 ± 0.012	0.099 ± 0.003	(C. M. Wu and Liou, 1992)
15	1299	1303	2,2,6-Trimethyl cyclohexanone		0.302 ± 0.001		Yamaguchi and Shibamoto (1981)
16	1300	1300	Tridecane		tr		Trendafilova et al. (2022)
17	1307	1258	Isocumene	0.204 ± 0.001			Vernon and Suratman (1983)
18	1331	1332	*(E)*-2-Heptenal		0.101 ± 0.002	0.100 ± 0.001	Hashizume et al. (2007)
19	1344	1337	6-Methyl-5-hepten-2-one		0.608 ± 0.038	0.298 ± 0.017	Babushok et al. (2011)
20	1348	1361	2-Butoxy ethanol		0.106 ± 0.005	0.102 ± 0.009	Chung et al. (2005)
21	1360	1351	Hexanol	tr	0.486 ± 0.018	0.596 ± 0.044	Babushok et al. (2011)
22	1370	1332	1-Tridecene		0.089 ± 0.010		(Umano and Shibamoto, 1987)
23	1398	–	*Furan isomer*[a]	0.503 ± 0.023			
24	1400	1400	Tetradecane		0.189 ± 0.007	0.104 ± 0.001	Shukurlu et al. (2023)
25	1403	1401	*(Z)*-3-Hexen-1-ol	0.317 ± 0.004	2.101 ± 0.004	1.001 ± 0.005	Botelho et al. (2007)
26	1407	1391	2-Nonanone		0.101 ± 0.006	0.108 ± 0.004	Babushok et al. (2011)
27	1409	1392	Nonanal	0.106 ± 0.001	0.478 ± 0.007	0.199 ± 0.009	Babushok et al. (2011)
28	1410	1409	β-Lutidine			1.712 ± 0.047	Thomas and Bassols (1992)
29	1412	1411	*(E)*-2-Hexen-1-ol		0.107 ± 0.004	0.108 ± 0.04	(Umano and Shibamoto, 1987)
30	1416	1415	Ethyl octanoate		tr		Toda et al. (1982)
31	1430	1431	Tetrahydro linalool	0.101 ± 0.001	0.107 ± 0.001	0.109 ± 0.001	Ter Heide (1976)
32	1435	1423	Dimethyl tetradecane	0.317 ± 0.011	0.106 ± 0.002	1.020 ± 0.013	Trendafilova et al. (2020)
33	1443	1428	*(E)*-2-Octenal		0.186 ± 0.013	0.200 ± 0.014	Thakeow et al. (2008)
34	1450	1444	1-Octen-3-ol	0.110 ± 0.019	0.307 ± 0.011	3.607 ± 0.021	Babushok et al. (2011)
35	1454	1446	*trans*-Linalool oxide		0.103 ± 0.001		Babushok et al. (2011)
36	1463	1449	Heptanol		0.207 ± 0.001	0.208 ± 0.006	Babushok et al. (2011)
37	1467	–	1,5,5-Trimethyl-9-oxa-bicyclo[4.3.0]non-2-ene[a]	0.096 ± 0.001	0.304 ± 0.012		

(*continued on next page*)

Table 2 (*continued*)

No	RRI exp.	RRI $_{lit.}$	Compound	HD	MSD-SPME	MSD-SPME	Ref.
				Ess. Oil %	Leaves %	Flowers %	
38	1469	–	*(2E)*-3,5,5-Trimethyl-2-hexene[a]			0.504 ± 0.002	
39	1471	1471	*(E, Z)*-2,4-Heptadienal	0.107 ± 0.001	1.108 ± 0.007	1.108 ± 0.006	Chyau and Mau (2001)
40	1475	1475	2,6-Dimethyl-7-octen-2-ol		0.102 ± 0.001		Chang et al. (1989a)
41	1492	1492	α-Ylangene		0.303 ± 0.002	0.301 ± 0.002	Choi (2005)
42	1496	1495	2-Ethyl hexanol	0.096 ± 0.001	0.504 ± 0.001	0.407 ± 0.002	Shimoda et al. (1996)
43	1499	1496	Decanal	0.198 ± 0.001	0.486 ± 0.003	0.892 ± 0.003	Babushok et al. (2011)
44	1500	1500	Pentadecane	0.102 ± 0.001			Leffingwell and Alford (2005)
45	1502	1504	(*E, E*)-2,4-Heptadienal	0.096 ± 0.002	1.208 ± 0.003	0.809 ± 0.003	Elmore et al. (2005)
46	1506	1481	α-Cubebene	0.095 ± 0.002	0.204 ± 0.002		
47	1508	1509	α-Copaene	0.101 ± 0.021	0.689 ± 0.027	0.706 ± 0.021	Hazzit et al. (2006)
48	1511	–	4-Vinylpyridine[a]			7.603 ± 0.032	
49	1512	1505	Dihydroedulane II	0.200 ± 0.001			Trendafilova et al. (2020)
50	1514	1535	β-Burbonene	0.102 ± 0.002	0.208 ± 0.002	0.207 ± 0.002	Rochat et al. (2009)
51	1520	1529	*(E,Z)*-3,5-Octadien-2-one		0.701 ± 0.021	0.705 ± 0.022	Rochat et al. (2009)
52	1538	1536	Ethyl nonanoate		0.209 ± 0.001	0.212 ± 0.001	Callejon et al. (2008)
53	1540	1538	α-Gurjunene		0.200 ± 0.001	0.108 ± 0.002	Frizzo et al. (2001)
54	1541	1519	Benzaldehyde	0.308 ± 0.021	4.108 ± 0.023	1.809 ± 0.027	Babushok et al. (2011)
55	1546	1548	β-Cubebene	0.087 ± 0.002			Flamini et al. (2004)
56	1553	1545	3-Ethyl-4-methylpyridine			0.599 ± 0.002	Umano et al. (1995)
57	1559	1552	Octanol	0.197 ± 0.001	0.508 ± 0.001	0.602 ± 0.001	Babushok et al. (2011)
58	1571	–	2-*tert*-Butyl cyclohexanol[a]	0.100 ± 0.002		0.106 ± 0.001	
59	1588	1580	β-Ylangene		0.707 ± 0.003	0.500 ± 0.003	Shimizu et al. (2009)
60	1592	1590	*(E,E)*-3,5-Octadien-2-one			0.201 ± 0.002	Rochat et al. (2009)
61	1595	1559	Calarene	0.100 ± 0.002			Capetanos et al. (2007)
62	1600	1600	Hexadecane	0.108 ± 0.001	0.187 ± 0.001	0.188 ± 0.001	Tekin et al. (2018)
63	1608	1589	(*E, Z*)-2,6-Nonadienal	0.109 ± 0.002	0.204 ± 0.002		Senger-Emonnot et al. (2006)
64	1609	1613	Isobornyl acetate	0.101 ± 0.001			Stancher and Pertoldi (1967)
65	1614	1615	2-Undecanone	0.106 ± 0.001			Mallia et al. (2005)
66	1615	1631	β-Copaene	0.201 ± 0.008	0.607 ± 0.024	1.011 ± 0.022	Píry et al. (1995)
67	1618	1617	β-Caryophyllene	0.397 ± 0.025	1.203 ± 0.021	1.209 ± 0.026	Mahmood et al. (2004)
68	1619	1630	1-Hexadecene		0.205 ± 0.001		Pennarun et al. (2003)
69	1625	1628	2-Octen-1-ol			0.308 ± 0.002	Pozo-Bayón et al. (2007)
70	1626	–	4-Vinylaniline[a]	0.087 ± 0.001			
71	1628	1625	Aromadendrene	0.101 ± 0.001		0.101 ± 0.001	Hazzit et al. (2006)
72	1631	–	5-Ethenyl-2-methyl pyridine[a]			1.409 ± 0.034	
73	1646	1644	Thujopsene			0.206 ± 0.001	Wedge et al. (2009)

(*continued on next page*)

Table 2 (*continued*)

No	RRI exp.	RRI $_{lit.}$	Compound	HD	MSD-SPME	MSD-SPME	Ref.
				Ess. Oil %	Leaves %	Flowers %	
74	1648	1638	β-Cyclocitral	0.406 ± 0.001	0.702 ± 0.002	0.105 ± 0.001	Kaya et al. (1999a,b)
75	1654	1652	Menthol	0.100 ± 0.011			Formisano et al. (2007)
76	1660	1656	Nonanol	0.705 ± 0.021	0.603 ± 0.022	0.603 ± 0.020	Babushok et al. (2011)
77	1672	1674	Alloaromadendrene	9.023 ± 0.036	24.908 ± 0.033	2.007 ± 0.031	Priestap et al. (2002)
78	1673	1674	*(Z)*-β-Farnesene			1.786 ± 0.012	Kerrola and Kallio (1994)
79	1675	1674	Phenyl acetaldehyde		2.401 ± 0.003	0.404 ± 0.003	Perestrelo et al. (2006)
80	1695	1693	α-Humulene	0.402 ± 0.021	0.700 ± 0.023	1.098 ± 0.022	Joichi et al. (2005)
81	1702	–	10-Methyl-2-undecanone[a]	0.205 ± 0.001			
82	1706	1704	2-Dodecanone			0.188 ± 0.001	Ledauphin et al. (2006)
83	1710	1704	γ-Muurolen	1.607 ± 0.056	3.202 ± 0.049	5.604 ± 0.048	Asilbekova et al. (2019)
84	1719	1706	α-Terpineol	1.796 ± 0.026	4.101 ± 0.022	1.806 ± 0.024	Schepetkin et al. (2022)
85	1722	1716	Leden	0.201 ± 0.001	0.404 ± 0.001		Capetanos et al. (2007)
86	1725	1732	Borneol			0.206 ± 0.001	Hazzit et al. (2006)
87	1726	1712	Dodecanal	0.103 ± 0.003		0.304 ± 0.003	Babushok et al. (2011)
88	1734	1708	Zonaren	0.104 ± 0.002	0.302 ± 0.002	0.803 ± 0.002	(Süzgeç Selçuk et al., 2021)
89	1738	1726	Germacren D	0.298 ± 0.001	0.605 ± 0.001	0.683 ± 0.001	(G. Özek et al., 2018)
90	1741	1737	Valensen	0.201 ± 0.002		0.604 ± 0.002	Gölükcü et al. (2015)
91	1747	1750	α-Muurolene	0.302 ± 0.002	0.502 ± 0.003	1.206 ± 0.002	Mahmood et al. (2004)
92	1750	–	Methoxy phenyl oxime[a]		0.208 ± 0.007	1.701 ± 0.008	NIST Mass Spectrometry Data Center (2024)
93	1752	–	Nordasina-9,11-diene[a]	0.217 ± 0.001			
94	1766	1766	Decanol	0.498 ± 0.004	0.288 ± 0.003	0.405 ± 0.004	Bardakci et al. (2012)
95	1768	1769	*(E)*-2-Undecenal	0.308 ± 0.001			Lin et al. (2002)
96	1772	1772	Citronellol			0.301 ± 0.022	Choi and Sawamura (2000)
97	1777	1777	δ-Cadinene	1.204 ± 0.032	2.609 ± 0.036	4.885 ± 0.029	Dob et al. (2005)
98	1780	1736	Naphthalene	1.305 ± 0.042	9.407 ± 0.040	14.106 ± 0.045	Babushok et al. (2011)
99	1785	1785	γ-Cadinene	1.694 ± 0.021	2.799 ± 0.020	3.603 ± 0.019	Mendívil et al. (2006)
100	1800	1800	Octadecane		0.186 ± 0.001		Asilbekova et al. (2019)
101	1803	1802	Cubenene		0.101 ± 0.001	0.303 ± 0.002	Brophy et al. (1989)
102	1807	1815	α-Cadinene	0.383 ± 0.004	0.803 ± 0.003	1.307 ± 0.004	Zeng et al. (2011)
103	1824	1825	*(E,E)*-2,4-Decadienal			0.305 ± 0.001	Gocmen et al. (2004)
104	1829	1779	(*E,Z*)-2,4-Decadienal	0.404 ± 0.002			Lin et al. (2002)
105	1841	1836	β-Damascone	0.201 ± 0.001			Jirovetz et al. (2005)
106	1855	1842	(*E*)-β-Damascenone	0.307 ± 0.001			(Y.-F. G. Wu and Cadwallader, 2002)
107	1858	1839	Geraniol	0.206 ± 0.002	0.106 ± 0.003	0.217 ± 0.002	Babushok et al. (2011)
108	1864	1886	*cis*-Calamenene	1.495 ± 0.024	1.708 ± 0.023	3.002 ± 0.022	Zeng et al. (2011)
109	1870	1871	Undecanol	0.103 ± 0.001			Başer et al. (2006)

