ORIGINAL ARTICLE
DOI: 10.4274/tjps.galenos.2025.12247

Comprehensive Investigation of Phytochemical Constituents and Biological Activities of *Scabiosa pseudograminifolia* Hub.-Mor.

ÖĞÜT et al. Investigation of *Scabiosa pseudograminifolia* Hub.-Mor.

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25.09.2024
16.02.2025

Epub: 08.04.2025

Cite this article as: ÖĞÜT K, ÖZEK G, ÖZTÜRK N, TEKİN M, ÖZEK T. Comprehensive investigation of phytochemical constituents and biological activities of *Scabiosa pseudograminifolia* Hub.-Mor. Turk J Pharm Sci.

ABSTRACT
Objectives: This investigation involved a detailed study of the phytochemical composition and biological properties of the endemic species *Scabiosa pseudograminifolia* Hub.-Mor. (*Caprifoliaceae*) growing in Sivas province of Türkiye. The plant materials were used to obtain essential oils as well as n-hexane, methanol, aqueous extracts, and fatty acids.
Materials and Methods: The gas chromatography-flame ionization detection/mass spectrometry technique was used to analyze the chemical compositions of essential oils and fatty acids.
Results: The major volatiles identified in the essential oil were hexadecanoic acid (30.2%) and linalool (15.6%). The main leaf volatiles obtained with the mass selective detector-solid phase microextraction technique were (*Z*)-3-hexenal (34.0%) and 6-methyl-1-octanol (4.3%). In the flowers, (*Z*)-3-hexenal (29.1%), hexanol (7.7%), linalool (5.0%), and hexanal (4.4%) were identified as the major components. Non-adecanoic (21.9%, 21.6%) and hexadecanoic (20.8%, 18.3%) acids were identified as the major fatty acids in the leaf and flowers of *S. pseudograminifolia*, respectively.
The essential oil of *S. pseudograminifolia* exhibited the highest Trolox Equivalent Antioxidant Activity value (2.39±0.15 mM). SP_W and methanol extract (SP_M) exhibited the most potent free radical scavenging activity, with inhibitory concentration$_{50}$ (IC_{50}) values of 0.16±0.04 mg/mL and 0.19±0.03 mg/mL, respectively. SP_M exhibited a substantial capacity to inhibit the oxidation of β-carotene and linoleic acid, with an IC_{50} value of 0.73±0.001 mg/mL. SP_W exhibited the greatest Oxygen Radical Absorbance Capacity values (248.43±15.44 µmol TE/g extract). The SP_W exhibited the highest phenolic content (0.52±0.01 mgGAE/$g_{extract}$) and flavonoid content (0.081±0.002 mgQE/$g_{extract}$). The extracts demonstrated a weak inhibitory effects on the α-amylase enzyme. Chlorogenic acid was detected in significant amounts in the polar extracts of *S. pseudograminifolia*.
Conclusion: This research includes the first biological activity and phytochemical studies on *S. pseudograminifolia* Hub.-Mor. growing in Sivas province of Türkiye.
Keywords: *Scabiosa pseudograminifolia,* essential oil, phenolics, biological activity

INTRODUCTION
The genus *Scabiosa* L. is a member of the Dipsacaceae subfamily, which is a part of the *Caprifoliaceae* family.[1,2] Although it originated in the Mediterranean region and the Near East, it is a family that has spread to different regions, from Northern Europe to East Asia, from Central Africa to South Africa.[3] The genus *Scabiosa* encompasses a total of 80 species worldwide, including 43 found in Europe, while the remaining species are distributed across Africa and Asia.[4] About 34 *Scabiosa* species were recorded in the flora of Türkiye.[5] The nomenclature of the genus is derived from the Latin term "scabiosus or scabies," *Sarcoptes scabiei* L., known as the itching mite or scab beetle, which causes highly contagious parasitic skin infections. It is thought to be given the name *Scabiosa* due to the use of multiple species for its treatment.[6] Although the members of the genus *Scabiosa* are generally known as "Uyuz Otu" in Türkiye, they have recently been called "Yazı Süpürgesi, Gicikotu, Kavurotu, Puk, Zivan".[7]

The literature review revealed that different species of the genus *Scabiosa* are commonly employed in traditional medicine to treat specific health issues. Specific species of *Scabiosa* are extensively utilized across various industries, including food, medicinal products, and skincare;[8] *Scabiosa columbaria* L. is traditionally employed for treating diphtheria, and *Scabiosa comosa* Fisch. Ex Roem. and Schult. is utilized in Mongolian and Tibetan traditional medicine for the treatment of liver ailments.[9] Also, qingganjiuwei powder, consisting of nine herbal components including *S. comosa*, is frequently used as an anti-fibrosis agent for patients with chronic liver disease in Inner Mongolia. This medication is approved by the Inner Mongolia Region Drug Administration for the treatment of liver disorders.[10] *Scabiosa atropurpurea L.* has been employed as a diuretic agent for acne, while S. succisa L. has been utilized in the treatment of asthma, bronchial pneumonia, and influenza. Furthermore, the external application of herbs from this particular species has been suggested for the treatment of respiratory, urogenital, and some skin conditions, such as herpes, ringworm, and scabies, as well as ulcers.[11] *Scabiosa stellata* L. is utilized for the treatment of heel fissures.[12] *Scabiosa tschilliensis* Grüning is utilized for hepatic disorders.[13]

With the phytochemical studies of a few *Scabiosa* species, the presence of coumarins, flavonoids, iridoids, pentacyclic triterpenoids, iridoid glucosides, and monoterpenoid glucoindole alkaloids has been reported.[9,14-18] Depending on this phytochemical content, the *Scabiosa* genus has demonstrated antidiabetic, hepatoprotective, analgesic, anti-inflammatory, antioxidant, antibacterial, anti-melanogenesis**,** anti-tyrosinase and anti-parasitic properties.[10]

To the best of our knowledge, there is no study on *Scabiosa pseudograminifolia* Hub.-Mor. Therefore, we have conducted the first study on this plant, which grows in Türkiye, by investigating its phytochemical and biological activity profile.

MATERIALS AND METHODS

Chemicals

The chemicals utilized in this study: n-hexane, dimethyl sulfoxide (DMSO), methanol, ethanol, formic acid, hydrochloric acid, glacial acetic acid, Folin-Ciocalteu (FC) reagent, boron trifluoride (BF3) 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). 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, along with propylparaben as an internal standard, were acquired from Sigma-Aldrich (St. Louis, MO, USA) or Merck (GmbH, Darmstadt, Germany). The rest of the chemicals utilized in this study were obtained from Merck (Germany).

Instruments

An Agilent 5975 GC-MSD system manufactured byAgilent Technologies (Santa Clara, CA, USA) was used to conduct GC-MS analyses. The SPME technique for volatiles was conducted using a manual SPME holder (57330-U, SUPELCO, Bellefonte, PA) and the 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 (Greiner), and a 96-deep-well round-bottom polypropylene plate with a volume of 2.2 mL. The absorbance readings were recorded using a BioTek Powerwave XS microplate reader. 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 conducted using a Zorbax Eclipse XDB-C18 column (150 mm, 4.6 mm, 5 µm particle size), manufactured by Agilent in Waldbronn, Germany. The Human UP 9000 System (18 mW)'s 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 C18 Ultrasphere column (INERTSIL, Waldbronn, Germany), (100x4.6 mm i.d. 3 microns) was used to analyze phenolic acids.

Plant material

The plant material *S. pseudograminifolia* Hub.-Mor. was collected at Sivas, Kangal-Gürün junction. The identification of the plant was conducted by Prof. Dr. Mehmet Tekin (Trakya University Faculty of Pharmacy, Department of Pharmaceutical Botany) and it was registered in Trakya University Faculty of Pharmacy Herbarium (code: 1621).

Hydrodistillation of essential oil

The essential oil was obtained from air-dried plant parts by hydrodistillation for 3 hours using Clevenger-type equipment, following the techniques outlined in the European Pharmacopoeia.[20] The essential oil yield was calculated on a moisture-free basis. The oil underwent dehydration using anhydrous sodium sulfate and was thereafter preserved in amber glass vials at 4 °C until the gas chromatographic and biological activity analyses.

