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Silene vulgaris subsp. *macrocarpa* leaves and roots from Morocco: assessment of the efficiency of different extraction techniques and solvents on their antioxidant capacity, brine shrimp toxicity and phenolic characterization

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ABSTRACT

This study was undertaken to investigate the effect of various solvents and techniques on the extractability of antioxidant compounds, particularly phenolics, from leaves and roots of *Silene vulgaris* subsp. *macrocarpa* grown wild in Morocco. Maceration and hot extraction with methanol or water and Soxhlet ethanol extraction were utilized. Aimed at establishing the potential safety of the extracts, *Artemia salina* lethality bioassay was performed. All the extracts were found to be non-toxic, except for the leaf Soxhlet ethanol. The antioxidant potential of the extracts was evaluated *in vitro* by DPPH, reducing power, and ferrous ions chelating activity assays. The leaf extracts displayed noticeable radical scavenging and chelating activities, and maceration with methanol (Mac-MeOH) resulted the most suitable extraction method for an effective recovery of antioxidants; further, the root Mac-MeOH extract demonstrated good chelating properties (IC_{50} = 335.49 ± 0.70 μg/mL). Thus, leaf and root Mac-MeOH extracts were subjected to phytochemical investigations. The total phenolic, flavonoid and condensed tannin content was determined spectrophotometrically. Thirteen polyphenolic compounds were positively identified, by HPLC-PDA-ESI-MS, in the leaf extract for the first time, with *p*-coumaric acid derivatives being the most abundant ones (81%), whereas only catechin and procyanidin B1 were found in the root extract.

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Introduction

The genus *Silene*, the largest of the Caryophyllaceae family, comprises more than 700 annual, biennial and perennial species distributed in the East Mediterranean, central Asia, Italy, Turkey, Iran, Iraq, Russia, England and Spain (Karamian and Ghasemlou 2013; Hoseini et al. 2017). The genus consists mainly of herbaceous plants and, more rarely, small shrubs or subshrubs (Hussein et al. 2017).

Silene vulgaris (Moench) Garcke is a herbaceous perennial species, sometimes cespitose and woody at the base. According to the Flora Europea, this species comprises eight different taxa, including the subsp. *macrocarpa* Turrill (Chater and Walters 1964).

Silene vulgaris (Moench) Garcke subsp. *macrocarpa* Turrill is characterized by long stolons, stems high up to 50 cm, leaves narrowly lanceolate (1–4 × 0.7–1.6 cm), flowers pink or greenish, gathered in dichasial cymes several-flowered, bracts scarious. The fruit is a capsule (10–13 mm) with narrow neck and erect or patent teeth (Chater and Walters 1964).

The use of *S. vulgaris* in folk medicine for the treatment of bladder diseases and urinary tract infection is reported (Kurt et al. 2018). Young shoots and leaves are considered as good remedy for bronchitis and asthma (Chandra and Rawat 2015). In Southern Italy it is traditionally employed as an antianemic (Conforti et al. 2011). In Morocco, *S. vulgaris* (vernacular name: Tighighacht) leaf powder or poultice is utilized as a remedy for eczema, psoriasis, wounds and pruritus (Boukhira et al. 2015).

Silene vulgaris is an edible plant; especially young shoots and leaves of this species are known to be used as foodstuff in Austria, Spain, Italy and in other Mediterranean countries, including Morocco, as well as in many different regions of Turkey (Rivera et al. 2005; Tardío et al. 2005; Lentini and Venza 2007; Hadjichambis et al. 2008; Boukhira et al. 2015; Kurt et al. 2018; Zengin et al. 2018). *Silene vulgaris* is traditionally utilized to enhance aromatic properties of Otlu cheese, a very famous brined cheese produced in the eastern region of Turkey (Dagdelen et al. 2014).

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Table 1. Percentage yields, Free radical scavenging activity (DPPH test), Reducing power, and Fe^{2+} chelating activity of *Silene vulgaris* subsp. *macrocarpa* leaf and root extracts.