(*continued on next page*)

Table 2 (*continued*)

No	RRI exp.	RRI $_{lit.}$	Compound	HD	MSD-SPME	MSD-SPME	Ref.
				Ess. Oil %	Leaves %	Flowers %	
110	1872	1867	*(E)*-Geranyl acetone	0.598 ± 0.002	0.392 ± 0.002	0.306 ± 0.002	Kundakovic et al. (2007)
111	1873	1870	*(Z)*-Cinnamaldehyde			0.205 ± 0.001	(Möllenbeck et al., 1997)
112	1878	1834	6-Methyl-γ-ionone	0.202 ± 0.001	0.202 ± 0.002	0.408 ± 0.001	Avato et al. (2004)
113	1884	–	3-Hydroxy-2,4,4-trimethylpentyl-2-methyl propanoate[a]	0.301 ± 0.002	0.481 ± 0.002	0.603 ± 0.002	
114	1889	–	2,2,4-Trimethyl-3-carboxyisopropyl isobutyl pentanoate[a]	0.403 ± 0.019	0.208 ± 0.012	0.188 ± 0.011	
115	1898	1894	2,2-Dimethyl-1-(2-hydroxy-1-methylethyl)propyl-2-methyl propanoate[a]	0.194 ± 0.001	0.307 ± 0.002	0.409 ± 0.002	Shiratsuchi et al. (1994)
116	1931	1942	β-Calacorene	0.102 ± 0.001	0.101 ± 0.001	0.107 ± 0.001	Noorizadeh and Farmany (2010)
117	1937	1904	Phenylethyl alcohol		0.504 ± 0.027	0.777 ± 0.022	Babushok et al. (2011)
118	1950	1941	α- Calocorene	0.304 ± 0.002	0.203 ± 0.001	0.408 ± 0.001	Polatoğlu et al. (2017)
119	1964	1935.5	(*E*)-β-Ionone	1.096 ± 0.018	0.884 ± 0.015	0.302 ± 0.014	Babushok et al. (2011)
120	1970	1970	Dodecanol	1.703 ± 0.001	0.502 ± 0.001	0.176 ± 0.001	Ledauphin et al. (2004)
121	2000	1995	*(E)*-5,6-Epoxy-β-ionone		0.389 ± 0.002		Castioni and Kapetanidis (1996)
122	2025	2001	Isocaryophyllene oxide	1.103 ± 0.001			Suleimenov et al. (2003)
123	2030	–	1-Cyclopropyl-3,4-dimethyloxyeugenol[a]			0.506 ± 0.014	
124	2039	2041	Pentadecanal	0.295 ± 0.001	0.207 ± 0.001	0.101 ± 0.001	Cros et al. (2005)
125	2042	–	1-(4-*tert*-Butylphenyl) propan-2-one[a]	0.101 ± 0.002			
126	2046	2037	Salvial-4(14)-en-1-one	0.704 ± 0.011	0.381 ± 0.012	0.200 ± 0.011	(Suleimenov et al., 2003)
127	2055	2060	Ledol	0.505 ± 0.024			Sagrero-Nieves et al. (1997)
128	2062	2037	Isoamyl salicylate		0.102 ± 0.001		Chang et al. (1989b)
129	2071	2088	4aβ,7α,7aβ-Nepetalactone		0.208 ± 0.002		İşcan et al. (2011)
130	2082	2071	Humulene epoxide II	0.906 ± 0.012		0.407 ± 0.012	Bardakci et al. (2012)
131	2090	2074	1,10-*di-epi*-Cubenol	0.307 ± 0.001			Weyerstahl et al. (1998)
132	2096	2088	1-*epi*-Cubenol	0.298 ± 0.002			Kaya et al. (1999a,b)
133	2099	2052	Junenol	1.301 ± 0.011	0.201 ± 0.001	0.188 ± 0.001	Wong et al. (2006)
134	2100	2100	Heneicosane	0.405 ± 0.024		0.901 ± 0.026	Schepetkin et al. (2023)
135	2106	–	Dibutyl maleate[a]	1.502 ± 0.002		0.212 ± 0.002	
136	2109	–	3-Methyl-2-(3,7,11-trimethyldodecyl) furan[a]			0.100 ± 0.001	
137	2111	–	*(Z)*-2-Butenedioic acid, bis(2-methylpropyl) ester[a]		0.598 ± 0.004		
138	2113	2110	Viridiflorol	0.089 ± 0.001			Bertoli et al. (2004)
139	2130	2131	Hexahydro farnesyl acetone	4.107 ± 0.003	0.501 ± 0.002	3.782 ± 0.003	Formisano et al. (2007)
140	2134	2097	Amyl salicylate	0.101 ± 0.001			Soria et al. (2009)
141	2144	2140	Spathulenol	0.890 ± 0.002	0.287 ± 0.001	0.089 ± 0.001	Kundakovic et al. (2007)
142	2147	2120	*(Z)*-3-Hexen-1-ol benzoate	0.297 ± 0.011	0.201 ± 0.012	0.104 ± 0.011	Bisio et al. (1998)
143	2160	2168	Tetradecanol		0.203 ± 0.001	0.182 ± 0.001	Vellutini et al. (2005)
144	2170	2159	Nonanoic acid (=*Pelargonic acid*)	0.596 ± 0.031			Trendafilova et al. (2020)
145	2175	2104	6-*epi*-Cubenol	1.406 ± 0.002			Erdurak et al. (2006)

(*continued on next page*)

Table 2 (*continued*)

No	RRI exp.	RRI $_{lit.}$	Compound	HD	MSD-SPME	MSD-SPME	Ref.
				Ess. Oil %	Leaves %	Flowers %	
146	2182	2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone	0.311 ± 0.011	0.202 ± 0.001	0.287 ± 0.014	Baser et al. (2004)
147	2185	2184	Eugenol		0.182 ± 0.001	0.503 ± 0.003	Trendafilova et al. (2020)
148	2191	2187	T-Cadinol	0.486 ± 0.021			Süzgeç Selçuk et al. (2021)
149	2202	2182	2-Methoxynaphthalene		0.381 ± 0.002	0.978 ± 0.002	Strangl et al. (2019)
150	2210	2209	τ-Muurolol	1.400 ± 0.017			Schepetkin et al. (2023)
151	2221	2222	Torreyol	0.608 ± 0.001			Trendafilova et al. (2020)
152	2235	2239	Carvacrol		0.201 ± 0.013		Shukurlu et al. (2023)
153	2240	2229	Hexyl salicylate	0.287 ± 0.011	0.101 ± 0.012		Castioni and Kapetanidis (1996)
154	2265	2255	α-Cadinol	2.203 ± 0.001	0.103 ± 0.001	0.201 ± 0.002	Schepetkin et al. (2023)
155	2266	2256	Cadalene		tr		Lourens et al. (2004)
156	2269	2263	Decanoic acid(=*Capric acid*)	1.458 ± 0.023			Trendafilova et al. (2020)
157	2282	2266	4,7-Dimethyl-1-tetralone	0.188 ± 0.012			Weyerstahl et al. (1998)
158	2299	2299	Sebacic acid, di(2-hexyl) ester[a]		0.288 ± 0.011		
159	2300	2300	Tricosane	0.567 ± 0.002	0.109 ± 0.011	1.666 ± 0.016	(G. Özek et al., 2018)
160	2319	–	2-Ethylhexyl salicylate[a]	0.289 ± 0.001			
161	2339	2320	14-Nor-cadin-5-en-4-one *(isomer-1)*	0.588 ± 0.016			Başer et al. (1998)
162	2351	2345	Galaxolide I	0.382 ± 0.013			Bardakci et al. (2012)
163	2373	2377	Hexadecanol	1.489 ± 0.025			Umano and Shibamoto (1987)
164	2379	2346	Eudesma-4(15),7-dien-1-β-ol	0.579 ± 0.032			Bendiabdellah et al. (2012)
165	2382	2309	Hexyl cinnamic aldehyde	0.883 ± 0.002		0.092 ± 0.002	Vinogradov (2004)
166	2412	2320	14-Nor-cadin-5-en-4-one *(isomer-2)*	0.287 ± 0.001			Başer et al. (1998)
167	2428	2419	4-Isopropyl-6-methyl-1-tetralone	0.385 ± 0.022			Kaya et al. (1999a,b)
168	2451	2502	Dodecanoic acid (=*Lauric acid*)	11.000 ± 0.015			Trendafilova et al. (2020)
169	2500	2500	Pentacosane	0.789 ± 0.032		0.285 ± 0.021	(G. Özek et al., 2018)
170	2580	2581	Octadecanol	1.392 ± 0.012			Umano et al. (2000)
171	2589	2573	Tridecanoic acid (=*Tridecylic acid*)	0.588 ± 0.010			Pino et al. (2003)
172	2611	2622	Phytol	2.785 ± 0.028			Schepetkin et al. (2023)
173	2640	–	2-Benzylidene-1-heptanol[a]	0.306 ± 0.001			
174	2694	2670	Tetradecanoic acid (=*Myristic acid*)	10.592 ± 0.033			Trendafilova et al. (2020)
175	2798	2822	Pentadecanoic acid (=*Pentadecylic acid*)	2.102 ± 0.020			Trendafilova et al. (2020)
176	2837	2304	14-Pentadecanolide	0.482 ± 0.002			Wedge et al. (2009)
			Total	**90.873**	**96.605**	**95.814**	

RRI: Relative Retention Index calculated for n-alkanes C_7–C_{40}.
*: ≥0.1 %; HD: hydrodistillation; tr: trace.
[a] identified from the Wiley mass spectra library".

and 25.39 %.

3.3. Total phenolic, flavonoid contents, and biological activity

The results of these analyses are summarized in Table 4, which presents a comparative evaluation of the total phenolic and flavonoid content, as well as the antioxidant and enzymatic inhibition activities of *S. hololeuca* essential oil and extracts.