Microsteam distillation-solid phase microextraction (MSD-SPME) of the volatiles

In the experiment, 1.0 g of the leaf and flower parts were separately added to a 25 mL flask containing 3.0 mL of water.[21] A distillation head with a septum for SPME holder needle entrance and a condenser was attached to a flask that was specifically designed for refluxing rather than distillation. In the pre-experiment, the fibre underwent conditioning at a temperature of 250 °C for 15 minutes. The electric heater was used gradually for the evaporation of volatiles from the sample. Once the evaporation started, the fibre was removed through the needle and placed in the headspace above the samples. The MSD-SPME process was conducted at the boiling point of water. The equilibrium time refers to the interval between the introduction of SPME fibre into the flask and the commencement of the extraction process. A sufficient extraction time of 3.0 minutes was employed following the establishment of equilibrium. Following the extraction time, the carefully loaded SPME fibre was withdrawn into the needle. Later, the needle was meticulously separated from the plug and employed for thermal desorption at the inlet port of gas chromatography-mass spectrometry (GC/MS) equipment.

Lipid extraction and fatty acid derivatization

Fatty acid research involved a series of consecutive steps, which included preparing the sample, extracting total lipids, methylating fatty acids, and then analyzing the fatty acid methyl esters using GC-MS/flame ionization detection (FID).[22] 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 water-soluble chemicals were retained in the upper layer of methanol-water. In the 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 to the mixture and vortexed. Following that, the organic solvent phase was filtered through a special filter of the kit. 200 µL of the extract was dried under nitrogen gas and then subjected to transesterification with BF3-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 minutes. The uppermost layer, hexane, was transported in a vial, concentrated under nitrogen gas, and thereafter injected into the GC-MS/FID system.

Preparation of extracts

The extracts of *S. pseudograminifolia* were prepared by fractionating the same powdered plant material with n-hexane, methanol, and water (plant material/solvent ratio 1:10). For each extract, the maceration process involved continuous shaking for 48±2 hours under ambient conditions. The supernatants obtained were filtered using Whatman filter paper. Subsequently, the organic solvents were removed from the filtrates using reduced pressure to obtain dry extracts. However, the aqueous extracts were dried using the lyophilization technique. The dried extracts were stored in amber glass vials at 4 °C until further analysis.[23,24]

The dried extracts were solved in 10% DMSO-methanol (10 mg/mL) and utilized as stock solutions prior to biological activity screening, total phenolic content assessment, and total flavonoid content evaluation

GC/MS analysis

The GC-MS analysis was conducted using the previously stated settings.[25] An Agilent Innowax FSC column, 60 m x0.25 mm, with a film thickness of 0.25 m was used, along with a carrier flow rate of 0.8 mL/min. The GC oven was initially set at 60 °C for 10 minutes. It was then gradually increased to 220 °C at a rate of 4 ° C per minute and held at that temperature for 10 minutes. Finally, the temperature was increased to 240 °C at a rate of 1°C per minute. The split ratio was adjusted to 40:1, while the injector temperature was set to 250 °C. The MS spectra were recorded at 70 eV, covering a mass range of 35 to 450 *m/z*.

Gas chromatography analysis

The GC assay was conducted with an Agilent 6890N GC equipment. To achieve an equivalent elution sequence as observed in GC-MS, the elution line was divided between MS and FID detectors, and a single injection was conducted using the same column and suitable operational parameters. The temperature of the FID was set at 300 °C. The contents of the essential oil and fatty acid methyl esters were determined 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".[26] Confirmation was accomplished by utilizing the exclusive "Başer Library of Essential Oil Constituents" database, which was acquired through chromatographic investigations conducted on pure compounds under identical equipment and conditions. For confirmation of identified compounds, each of the compounds' relative retention indices was calculated with a C8-C40 n-alkane standard mixture (Fluka, Buchs, Switzerland). The FID chromatograms were used to calculate the relative percentage of the separated individual compounds without normalization.

Reverse phase high performance liquid chromatography (RP-HPLC) analysis

The extracts of *S. pseudograminifolia* were subjected to RP-HPLC analysis to get profile of the phenolic acids. The chromatographic separation was conducted using two solvent systems: (A) a mixture of methanol, water, and formic acid in a ratio of 10:88:2 *(v/v/v)*, (B) a mixture of methanol, water, and formic acid in a ratio of

90:8:2 *(v/v/v)*, as reported previously.[27] The study employed a gradient elution program in the following manner: from 15-20 min, to 85% A; from 20-30 min, to 50% A; from 30-35 min, to 0% A; and from 36-42 min, back to 100% A. The flow-rate was 1 mL/min and the injection volume was 10 µL. Signals were detected at 280 nm. The relevant extracts were dissolved in a mixture of methanol and water (1:1 *v/v*) and injected into the HPLC. The peaks were identified using the following method: the separate phenolic acid standards were dissolved. The rate of peak normalization (peak area/peak retention time) of the relevant phenolic acids, was determined by calculating the integrated peak areas and their corresponding retention times. The quantities of these phenolic acids were then measured in the associated extracts using their calibration curves.

Total phenol content

The extracts of *S. pseudograminifolia* were evaluated for the total phenolic content measured as GA equivalent (GAE) using an FC reagent, according to previous procedure.[28] Methanol was used to prepare the stock solutions of the extracts and GA. The experimental procedure involved the combination of 20 µL of the sample solution (extract/GA), 1560 µL of ultrapure water, and 100 µL of FC reagent into a 96 deep-well plate. Following an incubation period of 1-8 minutes, 300 µL of a sodium carbonate solution (20%) was added to the mixture. The mixture was subjected to a 2-hour incubation period (at 25 °C in the dark).

Subsequently, 300 µL of the mixture was put into a 96-well microplate. The absorbance readings at a wavelength of 760 nm were subsequently compared to a GA 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 GA 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 [insert numerical value here]. The findings were quantified as mg GAE/g extract.

Total flavonoid content

The extracts of *S. pseudograminifolia* were evaluated for the total flavonoid content measured as quercetin equivalent (QE) using aluminum chloride as reagent. In the experiment, 80 µL of the sample solution (extract/quercetin), 80 µL of AlCl3, 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 minutes, 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.[29] 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 mg QE/g extract.

Trolox Equivalent Antioxidant Activity (TEAC) Test

The antioxidant potential of the extracts and essential oils of *S. pseudograminifolia* was assessed using ABTS+•, respectively, and calculated as Trolox equivalent.[30] At first, a solution of ABTS+• (7 mM) and potassium persulfate was prepared in pure water. Following a 16-hour incubation period in the dark, the aliquot of the solution was diluted with absolute ethanol until it reached an absorbance range of 0.700-0.800 at a wavelength of 734 nm. The extracts, essential oil (2 mg/mL), and Trolox (five dilutions ranging from 3.0 to 0.125 mM) were made in methanol (with 10% DMSO) as stock solutions. The experiment involved combining 10 µL sample (essential oil, extract, Trolox) with a 990 µL ABTS solution in a 96-deep well plate. Following a 30-minute incubation in the dark, a decrease in absorbance was recorded at a wavelength of 734 nm using a microplate reader. The ABTS•+ scavenging activity of the samples was quantified as TEAC and was determined using a linear equation calculated for Trolox (y=29.997x-0.6918). The calibration curve

Free Radical Scavenging Effect (DPPH) Test

The samples' ability to scavenge DPPH radicals was assessed using a modified version of the Brand-Williams method.[31] The extracts, essential oil (2 mg/mL), and standard inhibitor (0.1 mg/mL) were prepared in methanol with 10% DMSO as stock solutions. 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-well flat bottom plate cells. The mixtures were incubated in the dark for 30 minutes. The reduction in absorbance at a wavelength of 517 nm was recorded with the microplate reader. GA solution was employed in this test as the positive control. The experiments were conducted 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 includes all reagents except the test substance, is indicated as AbsControl. Abssample represents the absorbance of the sample after the addition of DPPH (Version 12.0).