S. vulgaris subsp. *macrocarpa* extracts	Yields (w/w %)		DPPH test IC_{50} (μg/mL)		Reducing power ASE/mL		Fe^{2+} chelating activity IC_{50} (μg/mL)	
	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
Mac-MeOH	27.5	12.8	70.00 ± 0.13	>1000	6.59 ± 0.95	117.51 ± 3.02	238.29 ± 0.36	335.49 ± 0.70
Mac-H_2O	8.9	21.4	>1000	>1000	29.58 ± 0.95	32.38 ± 2.87	924.14 ± 11.25	–
Hot-MeOH	5.9	9.4	326.76 ± 0.28	>1000	28.44 ± 1.36	94.49 ± 2.97	268.10 ± 6.30	823.98 ± 10.09
Hot-H_2O	7.1	6.2	219.73 ± 0.24	>1000	33.59 ± 1.97	284.60 ± 3.83	755.51 ± 16.64	–
Sox-EtOH	2.1	4.8	932.44 ± 2.75	332.61 ± 1.06	21.67 ± 1.51	19.32 ± 2.58	>1000	>1000
Standard			BHT		BHT		EDTA	
			48.47 ± 0.44		1.97 ± 0.08		6.68 ± 0.04	

Values are expressed as the mean ± SD ($n = 3$).

To the best of our knowledge, only few publications concerning the phytochemical composition and biological properties of *S. vulgaris* subsp. *macrocarpa* are available. In particular, Vardavas et al. (2006a, 2006b) analyzed the lipid concentrations (total monounsaturated, polyunsaturated and saturated lipid content, total fat content, total ω-3 and ω-6 fatty acid composition and ω-6/ω-3 ratio), the carotenoid, L-ascorbic acid, phylloquinone, γ-tocopherol and α-tocopherol and total polyphenol content of *S. vulgaris* subsp. *macrocarpa*, commonly utilized in the traditional diet of Crete (Greece). Kurt et al. (2018) previously studied the anticholinesterase, antioxidant, antiaflatoxigenic activities of different extracts (hexane, ethanol and water) obtained by maceration of the whole plant of *Silene vulgaris* var. *macrocarpa* collected in Turkey.

In continuation of our studies on species utilized in traditional medicine growing wild in Morocco (Mohti et al. 2019), this work aimed at establishing the most efficient solvent and technique for the extraction of antioxidant constituents, in particular phenolics, from two different organs, leaves and roots, of *S. vulgaris* subsp. *macrocarpa*. In order to assess the possible use of *S. vulgaris* subsp. *macrocarpa* as a safe source of antioxidant agents, the toxicity of all the extracts was investigated by the *Artemia salina* lethality bioassay. Furthermore, the quali-quantitative characterization of the polyphenolic compounds contained in the leaf and root extracts obtained by maceration with methanol, which displayed the best antioxidant efficacy, was performed.

Material and methods

Chemicals

$FeCl_2$ was obtained from Carlo Erba (Milan, Italy). LC-MS grade water (H_2O), acetonitrile (ACN), catechin, caffeic acid, *p*-coumaric acid, vanillic acid and rutin were obtained from Merck Life Science (Merck KGaA, Darmstadt, Germany). Unless indicated otherwise, all chemicals were purchased from Sigma-Aldrich (Milan, Italy).

Plant material and extraction

Silene vulgaris (Moench) Garcke subsp. *macrocarpa* Turrill was collected in Ait Ayach near to Midelt (Morocco). The taxonomic identification of the plant was confirmed by Prof. Jalal el Oualidi, Institute of Scientific Research, University Mohammed V, Rabat, Morocco. A voucher specimen is deposited in the herbarium of the Institute of Scientific Research, Rabat, Morocco, under accession number n° RAB110965.