Within the scope of the present research, the antioxidant activities of the essential oil of S. hololeuca and its n-hexane, methanol, and water

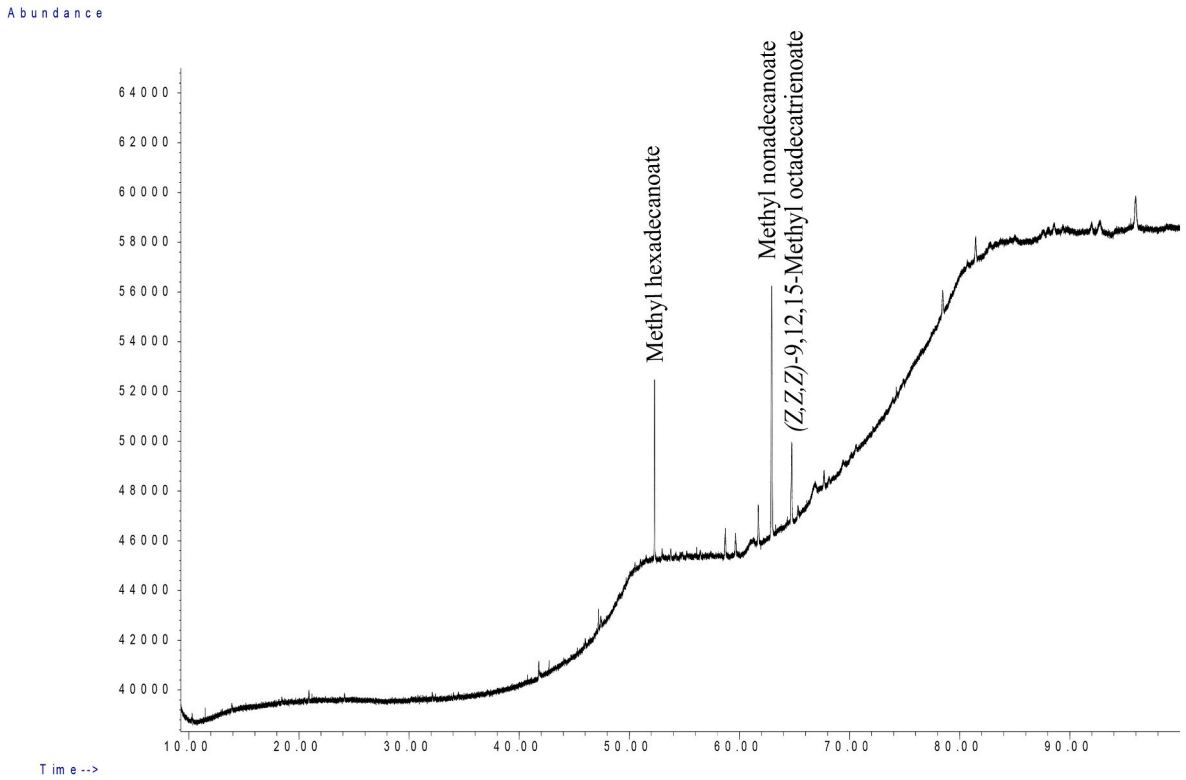


Fig. 4. Chromatogram of methyl esters of fatty acids of lipids obtained from *S. hololeuca* leaves.

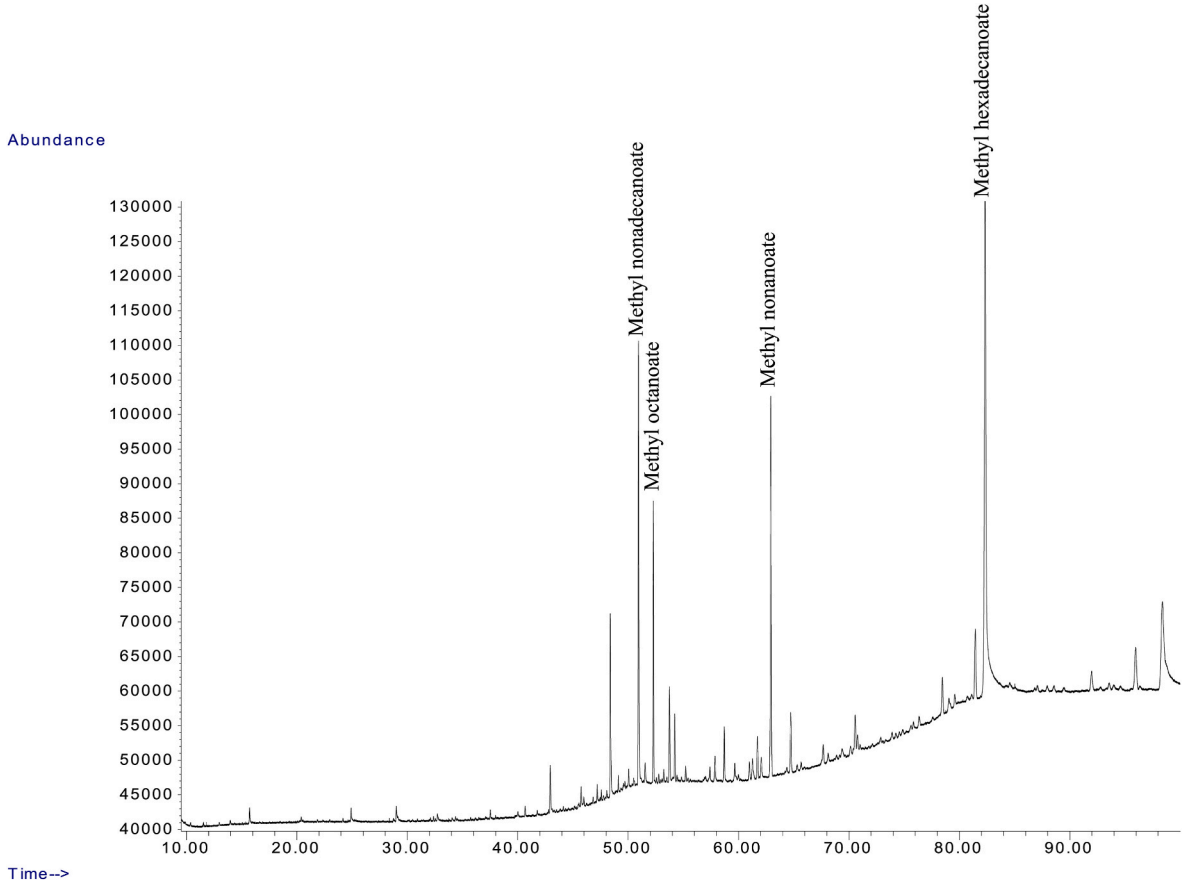


Fig. 5. Chromatogram of methyl esters of fatty acids of lipids obtained from *S. hololeuca* flowers.

Table 3
Different parts of *S.hololeuca* fatty acid methyl esters (FAME) analysis results.

"No	RRI#	Compound*	Leaves	Flowers
			%	
1	1194	Methyl hexanoate (=*Caproic acid methyl ester*); (6:0)	–	1.202 ± 0.012
2	1296	Methyl heptanoate (=*Enantic acid methyl ester*); (7:0)	–	0.503 ± 0.032
3	1396	Methyl octanoate (= *Caprylic acid methyl ester*); (8:0)	–	5.342 ± 0.002
4	1536	Methyl nonanoate (=*Pelargonic acid methyl ester*); (9:0	–	13.501 ± 0.017
5	1602	Methyl decanoate (=*Capric acid methyl ester*); (10:0)	–	3.112 ± 0.026
6	1815	Methyl dodecanoate (=*Lauric acid methyl ester*); (12:0)	–	0.708 ± 0.012
7	2016	Methyl tetradecanoate (=*Myristic acid methyl ester*); (14:0)	1.791 ± 0.010	0.521 ± 0.023
8	2223	Methyl hexadecanoate (=*Palmitic acid methyl ester*); (16:0)	20.103 ± 0.046	41.299 ± 0.011
9	2330	Methyl heptadecanoate (=*Margaric acid methyl ester*); (17:0)	–	0.498 ± 0.021
10	2436	Methyl octadecanoate (=*Stearic acid methyl ester*); (18:0)	4.691 ± 0.036	1.795 ± 0.018
11	2455	*(Z)*-9-Methyl octadecenoate (=*Oleic acid methyl ester*); (18:1); **ω-9**	3.207 ± 0.022	0.504 ± 0.024
12	2509	*(Z,Z)*-9,12-Methyl octadecadienoate (=*Linoleic acid methyl ester);* (18:2); **ω-6**	6.702 ± 0.015	1.608 ± 0.005
13	2542	Methyl nonadecanoate (=*Nonadecylic acid methyl ester*); (19:0)	44.903 ± 0.001	14.502 ± 0.020
14	2560	*(Z,Z,Z)*-9,12,15-Methyl octadecatrienoate (=*α-Linolenic acid methyl ester*); (18:3); **ω-3**	15.584 ± 0.003	2.500 ± 0.009
15	2642	Methyl eicosanoate (=*Arachidic acid methyl ester*); (20:0)	3.001 ± 0.036	0.607 ± 0.001
16	2843	Methyl docosanoate (=*Behanic acid methyl ester*); (22:0)	–	1.982 ± 0.014
17	3050	Methyl tetracosanoate (=*Lignoceric acid methyl ester*); (24:0)	–	1.403 ± 0.041
		Total	**99.982**	**91.587**
		Total saturated fatty acids	**74.489**	**86.975**
		Total unsaturated fatty acids	**25.493**	**4.612**

RRI#: Relative retention index of the methyl/ethyl derivative of the compound; *: Fatty acid in the underivatized form ≥0.5 %".

extracts were evaluated. The test results included an analysis of the DPPH free radical scavenging effect, Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), and β-carotene peroxidation inhibition assay.

In literature there are limited data on the phenolic and flavonoid levels of *Scabiosa* species. The total phenolic content of *S. arenaria* Forssk ranged from 34.77 to 269.09 mgGAE/$g_{extract}$, with flavonoid compounds measured between 0.81 and 10.9 mgQE/$g_{extract}$. The extracts and fractions derived from *S. atropurpurea* subsp. *maritima* (L.) Arcang exhibited a total phenolic content ranging from 17.7 to 186.75 mgGAE/$g_{extract}$. Additionally, the total flavonoid concentration varied between 4.38 and 208.69 mg catechin equivalent/g. The investigation of the methanolic extract of *S. sicula* M. Bieb revealed a total phenolic concentration of 2.67 mgGAE/$g_{extract}$. Polyphenolic content in methanolic extracts of *S. columbaria* subsp. *columbaria* var. *columbaria* L. from Türkiye varied between 269.833 and 640.111 μgGAE/mL, and flavonoid content varied between 6.060 and 13.527 μg QAE/mL (Albayrak et al., 2024). This study showed that the methanol extract of *S. hololeuca* had the highest levels of phenols and flavonoids, with concentrations of 0.59 ± 0.01 mgGAE/$g_{extract}$ and 0.09 ± 0.006 mgQE/$g_{extract}$, respectively.

According to the literature, the aqueous extract of *S. arenaria* has been reported to exhibit potent DPPH free radical inhibition, with an IC50 value of 0.18 mg/mL (Hafsa et al., 2009).The crude extracts of S. tschiliensis Grüning demonstrated DPPH-scavenging activity, with an IC50 value of 25.68 ± 1.21 μg/mL. The DPPH-scavenging capacity of these plants was significantly higher than that of other *Scabiosa* species, including *S. comosa* Fisch. ex Roem. & Schult. and *S. arenaria* (Wang et al., 2017)

Literature reports indicate that the IC_{50} values for the crude extract range between 22.42 and 415.23 mg/mL. The crude extract obtained from the leaves exhibited the highest level of DPPH-scavenging activity compared to other plant parts (Ben Jalloul et al., 2022). The present study revealed that the IC_{50} values of the extracts varied between 0.24 and 2.28 mg/mL, while the essential oil showed no activity against DPPH free radicals.