Lipid Peroxidation Inhibition (β-Carotene Bleaching Test)

The present study aimed to assess the inhibitory impact of extracts and essential oils on lipid peroxidation using the β-carotene bleaching test with some modifications.[32] 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 (at a temperature of 40 °C) and then nitrogen gas. Following that, oxygenated ultrapure water (50 mL) was added and the mixture was vortexed. Before each experiment, the emulsion solution was freshly prepared 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 minutes for a total of 105 minutes at a wavelength of 492 nm. The experiments were conducted three times. The results of the experiment were calculated using the following formula:

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

Where AA is the antioxidant activity, $Abs_{0\ sample}$ and Abs_{120} sample are the absorbance values of the sample at 0 min and 120 min, and Abs_0 control and Abs_{120} control are the absorbance values of the control at 0 min and 120 min. In this context, AA represents the antioxidant activity, whereas $Abs_{0\ sample}$ and $Abs_{120\ sample}$ indicate the absorbance values of the sample at 0 minutes and 120 minutes, respectively. Similarly, $Abs_{0\ control}$ and $Abs_{120\ control}$ represent the absorbance values of the control at 0 minutes and 120 minutes.

Oxygen Radical Absorbance Capacity (ORAC) Test

The oxygen radical absorption capacity of the extracts and essential oil of *S. pseudograminifolia* was assessed using a microtiter assay[33] with certain modifications. A fluorescent probe was employed in the assay, while 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) was utilised 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 solution were combined and incubated for 30 minutes at 37 °C in the dark. The plate was shaken for 10 seconds following the addition of 25 µL of AAPH reagent after incubation. The fluorescence values were measured at 37 °C at 60-second intervals over a duration of 180 minutes. The excitation wavelength was 485 nm, and the emission wavelength was 535 nm. The under the curve (AUC) values were determined using the *SigmaPlot* program. The net AUC values were obtained using the following formula:

$$\mathrm{NET\ AUC} = \mathrm{AUC}_{sample} - \mathrm{AUC}_{blank}$$

The standard curve was derived by graphing the net AUC and the linear relationship between the concentration of Trolox and net AUC. The calculation of ORAC values was performed using Trolox equivalents. The experiments were conducted three times.

α-Amylase Inhibition Test

Following the previously reported assay,[34] the extracts and essential oil were tested for their capacity to inhibit the α-amylase enzyme. The inhibitor of the α-amylase enzyme employed in this study was acarbose. The extract and essential oil solutions were prepared in methanol (containing 10% DMSO) in appropriate quantities. For the experiment, 50 µL of the sample (extract/essential oil/acarbose) and 50 µL of an enzyme solution (0.8 U/mL in 20 mM of sodium phosphate buffer pH 6.9) were added to a 96-well plate. The mixture was then incubated for 10 minutes in the dark at 37 °C. Following the incubation period, 50 µL of a starch solution (0.05%) was added, and the resulting mixture was incubated for an additional 10 minutes in the dark at 37 °C. Following the incubation period, the reaction was terminated by adding 25 µL of HCl solution (1M). Finally, 100 µL of I_2/KI reagent solution was added to the wells. In the absence of an enzyme solution, a buffer solution was added to the blank samples. All the reagents were present in the control wells, except for the sample. The plate reader was used to record absorbance readings at a wavelength of 630 nm. The calculation of the inhibition was done using the following formula:

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

RESULTS AND DISCUSSION

The phytochemical profile of *S. pseudograminifolia* and its *in vitro* antioxidant and anti-α-amylase effects have been determined for the first time within the scope of this investigation. All these studies have contributed to closing the knowledge gap regarding *S. pseudograminifolia*. The leaves of *S. pseudograminifolia* have

undergone extraction using solvents of different polarity, namely n-hexane, methanol, and aqueous solution. Table 1 displays the extract yields.

Chemical composition of essential oil and volatile constituents

The constituents that make up the essential oil from *S. pseudograminifolia* aerial parts have been identified using GC-MS and GC-FID techniques. In addition to this, the combined MSD-SPME technique was utilized to investigate the plant's volatile constituents. The plant's leaves and flowers were analysed separately for this purpose. The hydrodistilled essential oil compounds, in the order in which they were eluted on the HP-Innowax FSC column, along with their relative percentages, retention indices, and method of identification, are listed in Table 2, as well as volatile constituents extracted with the MSD-SPME technique.

In the GC-MS study of *S. pseudograminifolia,* seventeen compounds accounted for 89.9% of the total identified components. Hexadecanoic acid (30.2%), linalool (15.6%), and dodecanoic acid (10.9%) are the main components of the essential oil from the of *S. pseudograminifolia* aerial parts derived through the hydrodistillation.

We also used the MSD-SPME technique to examine volatile components of leaves and flowers. From the leaves of *S. pseudograminifolia*, we found 68 volatile constituents, which made up 96.7% of all the components found using the MSD-SPME. The main volatile components were *(Z)*-3-hexenal (34.0%), 6-methyl-1-octanol (4.3%), 4-methyl-(2*E*) undecene# (3.6%), and linalool (3.1%). Additionally, from the flowers of *S. pseudograminifolia*, we found 72 volatile constituents, which made up 95% of all the components found using the MSD-SPME. The main volatile components were *(Z)*-3-hexenal (29.1%), hexanol (7.7%), linalool (5%), and hexanal (4.4%). Table 2 presents these findings.

TABLE 2

The volatile components of the aerial parts was compared to that of leaves and flowers, revealing that they did not have the same major compounds. In addition, it has been observed that the chemical composition of *S. pseudoraminifolia* differs from that of other *Scabiosa* species.

In the published literature, there are few gas chromatographic investigations of esseantial oils from aerial parts *Scabiosa* species. In *S. columbaria* subsp. *columbaria* var. *columbaria* L. the major volatile compounds were identified as 4-octadecenal (30.0%) in the flower and carvone (35.44%) in the leaf.[88] The main components of the essential oil were tricosane (15.5%), rosifoliol (15.3%), (*E*)-caryophyllene (10.7%), and α-humulene (7.9%) in the aerial parts of *S. flavida* Boiss. & Hausskn.[89] In the leaf essential oil of *S. maritima* L., the main components were hexahydrofarnesyl acetone (42.0%) and dodecanoic acid (17.2%). In the inflorescence of *S. maritima,* the main components of essential oil were 3-vinyl pyridine (23.5%) and hexahydrofarnesyl acetone (19.4%).[90] α-Thujone (34.4%), camphor (17.5%), and β-thujone (15.29%) constituted the major compounds of the fruit oil of *S. arenaria* Forssk from Tunisia, while chrysanthenone (23.4%), together with camphor (12.9%) and α-thujone (10.7%), were the main constituents essential oil of the leaf and stem. In the case of the flower oil, also chrysanthenone (38.5%), camphor (11.7%), and α-thujone (9.5%) were reported as the major compounds.[4]

Fatty acid chemical composition

This research enabled the initial assessment of the fatty acid composition of *S. pseudograminifolia*. The lipids of *S. pseudograminifolia* were isolated from the leaves and flowers with microscale techniques and subjected to transesterification with BF_3 reagent for subsequent GS-FID/MS analysis. Results of chromatographic separation and identification of the methyl ester derivatives are presented in Figures 4 and 5, and Table 3, respectively.

In our study, a total of 20 fatty acids comprising 99.8% of the oil in *S. pseudograminifolia* leaves were identified. The major fatty acids were methyl nonadecanoate (21.9%), methyl hexadecanoate (20.8%), and *(Z,Z,Z)*-9,12,15-methyl octadecatrienoate. Also, the flowers of *S. pseudograminifolia* included a total of 23 fatty acids, which comprised 78.3% of the oil. The major fatty acids were methyl hexadecanoate (18.3%), *(Z,Z)*-9,12-methyl octadecadienoate (12.4%), and *(Z,Z,Z)*-9,12,15-methyl octadecatrienoate (12.2%).

Based on the literature, *Scabiosa* species' leaves included a total of 19 fatty acids, ranging from lauric acid (C12:0) to nervonic acid (C24:1n9). The primary 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 (18:3n6; 0.73-13.95%). Arachidonic acid methyl ester (C20:4n6) was found in small amounts among the species that were examined. The overall proportion of saturated fatty acids in the species under investigation was calculated to range from 48.97% to 80.11%. In contrast, the proportion of unsaturated fatty acids was estimated to range from 13.62% to 25.39%.[91]

Total phenolic, flavonoid contents and biological activity results

The phenolic compounds make an important contribution to the total antioxidant potential of natural products. So, basically, their phenolic contents were specified, and the characterization of the extracts was to be determined. The total phenolic contents of *S. pseudograminifolia* extracts were assessed using a FC reagent, and the results were presented in terms of GA equivalent. The total flavonoid content of *S. pseudograminifolia* extracts was assessed as the QEs. The results of spectrophotometric assays are presented in Table 4.