In order to extract the antioxidant compounds from *S. vulgaris* subsp. *macrocarpa* different methods and solvents were utilized. For this purpose, air dried and powdered leaves and roots (10 g) were subjected to: maceration under stirring for 24 h with methanol (100 mL) or distilled water (100 mL) (Mac-MeOH and Mac-H_2O extracts), hot extraction for 2 h with 100 mL of boiling methanol or distilled water (Hot-MeOH and Hot-H_2O extracts), and Soxhlet using 150 mL of ethanol (Sox-EtOH extract). The extractive solutions were freed of solvent under reduced pressure at 45 °C, using a rotary evaporator. The dried crude extracts were weighed and the yields, referred to 100 g of dried plant material, were calculated. The extract yields are given in Table 1.

Artemia salina Leach lethality bioassay

Artemia salina Leach (brine shrimp) lethality bioassay is a useful method to assess the toxicity of plant extracts. The potential toxicity of *S. vulgaris* subsp. *macrocarpa* extracts was investigated on brine shrimp larvae by determining the median Lethal Concentration (LC_{50}), according to the method previously reported (Taviano et al. 2018).

Brine shrimp cysts were hatched in artificial sea water (33 g sea salt/L deionized water) by incubation under a 60 W lamp, providing direct light and warmth (24–26 °C). Ten brine shrimp larvae were transferred to each sample vial, and artificial seawater was added to obtain a final volume of 5 mL. The extracts, opportunely dissolved and then diluted in artificial seawater, were tested at the final concentrations of 10, 100, 500 and 1000 μg/mL. After 24 h of incubation the numbers of surviving nauplii in each vial were counted. The experiments were conducted in triplicate for each concentration, and LC_{50} values were determined by the Litchfield and Wilcoxon method. Extracts are considered non-toxic if the LC_{50} is higher than 1000 μg/mL.

Antioxidant activity

Free radical scavenging activity

The antiradical activity of *S. vulgaris* subsp. *macrocarpa* extracts was assessed by the DPPH (1,1-diphenyl-2-picrylhydrazyl) test (Miceli et al. 2017). Briefly, 0.5 mL of solution containing different amounts of each extract (7.81–1000 μg/mL) was mixed with 3 mL of a freshly prepared methanol DPPH solution

(0.1 mM). The decolorizing process after 20 min was measured at 517 nm with a model UV-1601 spectrophotometer (Shimadzu). The scavenging activity was measured as the decrease in absorbance of the samples *versus* the DPPH standard solution and calculated by using the following equation:

$$\text{Radical scavenging activity } (\%) = [(A_0 - A_s)/A_0] \times 100$$

where A_0 is the absorbance of the control at 20 min, and A_s is the absorbance of the sample after 20 min. Butylated hydroxytoluene (BHT) was used as a positive control. In addition, the antiradical activity was expressed as 50% inhibitory concentration (IC_{50}), the concentration required to cause a 50% DPPH inhibition.

The results, obtained from the average of three independent experiments, are expressed as mean radical scavenging activity percentage (%) ± standard deviation (SD) and mean IC_{50} ± SD.

Reducing power

The reducing power of *S. vulgaris* subsp. *macrocarpa* extracts was evaluated by spectrophotometric detection of Fe^{3+}-Fe^{2+} transformation method (Miceli et al. 2017). One milliliter of different concentrations of the extracts (7.81–1000 μg/mL) was mixed with 2.5 mL of a 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] and incubated in a water bath at 50 °C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added to the cooled mixture that was centrifuged at 1570 g for 10 min. The supernatant (2.5 mL) was then mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% fresh ferric chloride ($FeCl_3$) solution. The absorbance was measured at 700 nm after 10 min. Ascorbic acid and BHT were used as the positive controls; the results were obtained from the average of three independent experiments and are expressed as mean absorbance values ± SD and ascorbic acid equivalent (ASE)/mL ± SD.