The extracts of *S. hololeuca* demonstrated good inhibitory potential towards to ABTS radicals. The methanol extract of (2.51 ± 0.11) and water extract (0.87 ± 0.06) had the highest TEAC values, respectively. The TEAC value of the methanol extract from *S. sicula* was reported to be as 0.34 ± 0.01 μg/mL. This is the concentration of Trolox solution that exhibits the same antioxidant activity as a 1 mg/mL extract solution (Kılınç et al., 2020). The TEAC value for *S. arenaria* was reported as 0.56 mMTE/$g_{extract}$ (Hafsa et al., 2009). The flower and leaf parts of *S. columbaria* demonstrated the TEAC values as 267.381 ± 0.012 and 242.857 ± 0.003, respectively (Akar, 2021). According to reports, the methanolic extract of *S. atropurpurea* showed the highest activity in β-carotene peroxidation inhibition. The curves exhibited a high degree

Table 4
Total phenol, flavonoid contents and biological activity results for *S. hololeuca* extracts.

	Total phenol content (mgGAE/g $_{extract}$)	Total flavonoid content (mgQE/g $_{extract}$)	DPPH (IC_{50}, mg/mL[a])	TEAC (mM)	ORAC[b] (TEμmol)	β-Carotene peroxidation inhibition (IC_{50}, mg/mL)	α-Amylase inhibition (%)
Essential oil	–	–	NE	0.15 ± 0.01	NE	NE	20.72[c]
n-Hexane extract	0.057 ± 0.003	NE	2.28 ± 0.05	0.51 ± 0.03	NE	NE	13.25[d]
Methanol extract	0.585 ± 0.015	0.093 ± 0.006	0.24 ± 0.01	2.51 ± 0.11	217.6 ± 11.8	1.3 ± 0.02	20.02[e]
Water extract	0.122 ± 0.004	0.011 ± 0.0007	1.21 ± 0.05	0.87 ± 0.06	238.9 ± 10.4	1.4 ± 0.16	10.73[f]
BHA	–	–	–	–	–	0.01 ± 0.0005	–
GA	–	–	0.002 ± 0.0	–	–	–	–
Acr	–	–	–	–	–	–	0.016 ± 0.006

BHA: butylated hydroxyanisole; **GA**: gallic acid; **Acr:** Acarbose.
a) : IC_{50} values are half maximal inhibitory concentrations.
b) ORAC values are determined for essential oil and extracts at 0.1 mg/mL; **NE**: Noneffective; **%Inh.**: % inhibition value determined for extracts at 10 mg/mL.
c) IC_{50} was not calculated; the value is 5 mg/mL.
d) IC_{50} was not calculated; the value is 0.078125 mg/mL.
e) IC_{50} was not calculated; the value is 0.625 mg/mL.
f) IC_{50} was not calculated; the value is 0.625 mg/mL.

of similarity and demonstrated that their corresponding inhibition percentages were remarkably comparable, with values of 97.19 % and 100 %. The results indicate that the hexane and chloroform extracts demonstrate a moderate degree of antioxidant activity, with percentages of 64.17 % and 42.91 %, respectively (Ali-Rachedi et al., 2024). The inhibition values for the methanol extract were 1.3 ± 0.001 mg/mL and those for the water extract were 1.4 ± 0.16 mg/mL. The water extract of *S. hololeuca* exhibits the highest ORAC activity. The study revealed a significant positive connection between the antioxidant activity and the total phenolic content of each extract. The α-amylase enzyme-inhibiting effect of *S. hololeuca* extracts was found to be insufficient.

3.4. Analysis of silylated compounds

The compounds identified in the silyl derivatives of the *S. hololeuca* aerial extracts were analyzed using GC-MS/FID, and the detected compounds are presented in Table 5, with the reported values representing the mean of three independent measurements along with their standard deviations.

The literature on silylated compounds in extracts of the genus *Scabiosa* remains limited. In the analysis of 70 % ethyl alcohol extracts of *S. ochroleuca* L., prepared using an ultrasonic extraction unit and a microwave bath extractor, significant differences in compound composition were observed. The ultrasonic extract contained L-lactic acid (28.0 %), ethyl ester of 7-chloro-7-oxoheptanoic acid (16.3 %), and quinic acid as the major constituents. In contrast, the extract obtained using the microwave bath extractor exhibited L-lactic acid (25.2 %), ethyl ester of 7-chloro-7-oxoheptanoic acid (22.1 %), and quinic acid (10.8 %) as the predominant compounds.

In the our investigation and comparative analysis of silylated compounds across the three extracts, significant variations were observed in the most abundant constituents. The *n*-hexane extract predominantly contained phthalic acid, bis(2-ethylhexyl) ester (47.174 ± 0.087), Oleanolic acid - 2TMS (25.911 ± 0.056), and hentriacontane (4.104 ± 0.016), with 95.550 % of its composition identified. In the methanol extract, oleanolic acid - 2TMS (38.300 ± 0.057), ursolic acid - 2TMS (12.124 ± 0.020), and sucrose - 8TMS (7.609 ± 0.027) were found in the highest concentrations, with 89.071 % of its composition identified. Conversely, the water extract was distinguished by the dominance of quinic acid - 5TMS (19.036 ± 0.027), β-D-galactopyranoside, 1-*O*-methyl - 4TMS (5.838 ± 0.017), and α-glucopyranose - 5TMS (5.397 ± 0.003), and 89.093 % of its composition was identified. These findings from our study indicate that while the *n*-hexane and methanol extracts are rich in triterpenic acids and hydrophobic compounds, the water extract primarily consists of polar and sugar-derived compounds.

3.5. HPLC analysis

The phenolic acids (gallic, protocatechuic, caffeic, chlorogenic, *p*-coumaric, ferulic, and *o*-coumaric acids) in *S. hololeuca* extracts were analyzed using an RP-HPLC technique with gradient mobile system (Öztürk et al., 2007). Table 6 presents the HPLC results of *S. hololeuca* water and methanol extracts, where both the methanol and water extracts were analyzed in triplicate, and the values are expressed as the mean along with their standard deviations.

Various research examined the effects of polar and nonpolar solvents (Abad-García et al., 2007; Akowuah et al., 2005; Galanakis et al., 2013; Horax et al., 2010; Palma et al., 2002). Researchers have documented the extraction of higher molecular weight flavonoid glycosides and phenols using polar solvents (Oreopoulou and Tzia, 2007). Polar solvents were determined to be more successful in the efficient extraction of phenolic acids due to the polar characteristics of phenolic compounds. Apolar solvents, such as *n*-hexane, demonstrated reduced efficacy in the extraction of phenolic acids (Mukhopadhyay et al., 2006). The extraction of chemicals such as protocatechuic acid, vanillic acid, and syringic acid from winery wastes indicated that methanol was the most effective

Table 5
GC/MS analysis results of the silylated extracts obtained from the aerial parts of *S. hololeuca* with extracts.

No	RRI	Compound	Hexane %	Methanol %	Water %
1	1281	Glycerol - 3TMS		0.102 ± 0.011	
2	1506	Malic acid - 3TMS		0.299 ± 0.002	5.403 ± 0.012
3	1529	Erythritol - 4TMS		tr	
4	1694	α-D- Methyl xylopyranoside - 3TMS[a]			0.727 ± 0.014
5	1729	Levoglucosan - 3TMS			0.103 ± 0.004
6	1753	Xylitol - 5TMS		0.818 ± 0.006	0.711 ± 0.010
7	1767	2-Propylpentanoic acid. 2.3.4.6-tetra(trimethylsilyl)-1-glucopyranoside[a]		0.098 ± 0.001	
8	1797	Tagatofuranose - 5TMS (*isomer-2*)		0.705 ± 0.021	2.604 ± 0.018
9	1800	*Unknown*			1.202 ± 0.005
10	1806	Quinic acid - 4TMS		0.123 ± 0.003	
11	1843	α-Fructofuranose - 5TMS		0.206 ± 0.014	0.496 ± 0.013
12	1846	*Unknown*			2.304 ± 0.020
13	1849	*Unknown*			0.996 ± 0.016
14	1852	β-d-Fructofuranose - 5TMS (*isomer-2*)		0.093 ± 0.001	
15	1858	Fructopyranose - 5TMS		0.106 ± 0.001	
16	1885	α-d-Glucofuranose -5TMS		0.124 ± 0.002	0.824 ± 0.002
17	1890	α-D- Galactoside. 1-*O*-methyl - 4TMS[a]			0.404 ± 0.001
18	1901	Quinic acid - 5TMS		1.807 ± 0.015	19.036 ± 0.027
19	1917	Gluconolactone - 4TMS		0.103 ± 0.001	
20	1929	α-Glucopyranose-5TMS		1.206 ± 0.012	5.397 ± 0.003
21	1932	β-d- Galactopyranoside. 1-*O*-methyl - 4TMS		0.614 ± 0.009	5.838 ± 0.017
22	1942	β-Mannopyranose - 5TMS		tr	
23	1945	Glucaric acid-γ-lactone - 4TMS			0.404 ± 0.002
24	1949	*trans-p*-Coumaric acid - 2TMS		0.095 ± 0.001	
25	1971	Mannitol - 6TMS	0.104 ± 0.001	2.301 ± 0.004	1.027 ± 0.003
26	1987	γ-Lactone saccharic acid - 4TMS	0.107 ± 0.001	1.302 ± 0.001	3.498 ± 0.012
27	2003	*Unknown*			1.732 ± 0.020
28	2023	β-Glucopyranose - 5TMS		1.605 ± 0.025	5.021 ± 0.018
29	2049	Hexadecanoic acid (=*Palmitic acid*) - TMS	0.407 ± 0.013	0.106 ± 0.001	0.417 ± 0.005
30	2106	(*E*)-Ferulic acid - 2TMS		0.101 ± 0.024	0.799 ± 0.012
31	2113	Methoxycyclohexanepentol - 5TMS (*isomer-3*)		0.111 ± 0.001	
32	2127	*myo*-Inositol - 6TMS		0.693 ± 0.014	2.206 ± 0.015
33	2144	*Sucrose – TMS (derivative)*		0.904 ± 0.002	
34	2150	(*E*)-Caffeic acid - 3TMS		0.207 ± 0.001	1.103 ± 0.024
35	2179	Phytol-TMS		0.106 ± 0.010	
36	2217	(*Z.Z*)-9.12-Octadecadienoic acid (=*Linoleic acid*) - TMS		0.098 ± 0.001	

(*continued on next page*)

Table 5 (*continued*)