An assessment was conducted to determine the antioxidant activity of the essential oil of *S. pseudograminifolia* and its hexane, methanolic, and aqueous extracts. The test results included the DPPH free radical scavenging effect, TEAC, ORAC, and β-carotene peroxidation inhibition assay.

There is a scarcity of reports regarding phenolic and flavonoid contents of *Scabiosa* species. The overall phenolic content of *S. arenaria* varied from 34.77 to 269.09 mg GAE/$g_{extract}$, while the quantities of flavonoid compounds ranged from 0.81 to 10.9 mg QE/$g_{extract}$. Previously, it has been reported that the aqueosus methanol extract and fractions from *S. atropurpurea* subsp. *maritima* contained the total phenolic content ranged from 17.7 to 186.75 mg GAE/$g_{extract}$, and the total flavonoid content varied from 4.38 to 208.69 mg catechin equivalent/g. The methanolic extract of *S. sicula* was found to have a total phenolic content of 2.67 mg GAE/$g_{extract}$. In the methanolic extracts of *S. columbaria* subsp. *columbaria* var. *columbaria* from Türkiye the phenolic content ranged from 269.833 to 640.111 µg GAE/mL, while the flavonoid content ranged from 6.060 to 13.527 µg QAE/mL.[14] In our study, we detected that the aqueous extract of *S. pseudograminifolia* exhibited the highest concentrations of phenols and flavonoids: 0.52±0.01 mg GAE/$g_{extract}$ and 0.081±0.002 mg QE/$g_{extract}$, respectively.

The literature demonstrated a strong suppression of DPPH free radicals in the aqueous extract of *S. arenaria*, with an inhibitory concentration $(IC)_{50}$ value of 0.18 mg/mL.[92] The crude extracts of *S. tschiliensis* exhibited DPPH-scavenging action, with an IC_{50} value of 25.68±1.21 µg/mL. They had much more DPPH-scavenging power than other plants from the *Scabiosa* genus that were studied, like *S. comosa* and *S. arenaria*.[93] According to the literature, the IC_{50} values for the crude extract of *S. atropurpurea* ranged from 22.42 to 415.23 mg/mL. The crude extract obtained from the leaves exhibited the most significant DPPH-scavenging activity compared to other plant parts.[94] In this study, we found that the extract's IC_{50} values ranged from 0.16 to 0.19 mg/mL, indicating no activity of the essential oil towards to DPPH free radicals. The TEAC value of the methanol extract from *S. sicula* was found to be 0.34±0.01 µg/mL. This is the concentration of Trolox solution that has the same antioxidant activity as a 1 mg/mL solution of the extract.[95] The TEAC value of *S. arenaria* was 0.56 mM Trolox/$g_{extract}$,[92] leaf parts of *S. columbaria,* it was 267.381±0.012 and 242.857±0.003, respectively.[88] The essential oil (2.39±0.15) and, aqueous extract (2.33±0.13) had the highest TEAC values, respectively, in contrast to DPPH activity. ORAC tests in *Scabiosa* species are rare and are the first in the literature to be performed in hexane, methanol, and water extracts, with the highest activity observed in water extract. In terms of β-carotene bleaching kinetics, it was observed that the methanolic fraction of *S. atropurpurea* and ascorbic acid (the reference compound) exhibited the highest level of efficacy in inhibiting β-carotene oxidation. These curves were nearly identical and showed their respective inhibition percentages were strikingly similar, with values of 97.19% and 100%. The findings indicate that the hexane and chloroform extracts exhibit a moderate level of antioxidant activity, with individual levels of 64.17% and 42.91%, respectively.[96] The inhibition values for the methanol extract were 0.730±0.001 mg/mL and those for the water extract were 1.4±0.2 mg/mL. 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. pseudograminifolia* was found to be insufficient.

HPLC analysis results

Some phenolic acids in the *S. pseudograminifolia* extracts have been determined using an RP-HPLC gradient system with a modified technique.[27] The HPLC results of *S. pseudograminifolia* aqueous and methanol extracts are shown in Table 5. The amounts are given in µg/$g_{extract}$.

Our results show that *S. pseudograminifolia* has significant amounts of chlorogenic acid, caffeic acid, protocatechic acid, *p*-coumaric acid, syringic acid, and ferulic acid (Table 5). Regarding the biological activity of extracts, the study revealed a significant positive connection between the activity and the total phenolic content of each extract. Therefore, in this investigation we have looked at the phenolic compounds in *S. pseudograminifolia*'s methanolic and aqueous extracts as a whole.

There are quite enough studies on the phenolic composition of *Scabiosa* species in the literature. These investigations have revealed that isoorientin and 4-O-caffeoylquinic acid are the primary compounds found in *S. stellata* extract.[97] Additionally, caffeoylquinic acid, rutin, ursolic acid, cyanuric acid, sinapic acid, luteolin, apigenin, quercetin, kaempherol, and tamarixetin have also been identified. Gallic, chlorogenic, caffeic, syringic, *p*-coumaric, sinapic, ferulic, catechin hydrate, epicatechin-3-*O*-gallate, luteolin-7-*O*-glucoside, isorhamnetin 3-*O*-glucoside, rutin, isoquercetin, myricetin, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, quercetin, naringenin, luteolin, isorhamnetin, and apigenin were detected in *S. atropurpurea* subsp. *maritima* hydromethanol extract.[94]

The methanol extracts from *S. columbaria* subsp. *columbaria* var. *columbaria* contained six phenolic compounds that were identified as the main ingredients. These were GA, catechin, 4-OH-benzoic acid, 4-OH-benzaldehyde, caffeic acid, and chlorogenic acid. The methanolic extract derived from *S. columbaria* subsp. *columbaria* contains GA and caffeic acid.[88]

CONCLUSION

This research includes the first biological activity and phytochemical studies on *S. pseudograminifolia* Hub.-Mor. growing in Sivas province of Türkiye.

The main constituents of the essential oil derived from *S. pseudograminifolia* were hexadecanoic acid (30.2%), linalool (15.6%), and dodecanoic acid (10.9%). The MSD-SPME method revealed that *(Z)*-3-hexenal (34.0%), 2-ethyl hexanol (3.2%), 6-methyl-1-octanol (4.3%), 4-methyl-(2*E*) undecene (3.6%), and linalool (3.1%) were were the major volatile components in the leaves of *S. pseudograminifolia*. Similarly, *(Z)*-3-hexenal (29.1%), hexanol (7.7%), linalool (5%) and hexanal (4.4%) were identified as the main volatile components in the flowers. Non-adecanoic (21.9%) and hexadecanoic (20.8%) acids were found as fatty acids in *S. pseudograminifolia*. The aqueous and methanol extracts exhibited a significant concentration of chlorogenic acid. The highest TEAC values were determined for essential oil and aqueous extract. The aqueous extract of *S. pseudograminifolia* exhibited the highest levels of mM, peroxidation inhibition and free radical scavenging potential. This may be attributed to the higher amount and number of phenolic compounds in the aqueus extract. The plant's ability to inhibit α-amylase enzyme is insufficient.
The determination of the phenolic and essential oil contents along with the biological activities of *S. pseudograminifolia* aerial part extracts, will contribute to both *in vitro* and *in vivo* biological activity tests of similar future studies.

Ethics
Ethics Committee Approval: Not required.
Informed Consent: Not required.

Footnotes
Authorship Contributions
Concept:
Design:
Data Collection or Processing:
Analysis or Interpretation:
Literature Search:
Writing:
Conflict of Interest: The authors declare no conflicts of interest.
Financial Disclosure: The authors declared that this study received no financial support.