Ferrous ions (Fe^{2+}) chelating activity

The Fe^{2+} chelating activity of *S. vulgaris* subsp. *macrocarpa* extracts was estimated by measuring the formation of the Fe^{2+}-ferrozine complex (Miceli et al. 2017). Briefly, 0.05 mL of 2 mM $FeCl_2$ was added to 1 mL of different concentrations of each extract (7.81–1000 μg/mL). The reaction was initiated by the addition of 0.1 mL of 5 mM ferrozine solution. Then, the mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. The control contains $FeCl_2$ and ferrozine, complex formation molecules. The percentage inhibition of ferrozine–Fe^{2+} complex formation was calculated by using the following equation:

$$\text{Chelating activity } (\%) = [(A_0 - A_s)/A_0] \times 100$$

where A_0 was the absorbance of the control, and As was the absorbance of the sample. Ethylenediaminetetraacetic acid (EDTA) was used as positive control. The results were obtained from the average of three independent experiments and are reported as mean chelating activity (%) ± SD and IC_{50} ± SD.

Phytochemical investigations on *S. vulgaris* subsp. *macrocarpa* leaf and root Mac-MeOH extracts

Total phenolic, flavonoid and condensed tannin content

The total phenolic, flavonoid and condensed tannin content of *S. vulgaris* subsp. *macrocarpa* leaf and root Mac-MeOH extracts was determined by colorimetric methods (Miceli et al. 2016).

The total phenolic content was measured by the Folin-Ciocalteu method. One hundred microliters of solution containing suitable concentration of each Mac-MeOH extract was mixed with 200 μL Folin-Ciocalteu reagent, 2 mL of distilled water, 1 mL of 15% sodium carbonate, and incubated at room temperature in the dark for 2 h. Then, the absorbance was measured by spectrophotometer at 765 nm. Gallic acid was used as a standard and the total phenolics were expressed as mg gallic acid equivalents (GAE)/g extract (dw) ± SD.

The total flavonoid content of the extracts was measured by using the aluminum chloride colorimetric assay. Five hundred microliters of each sample solution appropriately diluted was mixed with 1.5 mL MeOH, 100 μL of 10% aluminum chloride, 100 μL of 1 M potassium acetate and 2.8 mL of distilled water. The samples were incubated at room temperature in the dark for 30 min, and the absorbance of the reaction mixture was measured at 415 nm. Quercetin was used to make the calibration curve and total flavonoids were expressed as mg quercetin equivalents (QE)/g extract (dw) ± SD.

The condensed tannin content of the extracts was determined by using the vanillin method. Fifty microliters of each sample solution was mixed 1.5 mL of 4% vanillin in MeOH and 750 μL of concentrated hydrochloric acid. After incubation at room temperature in the dark for 20 min; the absorbance of the reaction mixture was measured at 500 nm. (+)-Catechin was used to make the calibration curve and condensed tannins were expressed as mg catechin equivalents (CE)/g extract (dw) ± SD.

The results of the spectrophotometric determinations were obtained from the average of three independent experiments.

Characterization of the polyphenolic compounds by HPLC-PDA-ESI-MS

A quali-quantitative investigation of the polyphenolic compounds contained in *S. vulgaris* subsp. *macrocarpa* leaf and root Mac-MeOH extracts was carried out by HPLC-PDA-ESI-MS. The latter consisted of a Shimadzu HPLC system (Kyoto, Japan) equipped with a CBM-20A controller, two LC-20AD pumps, a DGU-20A_3 degasser, a SIL-20AC autosampler, a SPD-M20A photo diode array detector (PDA) and a quadrupolar mass analyzer (LCMS-2020, Shimadzu, Kyoto, Japan), equipped with an ESI interface, in negative ionization mode. Data acquisition was performed by Shimadzu LabSolution software ver. 5.65.

Sample preparation. Twenty milligrams of *S. vulgaris* subsp. *macrocarpa* leaf and root Mac-MeOH extracts was dissolved in 1 mL of MeOH.

Chromatographic conditions. Analyses were carried out on a Ascentis Express C18, 15 cm × 4.6 mm I.D. with particle size of 2.7 μm (Merck Life Science, Merck KGaA, Darmstadt, Germany). The injection volume was 2 μL, mobile phase consisted of water/formic acid (99.9:0.1) (solvent A) and ACN/formic acid (99.9:0.1) (solvent B), the linear gradient profile was as follows: 0 min, 0% B, 5 min, 5% B, 15 min, 10% B, 30 min, 20% B, 60 min, 50% B, 70 min, 100% B, 71 min, 0% B . The flow-rate was 1 mL/min and it was split to 0.2 mL/min prior to MS detection.