No	RRI	Compound	Hexane %	Methanol %	Water %
37	2250	Octadecanoic acid (=*Stearic acid*) - TMS	0.119 ± 0.001		
38	2261	Phthalic acid. hexyl oct-3-yl ester[a]	0.099 ± 0.001		
39	2364	2-*O*-Glycerol-α-d-galactopyranoside - 6TMS		tr	
40	2421	*Unknown*		tr	1.409 ± 0.007
41	2429	*Sucrose – TMS (derivative)*		1.012 ± 0.029	3.7 ± 0.033
42	2448	Eicosanoic acid - TMS	0.106 ± 0.002	tr	
43	2471	Uridine - 3TMS			0.705 ± 0.022
44	2535	*Unknown*			1.3 ± 0.014
45	2543	*Sucrose – TMS (derivative)*		2.809 ± 0.013	
46	2547	Phthalic acid. bis(2-ethylhexyl) ester	47.174 ± 0.087		1.904 ± 0.046
47	2646	Docosanoic acid (=*Behenic acid*) - TMS	0.207 ± 0.001		
48	2700	Heptacosane	0.205 ± 0.002		
49	2713	Sucrose - 8TMS	0.108 ± 0.001	7.609 ± 0.027	
50	2800	Octacosane	0.306 ± 0.023		
51	2817	Trehalose - 8TMS	0.200 ± 0.001	2.107 ± 0.002	
52	2843	*neo*-Trehalose - 8TMS		0.777 ± 0.001	1.206 ± 0.004
53	2844	Tetracosanoic acid - TMS	0.333 ± 0.005		
54	2900	Nonacosane	1.601 ± 0.004		
55	2952	β.-Gentiobiose. octakis (trimethylsilyl) ether methyloxime (*isomer-2*)		0.289 ± 0.002	
56	2972	α-Gentiobiose - TMS		0.306 ± 0.001	
57	3000	Triacontane	0.333 ± 0.001		
58	3001	*Sucrose – TMS (derivative)*		0.921 ± 0.002	
59	3028	*Raffinose – TMS derivative*			4.448 ± 0.023
60	3031	*Raffinose – TMS derivative*			4.627 ± 0.011
61	3042	Hexacosanoic acid (=*Cerotic acid*) - TMS	0.302 ± 0.001		
62	3044	*Unknown*		2.403 ± 0.051	
63	3100	Hentriacontane (=*Untriacontane*)	4.104 ± 0.016		
64	3184	*crypto*-Chlorogenic acid - 6TMS	0.100 ± 0.001	2.522 ± 0.004	0.726 ± 0.001
65	3200	Dotriacontane	0.407 ± 0.024		
66	3244	Octacosanoic acid (=*Montanic acid*) - TMS	0.908 ± 0.003		
67	3252	*Unknown*			2.109 ± 0.022
68	3264	*Sucrose – TMS (derivative)*		2.857 ± 0.002	
69	3275	Campesterol - TMS	0.314 ± 0.005		
70	3300	Tritriacontane	3.023 ± 0.002		
71	3347	Triacontanol - TMS	0.102 ± 0.001		
72	3368	β-Sitosterol - TMS	2.487 ± 0.021	0.904 ± 0.020	
73	3378	Avenasterol - TMS	0.211 ± 0.003	0.098 ± 0.003	
74	3442	Triacontanoic acid - TMS	1.055 ± 0.001		
75	3602	Oleanolic acid (=*3-β-hydroxyolean-12-en-28-oic acid*) - 2TMS	25.911 ± 0.056	38.300 ± 0.057	3.304 ± 0.050
76	3619	*Unknown*	2.111 ± 0.021		
77	3647	Ursolic acid (=*3β-3-hydroxy-urs-12-en-28-oic acid*) - 2TMS	3.106 ± 0.024	12.124 ± 0.020	1.403 ± 0.031
Total			**95.550**	**89.071**	**89.093**

RRI: Relative retetntion indices.
tr: Trace (<0.1).
[a] Tentative identification from Wiley-NIST Library.

Table 6
The amounts of phenolic acid identified by reverse-phase HPLC in the extracts examined.

Phenolic Acid	Methanol Extract (μg/$g_{extract}$)	Water Extract (μg/$g_{extract}$)
p-Hydroxybenzoic acid	0.734 ± 0.001	0.439 ± 0.001
Caffeic acid	0.993 ± 0.012	0.187 ± 0.014
Chlorogenic acid	0.518 ± 0.102	0.523 ± 0.010
Syringic acid	0.744 ± 0.008	0.222 ± 0.003
p-Coumaric acid	0.469 ± 0.002	0.598 ± 0.003
o-Coumaric acid	1.032 ± 0.023	0.968 ± 0.016
Ferulic acid	0.272 ± 0.001	0.954 ± 0.005

solvent. The utilization of organic solvents was discouraged (Lafka et al., 2007). Thus, the utilization of polar solvents enabled the obtaining of phenolic acids with increased purity and yield, increasing their applicability in pharmaceutical and nutraceutical fields. The researches highlighted that polar solvents are more effective for extracting phenolic chemicals (Alara et al., 2021; Lee et al., 2009; Papoti and Tsimidou, 2009). So in this study, methanol and water extracts were used for phenolic acid analysis.

The literature search revealed that six phenolic compounds were the main constituents of the methanol extracts from *S. columbaria* subsp. *columbaria* var. *columbaria*. The compounds identified were gallic acid, catechin, 4-OH-benzoic acid, 4-OH-benzaldehyde, and chlorogenic acid (Akar, 2021). The analysis of *S. stellata* extract revealed that the primary compounds identified were isoorientin and 4-*O*-caffeoylquinic acid (Rahmouni et al., 2018). Furthermore, compounds that have been reported include caffeoylquinic acid, rutin, ursolic acid, cyanuric acid, sinapic acid, luteolin, apigenin, quercetin, kaempherol, and tamarixetin. Gallic acid, chlorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, sinapic acid, ferulic acid, catechin hydrate, epicatechin-3-*O*-gallate, luteolin-7-*O*-glucoside, and isorhamnetin, 3-*O*-glucoside, rutin, isoquercetin, myricetin, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, quercetin, naringenin, luteolin, isorhamnetin, and apigenin were identified in the hydromethanol extract of *S. atropurpurea* subsp. *maritima* (Ben Jalloul et al., 2022).

The results of our analyses indicate that *S. hololeuca* contains significant amounts of chlorogenic acid, caffeic acid, *p*-coumaric acid, syringic acid, and ferulic acid. The study findings indicated a significant positive correlation between the biological activity of the extracts and the total phenolic content of each extract. these results are consistent with the literature. Because of this, the phenolic compounds investigated in the methanolic and water extracts of *S. hololeuca* are being studied.

4. Conclusions

This study represents the first comprehensive investigation of the antioxidant and α-amylase inhibitory effects of *S. hololeuca* (*L. hololeuca*), an endemic plant species, through *n*-hexane, methanol, and water extracts.

The methanol extract exhibited the highest total phenol and flavonoid content, which correlates with its strong antioxidant activity. Both methanol and water extracts demonstrated significant antioxidant potential, likely due to the presence of phenolic acids, such as chlorogenic acid and *p*-coumaric acid, which were identified in the polar extracts. However, none of the tested extracts exhibited α-amylase inhibitory activity, indicating that *S. hololeuca* may not be effective for α-amylase-related metabolic disorders.

The essential oil composition of *S. hololeuca* was dominated by dodecanoic acid, tetradecanoic acid, and alloaromadendrene. Furthermore, SPME analysis indicated that (*E*)-2-hexenal and naphthalene were the main volatile compounds in the leaves, while naphthalene, 4-vinylpyridine, and γ-muurolene were predominant in the flowers. The primary fatty acids identified in the leaves and flowers were nonadecanoic acid and hexadecanoic acid.

Additionally, GC-MS analysis of silylated derivatives provided a deeper insight into the chemical profile of *S. hololeuca*. The derivatization process facilitated the identification of various organic acids, sugars, and triterpenic compounds, including oleanolic acid and ursolic acid, both of which are known for their bioactive properties. The methanol and water extracts contained substantial amounts of quinic acid and galactopyranoside derivatives, contributing to their polar nature and potential biological activities. Moreover, the limited number of studies on this subject in the literature underscores the significance of elucidating the chemical composition of other *Scabiosa* species through this research.

The findings suggest that *S. hololeuca*, a plant unique to Türkiye's flora, holds promise as a natural source for the food, pharmaceutical, and cosmetics industries due to its rich antioxidant content and diverse volatile compounds. The potential application of *S. hololeuca* in food and pharmaceutical formulations could provide a natural alternative to synthetic antioxidants. However, further toxicological and pharmacological studies are required to establish the safety and efficacy of this endemic species for wider industrial applications.

CRediT authorship contribution statement

Kübra Öğüt: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Data curation. **Gülmira Özek:** Writing – review & editing, Methodology, Data curation. **Nilgün Öztürk:** Writing – review & editing, Methodology. **Ömer Koray Yaylacı:** Resources. **Temel Özek:** Writing – review & editing, Validation, Supervision, Project administration, Methodology.

Statistical analysis

Three independent replicates of the extract were analyzed, with each replicate injected twice. The presented data represent the mean of the concordant values obtained for each sample, expressed as mean values ± standard deviation (MV ± SD).

Funding

The research was financially supported by Scientific Research Department of Anadolu University (BAP No: 2103S009).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Corresponding author reports equipment, drugs, or supplies was provided by Anadolu University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Abad-García, B., Berrueta, L.A., López-Márquez, D.M., Crespo-Ferrer, I., Gallo, B., Vicente, F., 2007. Optimization and validation of a methodology based on solvent extraction and liquid chromatography for the simultaneous determination of several polyphenolic families in fruit juices. J. Chromatogr. A 1154 (1–2), 87–96.

Akar, Z., 2021. Chemical compositions by using LC–MS/MS and GC–MS and antioxidant activities of methanolic extracts from leaf and flower parts of *Scabiosa columbaria* subsp. *columbaria* var. *columbaria* L. Saudi J. Biol. Sci. 28 (11), 6639–6644.

Akowuah, G.A., Ismail, Z., Norhayati, I., Sadikun, A., 2005. The effects of different extraction solvents of varying polarities on polyphenols of *Orthosiphon stamineus* and evaluation of the free radical-scavenging activity. Food Chem. 93 (2), 311–317.

Alara, O.R., Abdurahman, N.H., Ukaegbu, C.I., 2021. Extraction of phenolic compounds: a review. Curr. Res. Food Sci. 4, 200–214.

Albayrak, S., Aksoy, A., Yilmaz, M.A., Beyzi, E., 2024. Investigation of phytochemical, antioxidant, and antidiabetic potentials of *Scabiosa* L. (Caprifoliaceae) species with chemometric methods. Chem. Biodivers. 21 (2), e202301652.

Ali-Rachedi, F., Laoud, A., Meraghni, M., 2024. Antioxidant activity and acetylcholinesterase inhibitory of *Scabiosa atraupurpuria* sub maritima L. extract. Res. J. Biotechnol. 19, 7.

Andreica, A.-M., Balea, A., Pojar-Feneşan, M., Carpa, R., 2024. GC-MS comparative chemical composition of essential oils and volatile compounds of *Eryngium planum* L. using classical hydrodistillation, ultrasound-assisted hydrodistillation, and headspace solid-phase microextraction. Antimicrobial activity. Stud. Univ. Babes-Bolyai, Chem. 69 (1).

Asilbekova, D.T., Ozek, G., Ozek, T., Bobakulov, K.M., Baser, K.H.C., Sagdullaev, S.S., 2019. Essential oil and lipids from leaves of *Ferula kuhistanica*. Chem. Nat. Compd. 55, 993–998.

Avato, P., Raffo, F., Aldouri, N.A., Vartanian, S.T., 2004. Essential oils of *Varthemia iphionoides* from Jordan. Flavour Fragrance J. 19 (6), 559–561.

Babushok, V.I., Linstrom, P.J., Zenkevich, I.G., 2011. Retention indices for frequently reported compounds of plant essential oils. J. Phys. Chem. Ref. Data 40 (4).

Baderschneider, B., Luthria, D., Waterhouse, A.L., Winterhalter, P., 1999. Antioxidants in white wine (cv. Riesling): I. Comparison of different testing methods for antioxidant activity. Vitis 38 (3), 127–131.