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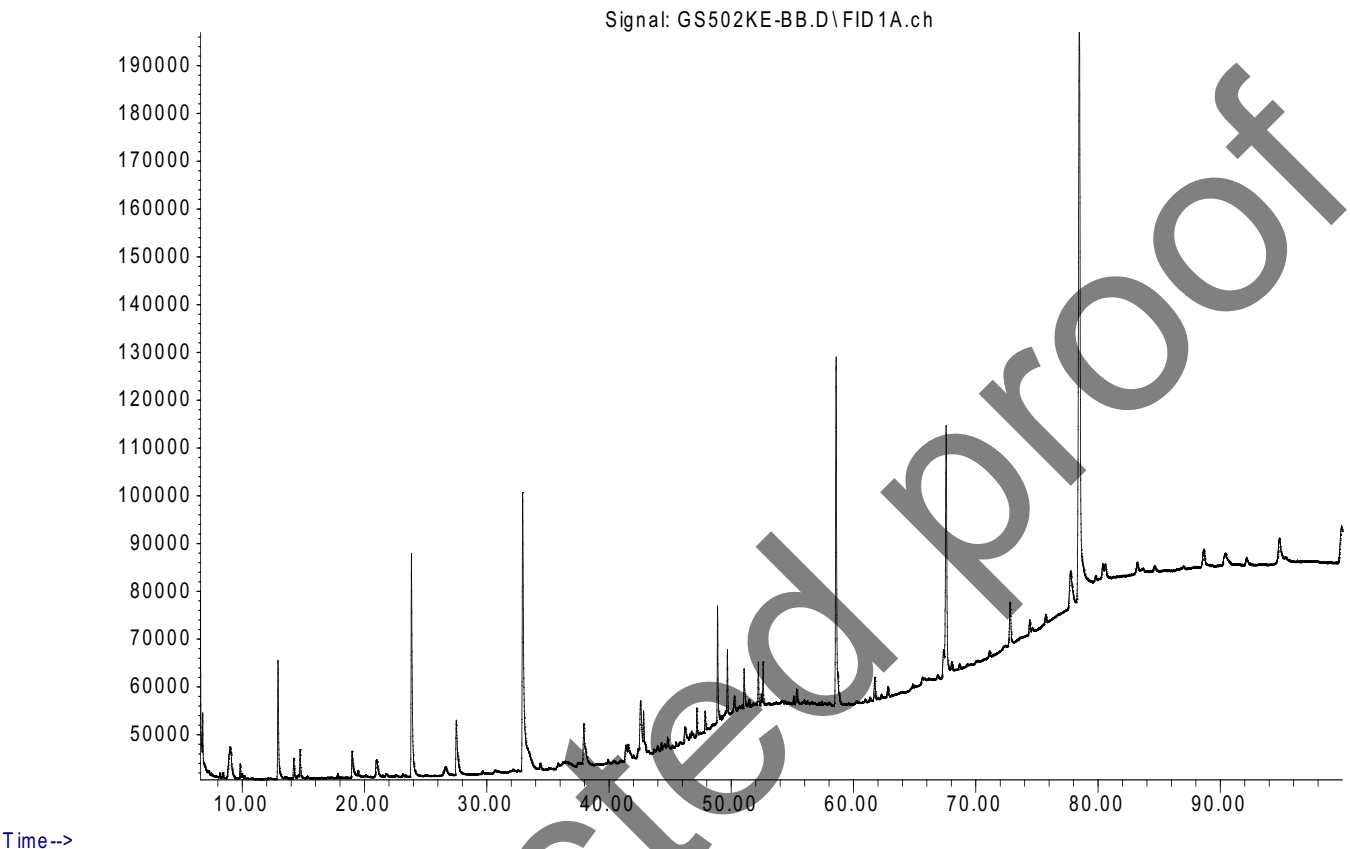

Figure 1. The chromatogram of essential oil obtained by hydrodistillation

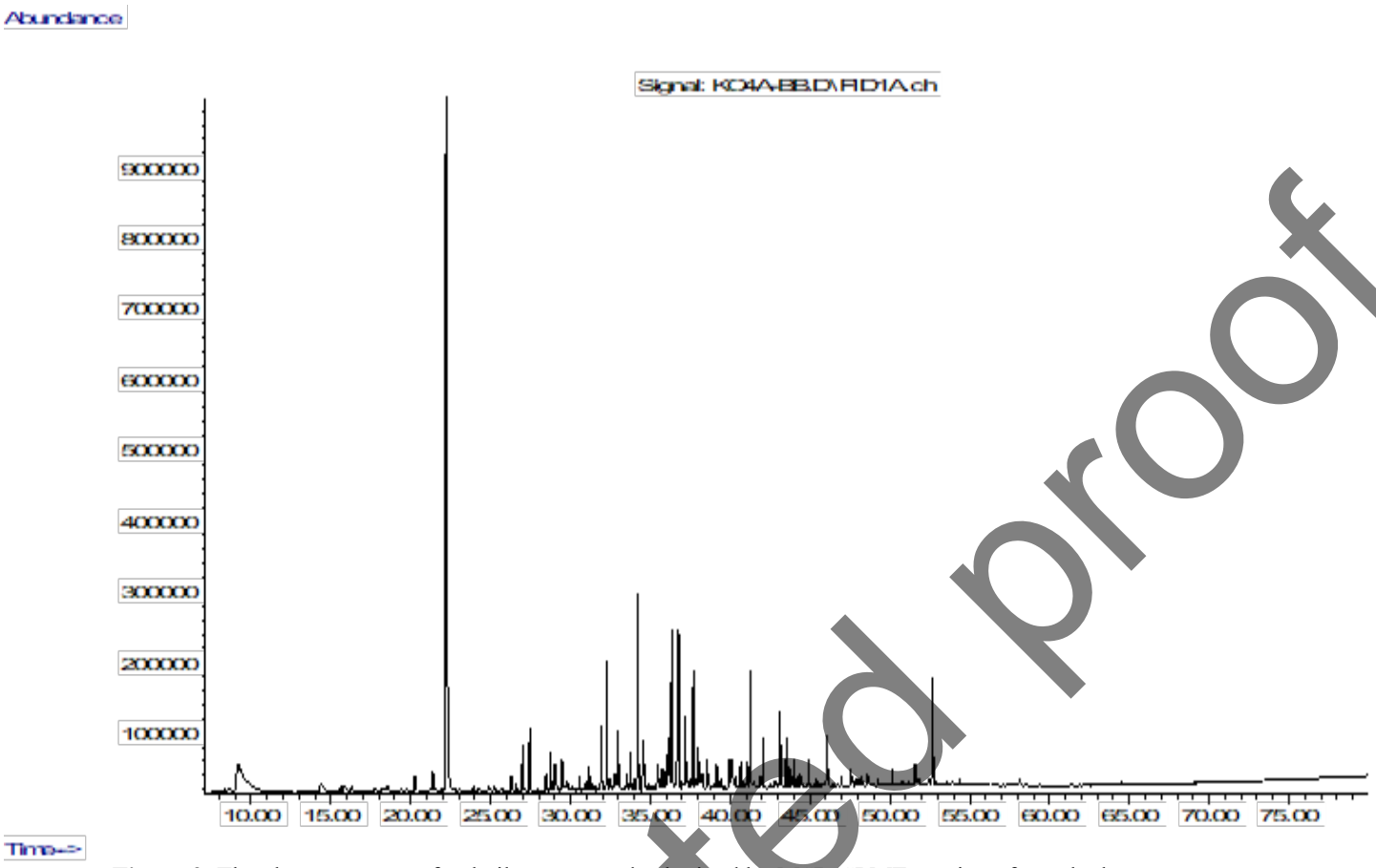

Figure 2. The chromatogram of volatile compounds obtained by MSD-SPME tecnique from the leaves
MSD-SPME: Microsteam distillation-solid phase microextraction