PDA conditions. The wavelength range was 200–400 nm and the chromatograms were extracted at 280 nm. Time constant was 25 ms and sample frequency 40 Hz.

MS conditions. The MS acquisition was performed using ESI, in negative mode, under the following conditions: mass spectral range 100–1200 *m/z*; interval: 0.5 sec; nebulizing gas (N_2) flow: 1.5 L/min; interface temperature: 350 °C Heat block: 300 °C, DL temperature: 300 °C; DL voltage –34 V; probe voltage 4.5 kV; Qarray voltage: 1.0 V, RF voltage: 90 V; detection gain 1.0 kV.

Quantitative determination was carried using calibration curves of five standards, namely catechin, caffeic acid, *p*-coumaric acid, vanillic acid and rutin. Standard calibration curves were prepared in a concentration range 0.1–50 mg/L with five different concentration levels. Triplicate injections were made for each level, and a linear regression was generated. The calibration curves with the external standards were obtained using concentration (mg/L) with respect to the area obtained from the integration of the PDA peaks at a wavelength of 278 nm for flavan-3-ol like compounds, 290 nm for benzoic acid-like compounds, 315 nm and 325 nm for cinnamic acid-like compounds, and 354 nm for flavonol-glycoside-like compounds.

The amount of each compound was finally expressed in mg/g of dried extract ± percent relative standard deviation (%RSD).

Results and discussion

Artemia salina Leach lethality bioassay

With the aim of establishing the potential safety of the extracts, the *A. salina* lethality bioassay was performed. *A. salina*, the brine shrimp, is an invertebrate utilized for the preliminary assessment of toxicity of bioactive compounds and plant extracts. The main advantages of using brine shrimps in toxicity testing are the rapidity, cost-effectiveness, easiness. This assay may be considered an alternative to the *in vitro* cell culture (Rajabi et al. 2015) and *in vivo* assays. A good correlation between the results of the oral acute toxicity determination in the murine model and this bioassay has been previously reported; thus, it represents a useful tool for predicting acute toxicity of plant extracts (Lagarto Parra et al. 2001).

Silene vulgaris subsp. *macrocarpa* extracts did not display any toxicity against brine shrimps ($LC_{50} > 1000$ μg/mL), except for the leaf Sox-EtOH extract, which exhibited low toxicity ($LC_{50} = 560.33 \pm 29.15$ μg/mL).

Antioxidant activity

In recent years, the antioxidant properties of numerous plant species have been the object of many scientific studies because of their potential safety and positive effects on human health (Acquaviva et al. 2018; Chekroun-Bechlaghem et al. 2019; Rahali et al. 2019). Many phytochemicals that are antioxidants have been isolated from different parts of plants, such as seeds, fruits, leaves, stems and roots (Caetano et al. 2011). Due to the presence of various antioxidant compounds with different chemical characteristics and polarities, the efficient recovery of antioxidants from plants depends not only on the extraction technique but also on the properly selected type of solvent.

Several studies conducted in the last decades indicate that polyphenolic compounds represent the main phytochemicals with antioxidant properties found in higher plants. The polyphenol group comprises a large number of heterogeneous compounds, whose chemical structure varies from simple to highly polymerized substances; thus the development of an effective method for the recovery of all phenolics from plant tissues is not simple (Jovanović et al. 2017). The most common procedure to extract phenolics is solid-liquid extraction; conventional techniques such as maceration and heat-assisted extraction can be applied for polyphenol extraction from different plant materials. Maceration is widely and successfully applied to extract thermolabile compounds, whereas high temperature involves faster polyphenol kinetics and improved extraction efficiency (Jovanović et al. 2017).