Bardakci, H., Demirci, B., Yesilada, E., Kirmizibekmez, H., Baser, K.H.C., 2012. Chemical composition of the essential oil of the subterranean parts of *Valeriana alliariifolia*. Record Nat. Prod. 6 (1), 89.

Başer, K.H.C., Kürkçüoğlu, M., Aytac, Z., 1998. Composition of the essential oil of *Salvia euphratica Montbret et Aucher ex Bentham var. euphratica* from Turkey. Flavour Fragrance J. 13 (1), 63–64.

Başer, K.H.C., Özek, G., Özek, T., Duran, A., Duman, H., 2006. Composition of the essential oils of *Rhabdosciadium oligocarpum* (Post ex Boiss.) Hedge et Lamond and *Rhabdosciadium microcalycinum* Hand.-Mazz. Flavour Fragrance J. 21 (4), 650–655.

Baser, K.H.C., Özek, T., Kırımer, N., Deliorman, D., Ergun, F., 2004. Composition of the essential oils of *Galium aparine L.* and *Galium odoratum* (L.) Scop. from Turkey. J. Essent. Oil Res. 16 (4), 305–307.

Bendiabdellah, A., El Amine Dib, M., Djabou, N., Allali, H., Tabti, B., Muselli, A., Costa, J., 2012. Biological activities and volatile constituents of *Daucus muricatus* L. from Algeria. Chem. Cent. J. 6, 1–10.

Ben Jalloul, A., Chaar, H., Tounsi, M.S., Abderrabba, M., 2022. Variations in phenolic composition and antioxidant activities of *Scabiosa maritima* (Scabiosa atropurpurea sub. maritima L.) crude extracts and fractions according to growth stage and plant part. South Afr. J. Bot. 146, 703–714.

Benyelles, B., Allali, H., Dib, M.E.A., Djabou, N., Tabti, B., Costa, J., 2014. Essential oil from *Rhaponticum acaule* L. roots: comparative study using HS-SPME/GC/GC–MS and hydrodistillation techniques. J. Saudi Chem. Soc. 18 (6), 972–976.

Bertoli, A., Menichini, F., Noccioli, C., Morelli, I., Pistelli, L., 2004. Volatile constituents of different organs of *Psoralea bituminosa* L. Flavour Fragrance J. 19 (2), 166–171.

Binder, R.G., Flath, R.A., Mon, T.R., 1989. Volatile components of bittermelon. J. Agric. Food Chem. 37 (2), 418–420.

Bisio, A., Ciarallo, G., Romussi, G., Fontana, N., Mascolo, N., Capasso, R., Biscardi, D., 1998. Chemical composition of essential oils from some *Salvia* species. Phytother Res.: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 12 (S1), S117–S120.

Botelho, G., Caldeira, I., Mendes-Faia, A., Clímaco, M.C., 2007. Evaluation of two quantitative gas chromatography–olfactometry methods for clonal red wines differentiation. Flavour Fragrance J. 22 (5), 414–420.

Brand-Williams, W., Cuvelier, M.-E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT–Food Sci. Technol. 28 (1), 25–30.

Brophy, J.J., Davies, N.W., Southwell, I.A., Stiff, I.A., Williams, L.R., 1989. Gas chromatographic quality control for oil of *Melaleuca terpinen-4-ol* type (Australian tea tree). J. Agric. Food Chem. 37 (5), 1330–1335.
Callejon, R.M., Morales, M.L., Ferreira, A.C.S., Troncoso, A.M., 2008. Defining the typical aroma of sherry vinegar: sensory and chemical approach. J. Agric. Food Chem. 56 (17), 8086–8095.
Capetanos, C., Saroglou, V., Marin, P.D., Simić, A., Skaltsa, H.D., 2007. Essential oil analysis of two endemic *Eryngium* species from Serbia. J. Serb. Chem. Soc. 72 (10), 961–965.
Carlson, S.E., Linder, H.P., Donoghue, M.J., 2012. The historical biogeography of *Scabiosa* (Dipsacaceae): implications for old world plant disjunctions. J. Biogeogr. 39 (6), 1086–1100.
Castioni, P., Kapetanidis, I., 1996. Volatile constituents from *Brunfelsia grandiflora* ssp. grandiflora: qualitative analysis by GC-MS. Sci. Pharm. 64 (1), 83–91.
Chang, L.P., Sheng, L.S., Yang, M.Z., An, D.K., 1989a. Retention index of essential oil in temperature-programmed capillary column gas chromatography. Yao Xue Xue Bao= Acta Pharmaceutica Sinica 24 (11), 847–852.
Chang, L.P., Sheng, L.S., Yang, M.Z., An, D.K., 1989b. Retention index of essential oil in temperature-programmed capillary column gas chromatography. Yao Xue Xue Bao= Acta Pharmaceutica Sinica 24 (11), 847–852.
Choi, H.-S., 2005. Characteristic odor components of kumquat (*Fortunella japonica* Swingle) peel oil. J. Agric. Food Chem. 53 (5), 1642–1647.
Choi, H.-S., Sawamura, M., 2000. Composition of the essential oil of *Citrus tamurana* Hort. ex Tanaka (*Hyuganatsu*). J. Agric. Food Chem. 48 (10), 4868–4873.
Chung, H.Y., Fung, P.K., Kim, J.-S., 2005. Aroma impact components in commercial plain sufu. J. Agric. Food Chem. 53 (5), 1684–1691.
Chyau, C.-C., Mau, J.-L., 2001. Effects of various oils on volatile compounds of deep-fried shallot flavouring. Food Chem. 74 (1), 41–46.
Cros, S., Lignot, B., Bourseau, P., Jaouen, P., Prost, C., 2005. Desalination of mussel cooking juices by electrodialysis: effect on the aroma profile. J. Food Eng. 69 (4), 425–436.
Dob, T., Berramdane, T., Chelgoum, C., 2005. Chemical composition of essential oil of *Pinus halepensis* Miller growing in Algeria. C. R. Chim. 8 (11–12), 1939–1945.
Ebadi-Nahari, M., Farnia, P., Nikzat, S., Mollaei, S., 2018. A chemotaxonomic evaluation of some *Scabiosa* L. species in Iran. Biochem. Systemat. Ecol. 81, 33–36.
Elmore, J.S., Nisyrios, I., Mottram, D.S., 2005. Analysis of the headspace aroma compounds of walnuts (*Juglans regia* L.). Flavour Fragrance J. 20 (5), 501–506.
Erdurak, C.S., Coşkun, M., Demirci, B., Başer, K.H.C., 2006. Composition of the essential oil of fruits and roots of *Ferulago isaurica* Peşmen and *F. syriaca* Boiss. (Umbelliferae) from Turkey. Flavour Fragrance J. 21 (1), 118–121.
European Pharmacopoeia Commission, 2023. Essential oils in herbal drugs. In: European Pharmacopoeia, eleventh ed. European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe, Strasbourg, France, pp. 323–324.
Flamini, G., Cioni, P.L., Morelli, I., 2004. Essential oils of *Galeopsis pubescens* and *G. tetrahit* from Tuscany (Italy). Flavour Fragrance J. 19 (4), 327–329.
Formisano, C., Rigano, D., Napolitano, F., Senatore, F., Arnold, N.A., Piozzi, F., Rosselli, S., 2007. Volatile constituents of *Calamintha origanifolia* Boiss. growing wild in Lebanon. Nat. Prod. Commun. 2 (12), 1934578X0700201213.
Frizzo, C.D., Serafini, L.A., Dellacassa, E., Lorenzo, D., Moyna, P., 2001. Essential oil of *Baccharis uncinella* DC. From southern Brazil. Flavour Fragrance J. 16 (4), 286–288.
Galanakis, C.M., Goulas, V., Tsakona, S., Manganaris, G.A., Gekas, V., 2013. A knowledge base for the recovery of natural phenols with different solvents. Int. J. Food Prop. 16 (2), 382–396.
Gillespie, K.M., Chae, J.M., Ainsworth, E.A., 2007. Rapid measurement of total antioxidant capacity in plants. Nat. Protoc. 2 (4), 867–870.
Gocmen, D., Gurbuz, O., Rouseff, R.L., Smoot, J.M., Fatih Dagdelen, A., 2004. Gas chromatographic-olfactometric characterization of aroma active compounds in sun-dried and vacuum-dried tarhana. Eur. Food Res. Technol. 218, 573–578.
Göger, G., Türkyolu, Ü., Gürşen, E.N., Yur, S., Karaduman, A.B., Göger, F., Tekin, M., Özek, G., 2021. Phytochemical characterisation of *Phlomis linearis* Boiss. & Bal and screening for anticholinesterase, antiamylase, antimicrobial, and cytotoxic properties. Turk. J. Chem. 45 (2), 387–399.
Gölükcü, M., Toker, R., Tokgöz, H., Turgut, D.Y., 2015. Farklı yöntemlerle elde edilen turunç (*Citrus aurantium* L.) kabuk yağlarının uçucu yağ bileşimleri. Derim 32 (2), 161–170.
Govaerts, R., Nic Lughadha, E., Black, N., Turner, R., Paton, A., 2021. The World Checklist of Vascular Plants, a continuously updated resource for exploring global plant diversity. Sci. Data 8 (1), 215.
Hafsa, M. Ben, Besbes, M., Mahjoub, M.A., Mighri, Z., 2009. Antimicrobial, antioxidant activities and total phenolic content of the acetonic, ethanolic and aqueous extracts of *Scabiosa arenaria* (Forsk). J. Med. Plants Nat. Prod 1, 59–68.
Halket, J.M., Waterman, D., Przyborowska, A.M., Patel, R.K.P., Fraser, P.D., Bramley, P. M., 2005. Chemical derivatization and mass spectral libraries in metabolic profiling by GC/MS and LC/MS/MS. J. Exp. Bot. 56 (410), 219–243.
Harmon, A.D., 2020. Solid-phase microextraction for the analysis of flavors. In: Techniques for Analyzing Food Aroma. CRC Press, pp. 81–112.
Hashizume, M., Gordon, M.H., Mottram, D.S., 2007. Light-induced off-flavor development in cloudy apple juice. J. Agric. Food Chem. 55 (22), 9177–9182.
Hazzit, M., Baaliouamer, A., Faleiro, M.L., Miguel, M.G., 2006. Composition of the essential oils of *Thymus* and *Origanum* species from Algeria and their antioxidant and antimicrobial activities. J. Agric. Food Chem. 54 (17), 6314–6321.
Hlila, M.B., Majouli, K., Mosbah, H., ben Jannet, H., Aouni, M., Selmi, B., Berhardt, L.V., 2018. Phytochemical and pharmacological properties of *Scabiosa* L. Genus. Adv. Medicine Biol. 105.
Horax, R., Hettiarachchy, N., Chen, P., 2010. Extraction, quantification, and antioxidant activities of phenolics from pericarp and seeds of bitter melons (*Momordica charantia*) harvested at three maturity stages (immature, mature, and ripe). J. Agric. Food Chem. 58 (7), 4428–4433.
İşcan, G., Köse, Y.B., Demirci, B., Can Başer, K.H., 2011. Anticandidal activity of the essential oil of *Nepeta transcaucasica* Grossh. Chem. Biodivers. 8 (11), 2144–2148.
Isidorov, V.A., 2020. GC-MS of Biologically and Environmentally Significant Organic Compounds: TMS Derivatives. John Wiley & Sons.
Javidnia, K., Miri, R., Javidnia, A., 2006. Constituents of the essential oil of *Scabiosa flavida* from Iran. Chem. Nat. Compd. 42, 529–530.
Jirovetz, L., Buchbauer, G., Stoyanova, A., Balinova, A., Guangjiun, Z., Xihan, M., 2005. Solid phase microextraction/gas chromatographic and olfactory analysis of the scent and fixative properties of the essential oil of *Rosa damascena* L. from China. Flavour Fragrance J. 20 (1), 7–12.
Joichi, A., Yomogida, K., Awano, K., Ueda, Y., 2005. Volatile components of tea-scented modern roses and ancient Chinese roses. Flavour Fragrance J. 20 (2), 152–157.
Jorge, T.F., Mata, A.T., António, C., 2016. Mass spectrometry as a quantitative tool in plant metabolomics. Phil. Trans. Math. Phys. Eng. Sci. 374 (2079), 20150370.
Kang, H.-R., Hwang, H.-J., Lee, J.E., Kim, H.R., 2016. Quantitative analysis of volatile flavor components in Korean alcoholic beverage and Japanese sake using SPME-GC/MS. Food Sci. Biotechnol. 25, 979–985.
Kaya, A., Başer, K.H.C., Demirci, B., Koca, F., 1999a. The essential oil of *Acinos alpinus* (L.) Moench growing in Turkey. Flavour Fragrance J. 14 (1), 55–59.
Kaya, A., Başer, K.H.C., Koca, F., 1999b. Essential oils of *Acinos troodi* (Post) Leblebici subsp. vardaranus Leblebici and subsp. grandiflorus Hartvig & Strid. Flavour Fragrance J. 14 (1), 50–54.
Kerrola, K.M., Kallio, H.P., 1994. Extraction of volatile compounds of angelica (*Angelica archangelica* L.) root by liquid carbon dioxide. J. Agric. Food Chem. 42 (10), 2235–2245.
Kılınç, H., Masullo, M., D'Urso, G., Karayildirim, T., Alankus, O., Piacente, S., 2020. Phytochemical investigation of *Scabiosa sicula* guided by a preliminary HPLC-ESI-MSn profiling. Phytochemistry 174, 112350.
Koprivnjak, O., Conte, L., Totis, N., 2002. Influence of olive fruit storage in bags on oil quality and composition of volatile compounds. Food Technol. Biotechnol. 40 (2), 129–134.
Kumar, N., Bharat, J.S., Kumar, A., Jaitak, V., 2024. Recent advancement in mass-based plant metabolomics: techniques, tools, and analytical approaches. Phytochem. Rev. 1–38.
Kundakovic, T., Fokialakis, N., Kovacevic, N., Chinou, I., 2007. Essential oil composition of *Achillea lingulata* and *A. umbellata*. Flavour Fragrance J. 22 (3), 184–187.
Lafka, T.-I., Sinanoglou, V., Lazos, E.S., 2007. On the extraction and antioxidant activity of phenolic compounds from winery wastes. Food Chem. 104 (3), 1206–1214.
Ledauphin, J., Basset, B., Cohen, S., Payot, T., Barillier, D., 2006. Identification of trace volatile compounds in freshly distilled Calvados and Cognac: carbonyl and sulphur compounds. J. Food Compos. Anal. 19 (1), 28–40.
Ledauphin, J., Saint-Clair, J.-F., Lablanquie, O., Guichard, H., Founier, N., Guichard, E., Barillier, D., 2004. Identification of trace volatile compounds in freshly distilled calvados and cognac using preparative separations coupled with gas chromatography−mass spectrometry. J. Agric. Food Chem. 52 (16), 5124–5134.
Lee, O.-H., Lee, B.-Y., Lee, J., Lee, H.-B., Son, J.-Y., Park, C.-S., Shetty, K., Kim, Y.-C., 2009. Assessment of phenolics-enriched extract and fractions of olive leaves and their antioxidant activities. Bioresour. Technol. 100 (23), 6107–6113.
Leffingwell, J.C., Alford, E.D., 2005. Volatile constituents of perique tobacco. J. Environ. Agric. Food Chem 4 (2), 899–915.
Lehbili, M., Magid, A.A., Kabouche, A., Voutquenne-Nazabadioko, L., Morjani, H., Harakat, D., Kabouche, Z., 2018. Triterpenoid saponins from *Scabiosa stellata* collected in North-eastern Algeria. Phytochemistry 150, 40–49.
Liang, L., Li, Y., Mao, X., Wang, Y., 2024. Metabolomics applications for plant-based foods origin tracing, cultivars identification and processing: Feasibility and future aspects. Food Chem. 139227.
Lin, J., Rouseff, R.L., Barros, S., Naim, M., 2002. Aroma composition changes in early season grapefruit juice produced from thermal concentration. J. Agric. Food Chem. 50 (4), 813–819.
Lourens, A.C.U., Reddy, D., Başer, K.H.C., Viljoen, A.M., Van Vuuren, S.F., 2004. In vitro biological activity and essential oil composition of four indigenous South African Helichrysum species. J. Ethnopharmacol. 95 (2–3), 253–258.
Mahmood, U., Kaul, V.K., Acharya, R., 2004. Volatile constituents of *Capillipedium parviflorum*. Phytochemistry 65 (14), 2163–2166.
Mallia, S., Fernández-García, E., Bosset, J.O., 2005. Comparison of purge and trap and solid phase microextraction techniques for studying the volatile aroma compounds of three European PDO hard cheeses. Int. Dairy J. 15 (6–9), 741–758.
Mendívil, E.A.S., Rodríguez, J.F.M., Espinosa, M.E., Fajardo, J.A.G., Vázquez, E.N.O., 2006. Chemical composition and fungicidal activity of the essential oil of *Thymus vulgaris* against *Alternaria citri*. E-Gnosis, 4, 0.
Möllenbeck, S., K, T., S, P., S, W., R, J., R, L., 1997. Chemical composition and analyses of enantiomers of essential oils from Madagascar. Flavour Fragrance J. 12 (2), 63–69.
Möller, R., Nürnberg, G., Albrecht, E., Ruth, W., Brockmann, G.A., Dannenberger, D., 2019. A method for analyzing fatty acids in cattle hair, with special emphasis on lauric acid and myristic acid. Eur. J. Lipid Sci. Technol. 121 (11), 1900143.
Mostafa, E.-N., Siahkola, N., 2016. Palynological study of some Iranian species of *Scabiosa* L. (Caprifoliaceae). Bangladesh J. Plant Taxon. 23 (2), 215.
Mouffouk, C., Hambaba, L., Haba, H., Mouffouk, S., Bensouici, C., Mouffouk, S., Hachemi, M., Khadraoui, H., 2018. Acute toxicity and in vivo anti-inflammatory effects and in vitro antioxidant and anti-arthritic potential of *Scabiosa stellata*. Orient. Pharm. Exp. Med. 18, 335–348.
Mukhopadhyay, S., Luthria, D.L., Robbins, R.J., 2006. Optimization of extraction process for phenolic acids from black cohosh (*Cimicifuga racemosa*) by pressurized liquid extraction. J. Sci. Food Agric. 86 (1), 156–162.