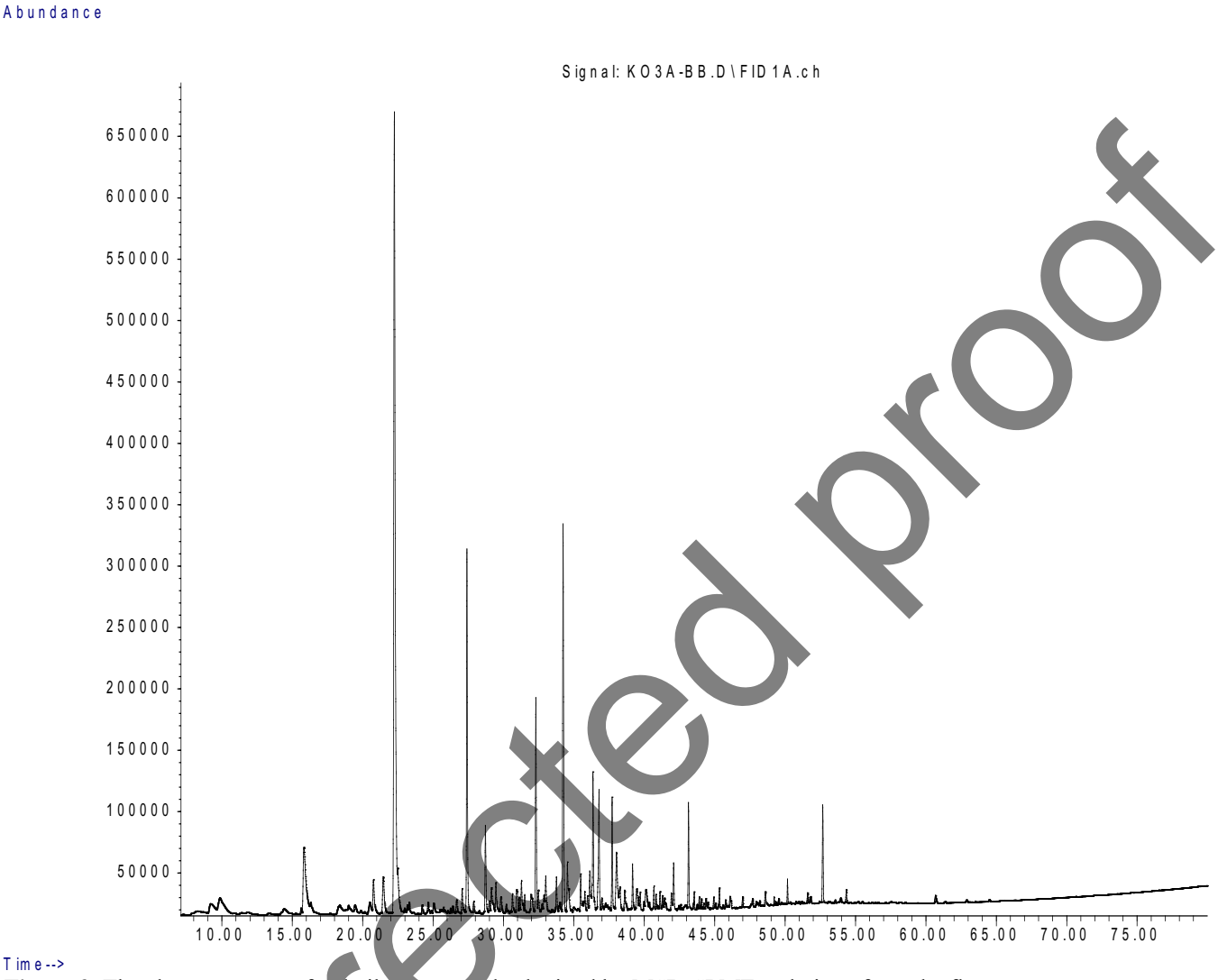

Figure 3. The chromatogram of volatile compounds obtained by MSD-SPME technique from the flowers
MSD-SPME: Microsteam distillation-solid phase microextraction

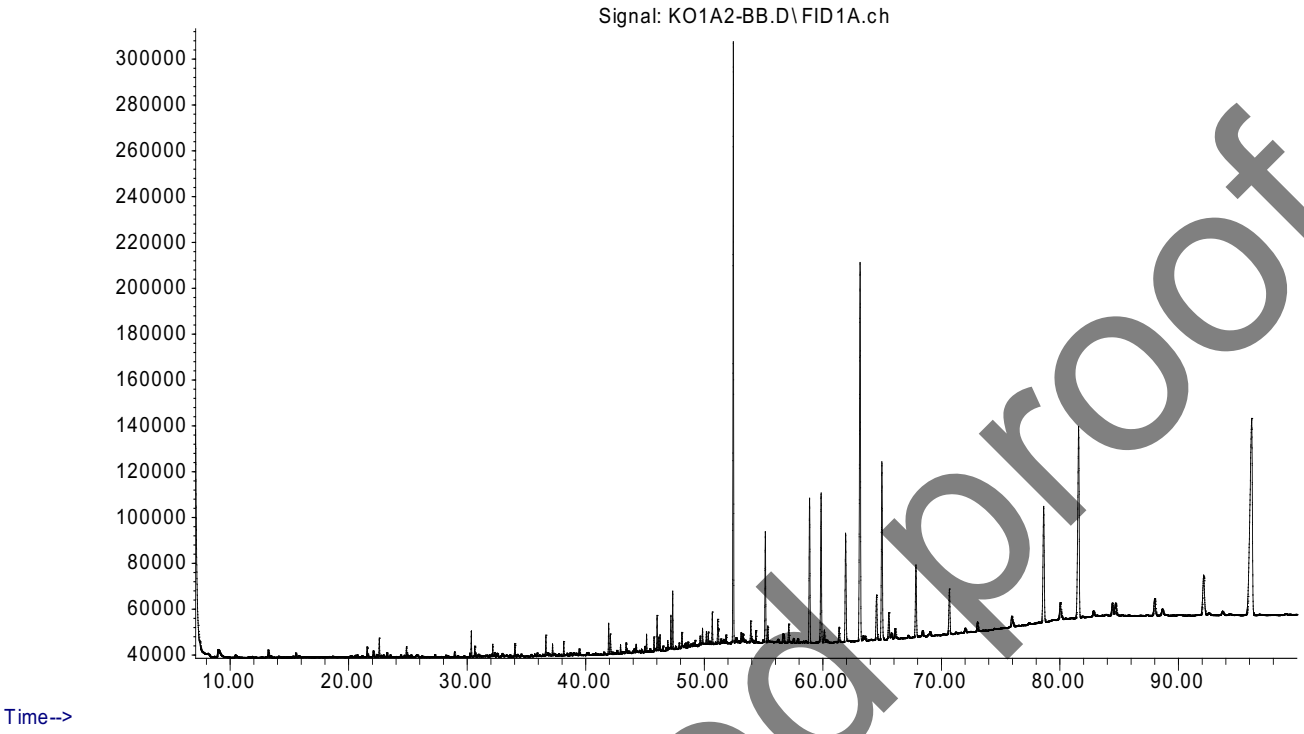

Figure 4. The chromatogram of fatty acid methyl esters of the lipids obtained from leaves