The selection of the solvent is one of the most important steps in the process of extraction. Generally, water and organic solvents such as ethanol, methanol, acetone, and ethyl acetate, and their mixtures are the most commonly used, due to wide range of phenolic compounds that these extractants can dissolve (Caetano et al. 2011; Wijekoon et al. 2011). Ethanol was found to be a good solvent for polyphenol extraction and safe for human consumption. Methanol resulted in most cases an efficient solvent system for the extraction of lower molecular weight polyphenols, whereas aqueous acetone is a suitable extraction solvent for higher molecular weight flavanols (Do et al. 2014). Furthermore, the results of various studies highlighted that aqueous extracts (water and hot water extracts) obtained from different plant species exhibited powerful antioxidant properties which were in most cases strongly related to their total polyphenol content (Kim et al. 2011; Wang and Xu 2014; Lou et al. 2016).

Starting from these assumptions, maceration and hot extraction with methanol or water and Soxhlet ethanol extraction were utilized to extract antioxidants, especially phenolics, from leaves and roots of *S. vulgaris* subsp. *macrocarpa*.

In order to provide a better assessment of the antioxidant capacity, the extracts were screened using three different *in vitro* model systems, including DPPH radical scavenging activity, reducing power and Fe^{2+} chelating activity assays.

In the DPPH test *S. vulgaris* subsp. *macrocarpa* extracts exhibited radical scavenging properties, that increase with the increasing concentrations. Particularly, the leaf extracts displayed good activity, being Mac-MeOH extract the most effective one. Based on IC_{50} values, the activity of the leaf extracts and the standard decreases in the order BHT > Mac-MeOH > Hot-H_2O > Hot-MeOH > Sox-EtOH > Mac-H_2O. The scavenging activity of the root extracts is weak compared to BHT, except for Sox-EtOH, and it decreases in the order BHT > Sox-EtOH > Hot-MeOH > Mac-H_2O > Mac-MeOH > Hot-H_2O (Supplemental Figure S1, Table 1).

As shown in Supplemental Figure S2 and in Table 1, *S. vulgaris* subsp. *macrocarpa* extracts, particularly those obtained from the roots, showed low reducing power compared to the standard. Among the extracts, the leaf Mac-MeOH exhibited the best activity, as confirmed also by the ASE/mL value. The reducing power of the extracts and standard decreases in the order BHT > Mac-MeOH > Sox-EtOH > Hot-MeOH > Mac-H_2O > Hot-H_2O and BHT > Sox-EtOH > Mac-H_2O > Hot-MeOH > Mac-MeOH > Hot-H_2O for leaves and roots, respectively.

In the Fe^{2+} chelating activity assay *S. vulgaris* subsp. *macrocarpa* leaf extracts showed good chelating properties, and the Hot-MeOH and Mac-MeOH were found to display the best activity, reaching about 90% and 80%, respectively, at the highest tested concentration; anyway, the obtained IC_{50} values indicate that Mac-MeOH resulted the most effective. Based on IC_{50} values the chelating activity of the extracts and standard decreases in the order EDTA > Mac-MeOH > Hot-MeOH > Hot-H_2O > Mac-H_2O > Sox-EtOH. Concerning the root extracts, the Mac-MeOH displayed the best activity, reaching about 85% at the highest tested concentration, whereas Mac-H_2O and Hot-H_2O extracts did not show any chelating effect (Supplemental Figure S3, Table 1).

The results of the antioxidant tests showed that the leaf extracts displayed noticeable radical scavenging and chelating activities, and maceration with methanol resulted the most suitable extraction system for obtaining an effective recovery of antioxidants; further, the root Mac-MeOH extract demonstrated good chelating properties.

Kurt et al. (2018) previously evaluated the antioxidant properties of different extracts (hexane, ethanol and water) obtained by maceration of the whole plant of *Silene vulgaris* var. *macrocarpa* grown in Turkey; the extracts were found to possess moderate radical scavenging ($ABTS^+$ assay) and cupric reducing antioxidant capacities (CUPRAC). Our results agree with those previously reported regarding the reducing properties, whereas they differ considerably from those of the radical scavenging assay.