Mutavski, Z., Jerković, I., Nikolić, N.Ć., Radman, S., Flanjak, I., Aladić, K., Šubarić, D., Vulić, J., Jokić, S., 2024. Comprehensive phytochemical profiling of *Ulva lactuca* from the Adriatic Sea. Int. J. Mol. Sci. 25 (21), 11711.

Nielsen, G.S., Larsen, L.M., Poll, L., 2003. Formation of aroma compounds and lipoxygenase (EC 1.13. 11.12) activity in unblanched leek (*Allium ampeloprasum* Var. Bulga) slices during long-term frozen storage. J. Agric. Food Chem. 51 (7), 1970–1976.

NIST Mass Spectrometry Data Center, 2024. NIST mass spectrometry data center, William E. Wallace, director, "retention indices". In: Linstrom, P.J., Mallard, W.G. (Eds.), NIST Chemistry WebBook, NIST Standard Reference Database Number 69. National Institute of Standards and Technology, Gaithersburg MD, 20899. https://doi.org/10.18434/T4D303. (Accessed 26 August 2024).

Noorizadeh, H., Farmany, A., 2010. Exploration of linear and nonlinear modeling techniques to predict retention index of essential oils. J. Chin. Chem. Soc. 57 (6), 1268–1277.

Oreopoulou, V., Tzia, C., 2007. Utilization of plant by-products for the recovery of proteins, dietary fibers, antioxidants, and colorants. In: Utilization of By-Products and Treatment of Waste in the Food Industry. Springer, pp. 209–232.

Otang-Mbeng, W., Sagbo, I.J., 2020. Anti-melanogenesis, antioxidant and anti-tyrosinase activities of *Scabiosa columbaria* L. Processes 8 (2). https://doi.org/10.3390/pr8020236.

Özek, G., Özek, T., Baser, K.H.C., Duran, A., Sagiroglu, M., 2006. Comparison of essential oil of *Xanthogalum purpurascens* Lallem. obtained via different isolation techniques. J. Essent. Oil Res. 18 (2), 181–184.

Özek, G., Tekin, M., Haliloğlu, Y., Başer, K.H.C., Özek, T., 2018. Chemical compositions of *Achillea sivasica*: different plant part volatiles, enantiomers and fatty acids. Record Nat. Prod. 12 (2), 142.

Özek, T., Özek, G., Baser, K.H.C., Duran, A., 2005. Comparison of the essential oils of three endemic Turkish *Heracleum* species obtained by different isolation techniques. J. Essent. Oil Res. 17 (6), 605–610.

Özek, T., Özek, G., Başer, K.H.C., Duran, A., Sagiroglu, M., 2008. Composition of the essential oils of *Angelica sylvestris* L. var. sylvestris isolated from the fruits by different isolation techniques. J. Essent. Oil Res. 20 (5), 408–411.

Öztürk, N., Tuncel, M., Tuncel, N.B., 2007. Determination of phenolic acids by a modified HPLC: its application to various plant materials. J. Liq. Chromatogr. Relat. Technol. 30 (4), 587–596.

Palma, M., Piñeiro, Z., Barroso, C.G., 2002. In-line pressurized-fluid extraction–solid-phase extraction for determining phenolic compounds in grapes. J. Chromatogr. A 968 (1–2), 1–6.

Papoti, V.T., Tsimidou, M.Z., 2009. Looking through the qualities of a fluorimetric assay for the total phenol content estimation in virgin olive oil, olive fruit or leaf polar extract. Food Chem. 112 (1), 246–252.

Pennarun, A.-L., Prost, C., Haure, J., Demaimay, M., 2003. Comparison of two microalgal diets. 2. Influence on odorant composition and organoleptic qualities of raw oysters (*Crassostrea gigas*). J. Agric. Food Chem. 51 (7), 2011–2018.

Perestrelo, R., Fernandes, A., Albuquerque, F.F., Marques, J.C., Câmara, J.D.S., 2006. Analytical characterization of the aroma of Tinta Negra Mole red wine: identification of the main odorants compounds. Anal. Chim. Acta 563 (1–2), 154–164.

Pino, J.A., Almora, K., Marbot, R., 2003. Volatile components of papaya (*Carica papaya* L., Maradol variety) fruit. Flavour Fragrance J. 18 (6), 492–496.

Píry, J., Príbela, A., Ďurčanská, J., Farkaš, P., 1995. Fractionation of volatiles from blackcurrant (*Ribes nigrum* L.) by different extractive methods. Food Chem. 54 (1), 73–77.