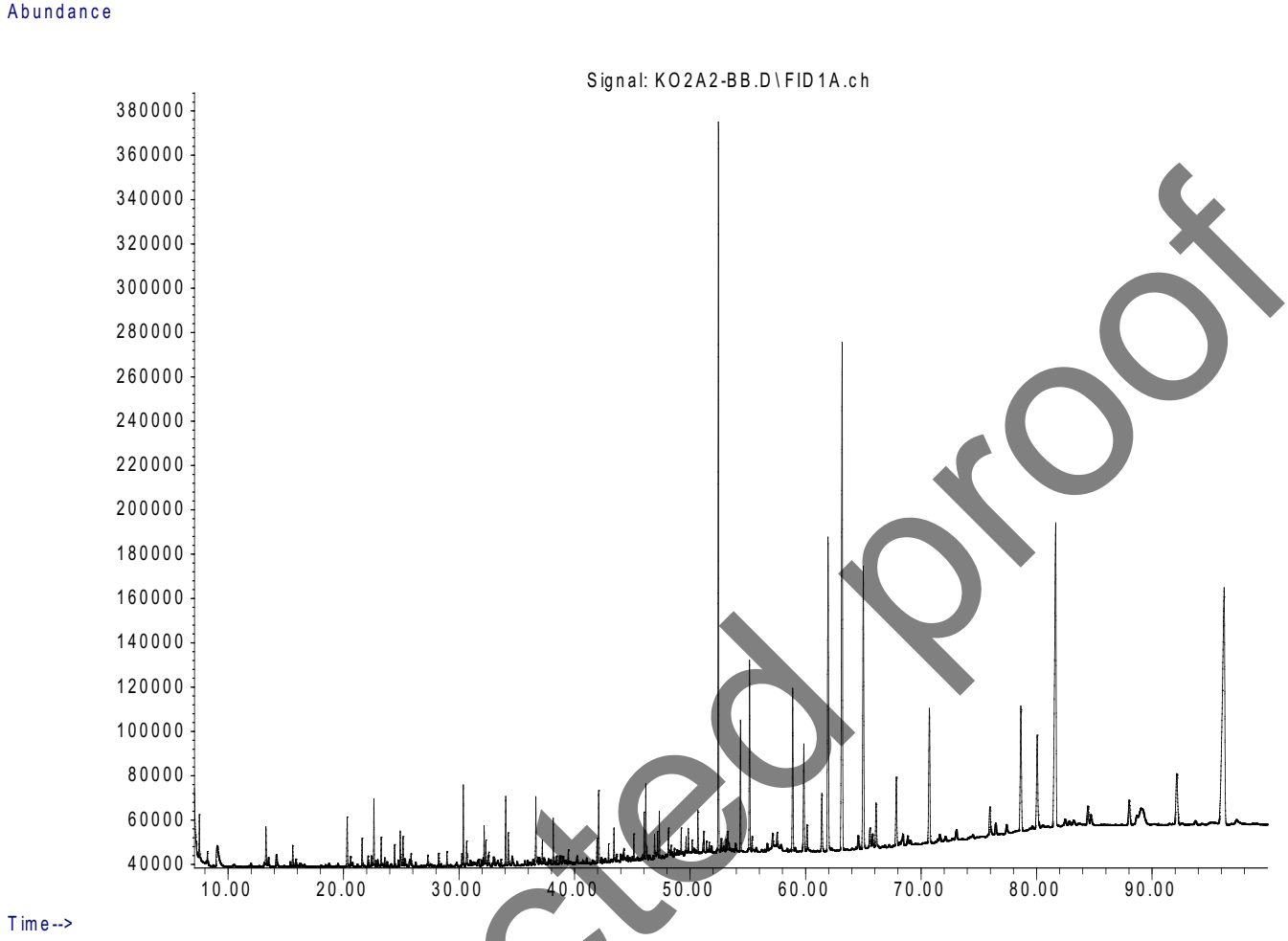

Figure 5. The chromatogram of fatty acid methyl esters of of the lipids obtained from flowers

Table 1. Yields of extracts			
Extract type	**Extract code**	**Amounts of extract, g**	**Yield*, %**
n-Hexane extract	SP_H	0.151	0.43
Methanol extract	SP_M	4.733	13.44
Aqueous extract	SP_W	3.057	8.68
Essential oil	SP_{EO}		0.02
*Yield was calculated based on air dried plant weight, SP:			

Table 2. Hydrodistilled essential oil compounds and volatile constituents obtained from *Scabiosa pseudograminifolia* aerial parts, leaves, and flowers using the MSD-SPME technique

$RRI_{exp.}$	$RRI_{lit.}$	**Compound***	**%**			**References**
			Hydrodistilled aerial parts	**MSD-SPME of leaves**	**MSD-SPME of flowers**	
945	945	2-Ethyl furan	0.3			[35]
950	949	2,4-Dimethylfuran	0.7			[36]
1047	1050	(*E*)-2-Butenal		0.8	0.5	[37]
1052	-	4-Methyl 2,4-pentadienal#	0.8			
1087	1098	Hexanal		0.5	4.4	[38]
1187	1187	Heptanal			0.6	[39]
1191	1149	*(E)*-3-Hexenal	1.2			[39]
1202	1198	Limonene		0.7	1.2	[40]
1211	1215	Isoamyl alcohol			1.5	[41]
1212	1211	1,8-Cineole		0.1		[40]
1223	1244	Amyl furan		0.1		[42]
1225	1225	*(Z)*-3-Hexenal		34.0	29.1	[40]
1230	1231	*(E)*-2-Hexenal			1.5	[43]
1251	1253	γ-Terpinene			0.1	[44]
1256	1231	Isocumene	8.0			[45]
1260	1262	*(E)*-β-Ocimene		0.2	0.2	[44]
1263	1260	Pentanol			0.2	[46]
1285	1280	*p*-Cymene		0.2	0.3	[47]
1295	1282	Terpinolene		0.2	0.3	[40]
1306	1287	Octanal		0.2	0.3	[40]
1319	1319	4-Nonanone			0.2	[48]
1331	1332	(*E*)-2-Heptenal			0.3	[37]
1332	1356	Allyl caproate		0.6		[49]
1344	1337	6-Methyl-5-hepten-2-one		1.3	0.6	[40]
1363	1351	Hexanol		1.7	7.7	[40]
1377	1340	*(E)*-3-Hexene-1-ol			0.2	[50]
1400	1400	Tetradecane		0.4		[42]
1403	1380	*(Z)*-3-Hexene-1-ol		0.9	2.0	[40]
1407	1391	2-Nonanone			0.1	[40]
1409	1392	Nonanal		1.1	0.6	[40]
1410	1400	*(E)*-2-Hexene-1-ol			0.7	[40]
1412	-	3-Octene-2-one#			0.4	[51]
1413	1388	4,8-Dimethyl-1,3,7-nonatriene		1.3		[42]
1443	1428	*(E)*-2-Octenal		0.4	0.4	[52]
1450	1444	1-Octen-3-ol		0.1	0.4	[40]
1454	1446	*trans*-Linalool oxide		0.2	0.3	[40]
1463	1449	Heptanol			0.2	[40]
1471	1479	*(E,Z)*-2,4-Heptadienal		1.9	0.4	[42]
1476	1461	Furfural			0.3	[40]
1496	1490	2-Ethyl hexanol		3.2	4.3	[53]
1499	1496	Decanal			0.3	[40]
1502	1497	*(E,E)*-2,4-Heptadienal		1.5	0.8	[53]
1514	1535	β-Burbonene		0.4		[54]

1520	1524	*(E,Z)*-3,5-Octadiene-2-one		0.9	0.8	[55]
1528	1515	Camphor		0.3	0.2	[40]
1541	1519	Benzaldehyde		1.8	3.9	[40]
1553	1543	Linalool	15.6	3.1	5.0	[40]
1559	1552	Octanol		1.3	1.1	[40]
1568	1573	*(E,E)*-3,5-Octadiene-2-one		0.5	0.6	[56]
1586	1566	(*E,E*)-2,6-Nonadienal			0.6	[47]
1589	1583	(*E,Z*)-2,6-Nonadienal			0.3	[57]
1600	1600	Hexadecane		0.5		[58]
1612	1614	Furfuryl alcohol		1.1		[59]
1619	-	4-Methyl-(2E)-undecene[#]		3.6	2.5	[39]
1630	-	6-Methyl-1-octanol[#]		4.3	2.5	[39]
1632	1638	β-Cyclocitral		1.7		[60]
1641	1616	1-Ethyl-1H-pyrrole-2-carbaldehyde[#]			0.2	[61]
1660	1656	Nonanol		2.9	2.1	[40]
1662	1665	Safranal		0.8		[62]
1663	1663	Phenylacetaldehyde		0.5	2.1	[63]
1678	1648	Acetophenone			0.7	[40]
1702	1670	Methyl chavicol		0.7	0.2	[40]
1719	1706	α-Terpineol	2.5	0.7	0.9	[64]
1726	1712	Dodecanal			0.5	[40]
1743	-	2-(1-Cyclopenten-1-yl) furan[#]		0.7		[39]
1748	-	Methoxy phenyl oxime[#]		1.0	0.8	[39]
1770	1751	Carvone		0.2	0.4	[65]
1775	1753	Ethyl benzaldehyde		0.3	0.3	[66]
1790	1762	Naphthalene		0.6	0.4	[67]
1796	-	Octenyl cyclopentene[#]		2.8		[39]
1797	2025	*(E)*-Cinnamaldehyde			0.2	[68]
1807	1797	Methyl acetophenone§		0.4	0.4	[69]
1815	1775	Cuminaldehyde		1.2	0.9	[70]
1816	1839	Geraniol	2.3			[40]
1824	-	2,2,4-Trimethyl-3-carboxyisopropyl-isobutyl pentanoate[#]	0.9			[39]
1842	1845	*(E)*-Anethole		2.5	2.0	[60]
1860	1867	*(E)*-Geranyl acetone		1.2	0.3	[71]
1872	-	2,2,4-Trimethyl pentane-1,3-diol diisobutyrate[#]			0.2	[39]
1876	-	2-Naphthalenol		0.3		[39]
1878	1880	1-Isobutyl 4-isopropyl 3-isopropyl-2,2-dimethylsuccinate		0.6		[58]
1886	1872	2-Methyl naphthalene		0.1		[72]
1894	1865	Benzyl alcohol		0.4	0.2	[40]
1941	1904	Phenylethyl alcohol			0.4	[40]
1964	1936	*(E)*-β-Ionone		1.6	0.2	[40]
1995	1996	Benzothiazole		0.3	0.2	[73]
2022	1992	Phenol			0.1	[40]
2023	1995	*trans*-β-Ionone-5,6-epoxide		0.5		[42]
2025	2028	Methyl eugenol		0.2	0.1	[74]
2036	2041	Isopropyl myristate		0.1		[75]
2037	2015	Pentyl octyl benzene		0.1		[76]
2041	2038	Phenyl ether		0.2		[77]
2065	2058	Anisaldehyde		0.2	0.2	[78]
2066	2072	Lilial		0.2	0.1	[79]
2130	2131	Hexahydro farnesyl acetone	3.0	0.4	0.4	[80]