Based on the obtained results, the Mac-MeOH extracts of both leaves and roots were selected for phytochemical investigations.

Phytochemical investigations on S. vulgaris subsp. macrocarpa leaf and root Mac-MeOH extracts

Total phenolic, flavonoid and condensed tannin content

The results of Folin-Ciocalteu assay showed that the total phenolic content of *S. vulgaris* subsp. *macrocarpa* leaf and root Mac-MeOH extracts was equal to 287.91 ± 1.97 mg GAE/g extract and 31.29 ± 1.12 mg GAE/g extract, respectively.

The total flavonoid content of the leaf extract was 121.67 ± 0.19 mg QE/g extract, whereas no flavonoids were found in root extract.

The condensed tannin content of the extracts, as evaluated by the vanillin assay, was found to be 18.33 ± 0.96 mg CE/g extract for the leaves and 2.67 ± 0.23 mg CE/g extract for the roots.

Determination of the polyphenolic compounds by HPLC-PDA-ESI-MS

To date, there is not information regarding the polyphenolic fingerprint in *S. vulgaris* subsp. *macrocarpa* leaf extracts, and only one work dealt with the determination of the anticholinesterase, antioxidant, antiaflatoxigenic activities of *S. vulgaris* var. *macrocarpa* collected in Turkey (Kurt et al. 2018). In addition, only one work reported the phytochemical investigation of *S. vulgaris* var. *vulgaris* (Zengin et al. 2018).

Figure 1 shows the HPLC-PDA chromatograms, extracted at a 280 nm wavelength, of the polyphenolic compounds occurring in the leaf and root Mac-MeOH extracts of *S. vulgaris* subsp. *macrocarpa*. Polyphenol identification was carried out by data coming from retention times, PDA and MS spectra. As can be appreciated, 13 polyphenolic compounds were positively identified and quantified in the leaves for the first time, as reported in Table 2 where for each compound, retention times, $[M-H]^-$ values and UV/Vis spectra are indicated. Most of them belong to the class of hydroxycinnamic acids and in particular are *p*-coumaric acid derivates. With regards to MS, when observed, also secondary fragments (daughters ions) arising from fragmentation of the $[M-H]^-$ are included; in all cases the detection of aglycones deriving from the loss of the sugar moiety was observed. On the other hand, only two polyphenolic compounds, namely catechin and procyanidin B1 were found in the roots (Table 2). Quantitative determination was accomplished by interpolation of calibration curves of the reference materials listed in Experimental Section. Among the polyphenolic compounds identified (Table 2), *p*-coumaric acid derivatives represent the most abundant ones (28.65 mg/g extract), accounting for roughly 81% of the whole polyphenolic content (35.49 mg/g extract). In all cases, %RSD values were below 3%.

Phenolic acids, especially hydroxycinnamic acids, are an important group of secondary plant metabolites with powerful antioxidant capacities. In addition, caffeic acid and *p*-coumaric acid have previously been shown to be effective antioxidants in different *in vitro* models including DPPH, reducing power, and metal chelating assays; antioxidant capabilities of these phenolics resulted very close or even stronger than those of synthetic antioxidant compounds such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Kiliç and Yeşiloğlu 2013; Masek et al. 2016). A study carried out by Chang et al. (2017) reported IC_{50} values of 0.97 μM and 6.65 μM in the DPPH test and of 655.3 μM and 1234.1 μM in the Fe^{2+} ions chelating activity assay for caffeic acid and *p*-coumaric acid, respectively. By contrast, Olennikov et al. (2011) highlighted in the DPPH test