Polatoğlu, K., Servi, H., Özçınar, Ö., Nalbantsoy, A., Gücel, S., 2017. Essential oil composition of endemic *Arabis purpurea* Sm. & *Arabis cypria* Holmboe (Brassicaceae) from Cyprus. J. Oleo Sci. 66 (1), 65–70.

Pozo-Bayón, M.A., Ruíz-Rodríguez, A., Pernin, K., Cayot, N., 2007. Influence of eggs on the aroma composition of a sponge cake and on the aroma release in model studies on flavored sponge cakes. J. Agric. Food Chem. 55 (4), 1418–1426.

Priestap, H.A., van Baren, C.M., Leo, Lira, Di, P., Prado, H.J., Neugebauer, M., Mayer, R., Bandoni, A.L., 2002. Essential oils from aerial parts of *Aristolochia gibertii* Hook. Flavour Fragrance J. 17 (1), 69–71.

Rahmouni, N., Pinto, D.C.G.A., Beghidja, N., Benayache, S., Silva, A.M.S., 2018. *Scabiosa stellata* L. phenolic content clarifies its antioxidant activity. Molecules 23 (6), 1285.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26 (9–10), 1231–1237.

Rochat, S., Egger, J., Chaintreau, A., 2009. Strategy for the identification of key odorants: application to shrimp aroma. J. Chromatogr. A 1216 (36), 6424–6432.

Rohloff, J., 2015. Analysis of phenolic and cyclic compounds in plants using derivatization techniques in combination with GC-MS-based metabolite profiling. Molecules 20 (2), 3431–3462.

Sagrero-Nieves, L., Bartley, J.P., Espinosa, G.B., Dominguez, X.A., Verde, J.S., 1997. Essential oil composition of *Aristolochia brevipes* Benth. Flavour Fragrance J. 12 (6), 401–403.

Schepetkin, I.A., Özek, G., Özek, T., Kirpotina, L.N., Khlebnikov, A.I., Quinn, M.T., 2022. Neutrophil immunomodulatory activity of (−)-borneol, a major component of essential oils extracted from *Grindelia squarrosa*. Molecules 27 (15), 4897.

Schepetkin, I.A., Özek, G., Özek, T., Kirpotina, L.N., Klein, R.A., Khlebnikov, A.I., Quinn, M.T., 2023. Composition and biological activity of the essential oils from wild horsemint, yarrow, and yampah from subalpine meadows in southwestern Montana: immunomodulatory activity of dillapiole. Plants 12 (14), 2643.

Senger-Emonnot, P., Rochard, S., Pellegrin, F., George, G., Fernandez, X., Lizzani-Cuvelier, L., 2006. Odour active aroma compounds of sea fig (Microcosmus sulcatus). Food Chem. 97 (3), 465–471.

Shimizu, Y., Imayoshi, Y., Kato, M., Maeda, K., Iwabuchi, H., Shimomura, K., 2009. Volatiles from leaves of field-grown plants and shoot cultures of Gynura bicolor DC. Flavour Fragrance J. 24 (5), 251–258.

Shimoda, M., Wu, Y., Osajima, Y., 1996. Aroma compounds from aqueous solution of haze (Rhus succedanea) honey determined by adsorptive column chromatography. J. Agric. Food Chem. 44 (12), 3913–3918.

Shiratsuchi, H., Shimoda, M., Imayoshi, K., Noda, K., Osajima, Y., 1994. Off-flavor compounds in spray-dried skim milk powder. J. Agric. Food Chem. 42 (6), 1323–1327.

Shukurlu, E.N., Özek, G., Özek, T., Vitalini, S., 2023. Chemical composition of different plant part from Lactuca serriola L.–focus on volatile compounds and fatty acid profile. Zeitschrift für Naturforschung C 78 (7–8), 285–291.

Soria, A.C., Sanz, J., Martínez-Castro, I., 2009. SPME followed by GC–MS: a powerful technique for qualitative analysis of honey volatiles. Eur. Food Res. Technol. 228 (4), 579–590.

Spietelun, A., Marcinkowski, Ł., de la Guardia, M., Namieśnik, J., 2013. Recent developments and future trends in solid phase microextraction techniques towards green analytical chemistry. J. Chromatogr. A 1321, 1–13.

Stancher, B., Pertoldi, M.G., 1967. Characterization of commercial materials used in synthetic essential oil production: Identification and gas-chromatographic determination of impurities. Rassegna Chimica 19 (3), 99–109.

Strangl, M., Ortner, E., Buettner, A., 2019. Evaluation of the efficiency of odor removal from recycled HDPE using a modified recycling process. Resourc. Conserv. Recycl. 146, 89–97.

Suleimenov, E. M., Atazharova, G. A., Demirci, B., Baser, K. H. C., & Adekenov, S. M. (2003). Essential oil composition of Artemisia lercheana and A. sieversiana of Kazakhstan flora in Recent problems of development of new medicines of natural origin in. In *Proceedings of symposium, St. Petersburg-Pushkin*.

Süzgeç Selçuk, S., Özek, T., Özek, G., Yur, S., Göger, F., Gurdal, M., Baser, K., 2021. The Leaf and the Gall volatiles of Salvia fruticosa Miller from Turkey: Chemical composition and biological activities. Records of Natural Products 15 (1).

Tamura, H., Kihara, S., Sugisawa, H., 1990. A new identification method for aliphatic compounds using linear equations of the GC retention index value. Agric. Biol. Chem. 54 (12), 3171–3176.

Tekin, M., Özek, G., Martin, E., Özek, T., Yılmaz, G., Başer, K.H.C., 2018. Essential oil characterization of *Cousinia sivasica* Hub.-Mor. Asteraceae. Biol. Divers. Conserv. 11 (2), 16–21.

Ter Heide, R., 1976. Studies on terpenes: III. Gas chromatography of acyclic monoterpene alcohols. Journal of Chromatography A 129, 143–154.

Thakeow, P., Angeli, S., Weißbecker, B., Schütz, S., 2008. Antennal and behavioral responses of Cis boleti to fungal odor of Trametes gibbosa. Chem. Sens. 33 (4), 379–387.

Thomas, A.F., Bassols, F., 1992. Occurrence of pyridines and other bases in orange oil. J. Agric. Food Chem. 40 (11), 2236–2243.

Toda, H., Yamaguchi, K., Shibamoto, T., 1982. Isolation and identification of banana-like aroma from banana shrub (Michellia figo Spreng). J. Agric. Food Chem. 30 (1), 81–84.

Trendafilova, A., Ozek, G., Yurb, S., Goger, F., Ozek, T., Rangelov, M., Aneva, I., 2022. Caracterización química, actividad antioxidante, potencial inhibidor de a-amilasa y acetilcolinesterasa de Angelica pancicii Vandas ex Velen. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 21 (4), 418–430.

Trendafilova, A., Todorova, M., Ozek, T., Ozek, G., Aneva, I., 2020. Volatile constituents of four Inula species of Bulgarian origin. Biochem. System. Ecol. 90, 104035.

Umano, K., Hagi, Y., Nakahara, K., Shoji, A., Shibamoto, T., 2000. Volatile chemicals identified in extracts from leaves of Japanese mugwort (Artemisia princeps Pamp.). J. Agric. Food Chem. 48 (8), 3463–3469.

Umano, K., Hagi, Y., Nakahara, K., Shyoji, A., Shibamoto, T., 1995. Volatile chemicals formed in the headspace of a heated D-glucose/L-cysteine Maillard model system. J. Agric. Food Chem. 43 (8), 2212–2218.

Umano, K., Shibamoto, T., 1987. Analysis of headspace volatiles from overheated beef fat. J. Agric. Food Chem. 35 (1), 14–18.

Vellutini, M., Baldovini, N., de Rocca Serra, D., Tomi, F., Casanova, J., 2005. β-Cyclolavandulyl and β-isocyclolavandulyl esters from *Peucedanum paniculatum* L., an endemic species to Corsica. Phytochemistry 66 (16), 1956–1962.

Vernon, F., Suratman, J.B., 1983. The retention index system applied to alkylbenzenes and monosubstituted derivatives. Chromatographia 17, 600–604.

Vinogradov, B.A., 2004. Production, composition, properties and application of essential oils, 2004, retrieved from. http://viness.narod.ru.

Vl, S., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299, 152–178.

Wang, J., Liu, K., Li, X., Bi, K., Zhang, Y., Huang, J., Zhang, R., 2017. Variation of active constituents and antioxidant activity in *Scabiosa tschiliensis* Grunning from different stages. J. Food Sci. Technol. 54, 2288–2295.

Wedge, D.E., Klun, J.A., Tabanca, N., Demirci, B., Ozek, T., Baser, K.H.C., Liu, Z., Zhang, S., Cantrell, C.L., Zhang, J., 2009. Bioactivity-guided fractionation and GC/MS fingerprinting of *Angelica sinensis* and *Angelica archangelica* root components for antifungal and mosquito deterrent activity. J. Agric. Food Chem. 57 (2), 464–470.

Weyerstahl, P., Marschall, H., Weirauch, M., Thefeld, K., Surburg, H., 1998. Constituents of commercial Labdanum oil. Flavour Fragrance J. 13 (5), 295–318.

Wong, K.C., Lim, T.B., Ali, D.M.H., 2006. Essential oil of *Homalomena sagittifolia* Jungh. Flavour Fragrance J. 21 (5), 786–788.

Wu, C.M., Liou, S.E., 1992. Volatile components of water-boiled duck meat and Cantonese style roasted duck. J. Agric. Food Chem. 40 (5), 838–841.

Wu, Y.-F.G., Cadwallader, K.R., 2002. Characterization of the aroma of a meatlike process flavoring from soybean-based enzyme-hydrolyzed vegetable protein. J. Agric. Food Chem. 50 (10), 2900–2907.

Yamaguchi, K., Shibamoto, T., 1981. Volatile constituents of green tea, *Gyokuro* (*Camellia sinensis* L. var Yabukita). J. Agric. Food Chem. 29 (2), 366–370.
Yur, S., Tekin, M., Göger, F., Başer, K.H.C., Özek, T., Özek, G., 2017. Composition and potential of *Tanacetum haussknechtii* Bornm. Grierson as antioxidant and inhibitor of acetylcholinesterase, tyrosinase, and α-amylase enzymes. Int. J. Food Prop. 20 (Suppl. 3), S2359–S2378.
Yu, Y., Lu, Z., Yu, N., Xu, W., Li, G., Shi, J., Xu, Z., 2012. HS-SPME/GC-MS and chemometrics for volatile composition of Chinese traditional aromatic vinegar in the Zhenjiang region. J. Inst. Brew. 118 (1), 133–141.
Zarate, E., Boyle, V., Rupprecht, U., Green, S., Villas-Boas, S.G., Baker, P., Pinu, F.R., 2016. Fully automated trimethylsilyl (TMS) derivatisation protocol for metabolite profiling by GC-MS. Metabolites 7 (1), 1.
Zengin, G., Sarikurkcu, C., Aktumsek, A., Ceylan, R., 2014. *Sideritis galatica* Bornm.: a source of multifunctional agents for the management of oxidative damage, Alzheimer's and diabetes mellitus. J. Funct.Foods 11, 538–547.
Zeng, Z., Xie, R., Zhang, T., Zhang, H., Chen, J.Y., 2011. Analysis of volatile compositions of *Magnolia biondii* pamp by steam distillation and headspace solid-phase micro-extraction. J. Oleo Sci. 60 (12), 591–596.
Zhou, W., Pawliszyn, J., 2024. Perspective on SPME-MS: green and high-performance methods for rapid screening. Anal. Chim. Acta 1291, 342244.