2144	2120	*(Z)*-3-Hexen-1-ol benzoate		0.1		[81]
2182	2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone		0.6	0.2	[42]
2194	2192	Nonanoic acid *(=Pelargonic acid)*	1.5			[82]
2202	2105	Thymol		0.2	0.2	[83]
2243	2240	Carvacrol	1.3	3.0	1.9	[83]
2451	2449	Dodecanoic acid *(=Lauric acid)*	10.9			[84]
2670	2670	Tetradecanoic acid *(=Myristic acid)*	9.1			[85]
2820	2822	Pentadecanoic acid *(=Pentadecylic acid)*	1.6			[86]
2877	2890	Hexadecanoic acid (*=Palmitic acid)*	30.2			[87]
		Total	89.9	96.7	94.7	

*:
#: Tentative identification from Wiley-NIST digital Library, MSD-SPME: Microsteam distillation-solid phase microextraction,
$RRI_{exp.}$:
$RRI_{lit.}$:

Table 3. Chemical composition of the fatty acids obtained from the leaves and flowers of the *Scabiosa pseudograminifolia*

RRI#	Compound*	Leaves	Flowers
1402	Methyl octanoate (*=Caprylic acid methyl ester);* (8:0)		0.3
1505	Methyl nonanoate (*=Pelargonic acid methyl ester);* (9:0)		0.3
1815	Methyl dodecanoate (*=Lauric acid methyl ester*); (12:0)	0.9	0.5
1980	*Unidentified* (MA:278)	0.4	0.2
2012	*Unidentified* (MA:278)	1.0	0.5
2016	Methyl tetradecanoate (*=Myristic acid methyl ester*); (14:0)	1.9	1.0
2051	Octanedioic acid (*=Suberic acid);* (8:0)		0.4
2125	Methyl pentadecanoate (*=Pentadecylic acid methyl ester*); (15:0)	0.5	0.5
2158	Nonanedioic acid (*=Azelaic acid*); (9:0)	1.0	1.0
2223	Methyl hexadecanoate (*=Palmitic acid methyl ester*); (16:0)	20.8	18.3
2251	*(Z)*-9-Methyl hexadecenoate (*=Palmitoleic acid methyl ester*); (16:1); ω-7		0.4
2330	Methyl heptadecanoate (*=Margaric acid methyl ester*); (17:0)	0.6	0.4
2436	Methyl octadecanoate *(=Stearic acid methyl ester)*; (18:0)	7.1	5.6
2455	*(Z)*-9-Methyl octadecenoate *(=Oleic acid methyl ester)*; (18:1); ω-9	7.8	4.0
2468	*(E)*-9-Methyl octadecenoate (*=Elaidic acid methyl ester*); (18:1); ω-9	0.6	0.9
2509	*(Z,Z)*-9,12-Methyl octadecadienoate (*=Linoleic acid methyl ester);* (18:2); ω-6	5.8	12.4
2542	Methyl nonadecanoate (*=Nonadecylic acid methyl ester*); (19:0)	21.9	
2572	*(Z,Z,Z)*-9,12,15-Methyl octadecatrienoate *(=α-Linolenic acid methyl ester); (18:3)*; ω-3	10.3	12.2
2642	Methyl eicosanoate *(=Arachidic acid methyl ester)*; (20:0)	4.4	3.1
2740	Methyl heneicosanoate *(=Heneicosilicic acid methyl ester)*; (21:0)	0.6	0.6
2843	Methyl docosanoate *(=Behanic acid methyl ester)*; (22:0)	8.3	6.5
2868	*(Z)*-13-Methyl dococenoate *(=Erucic acid methyl ester)*; (22:1); ω -9	1.2	5.0
2945	Methyl trichosanoate *(=Trichosilic acid methyl ester)*; (23:0)	0.8	0.5
3050	Methyl tetracosanoate *(=Lignoceric acid methyl ester);* (24:0)	3.9	3.7
	Total	99.8	78.3

RRI# : Relative retention index of the methyl/ethyl derivative of the compound

Table 4. TPC, TFC and biological activity results of *Scabiosa pseudograminifolia* extracts

Codes	TPC (mgGAE/g $_{extract}$)	TFC (mgQE/g $_{extract}$)	DPPH[a] (IC_{50}, mg/mL)	TEAC (mM)	ORAC[b] (TEμmol)	β-Carotene peroxidation inhibition (IC_{50}, mg/mL)	α-Amylase inhibition (%)
SP_{EO}	-	-	NE	2.39±0.15	32.6±6.8	NE	33.6
SP_{H}	0.11±0.06	NE	28.80±1.90[c]	0.26±0.14	NE	NE	NE
SP_{M}	0.5±0.01	0.067±0.008	0.19±0.03	2.21±0.20	134.0±11.0	0.730±0.001	NE
SP_{W}	0.52±0.01	0.081±0.002	0.16±0.04	2.33±0.13	248.4±15.4	1.4±0.2	NE
BHA	-	-	-	-	-	0.01±0.0005	-
GA	-	-	0.002±0.0	-	-	-	-
ACR	-	-	-	-	-	-	85.0

[a]: IC_{50} values are half maximal inhibitory concentrations, [b]: ORAC values are determined for essential oil and extracts at 0.1 mg/mL, [c]: IC_{50} was not calculated; the value was for 10 mg/mL, %Inh.: % inhibition value determined for the extracts at 10 mg/mL. IC_{50}: Inhibitory concentration 50, ORAC: Oxygen Radical Absorbance Capacity, TPC: Total phenol content, TFC: Total flavonoid content, SP_{EO}: The plant's essential oil, SP_{H}: The plant's hexane extract, SP_{M}: The plant's methanol extract, SP_{W}: The plant's aqueous extract code, BHA: Butylated hydroxyanisole, GA: Gallic acid, ACR: Acarbose, NE: Non-effective, TEAC: Trolox equivalent antioxidant activity, ORAC: Oxygen radical absorbance capacity

Table 5. RP-HPLC quantitative determination of phenolic acids in *Scabiosa pseudograminifolia* extracts

	Phenolic acid amounts (μg/$g_{extract}$)							
Extracts	**PA**	***p*-HBA**	**CA**	**ClA**	**SA**	***p*-CA**	**FA**	***o*-CA**
SP_{M}	0.11	0.12	0.05	4.06	0.07	0.44	0.30	0.53
SP_{W}	0.22	0.79	0.25	5.26	0.21	0.89	0.48	0.65

RP-HPLC: Reverse phase high performance liquid chromatography, SP_{M}: The plant's methanol extract, SP_{W}: The plant's aqueous extract code, PA: Protocatechic acid, *p*-HBA: *p*-Hydroxybenzoic acid, CA: Caffeic acid, ClA: Chlorogenic acid, SA: Syringic acid, *p*-CA: *p*-Coumaric acid, FA: Ferulic acid, *o*-CA: *o*-Coumaric acid