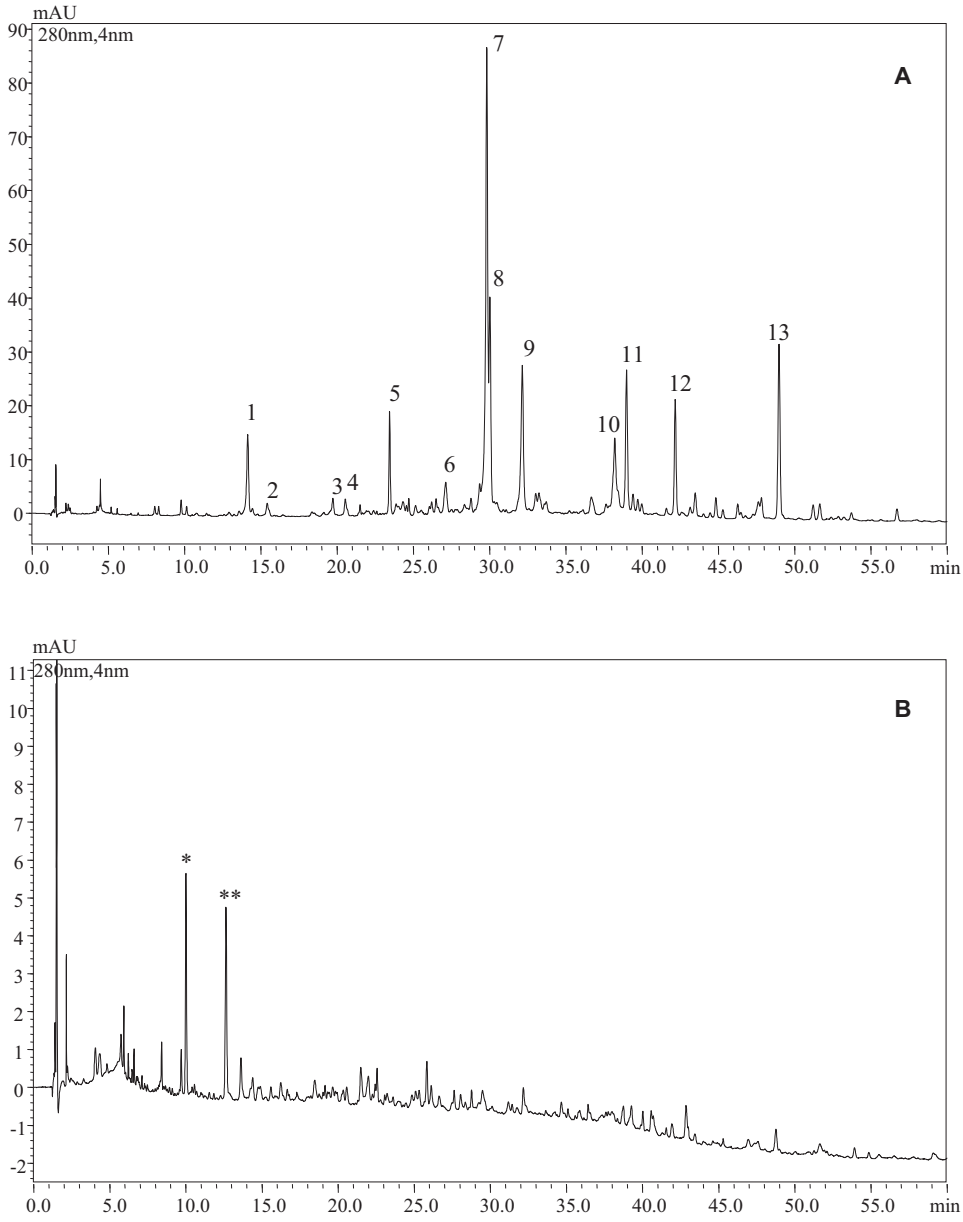

Figure 1. HPLC-PDA-ESI-MS polyphenolic fingerprint of *Silene vulgaris* subsp. *macrocarpa* leaf (A) and root (B) Mac-MeOH extracts. Column: Ascentis Express C18, 15 cm × 4.6 mm, 2.7 μm d.p. (ESI, negative ionization mode). For peak identification, see Table 2.

Table 2. HPLC-PDA-ESI-MS (negative ionization mode) polyphenolic fingerprint of *Silene vulgaris* subsp. *macrocarpa* leaf and root Mac-MeOH extracts. Column: Ascentis Express C18, 15 cm × 4.6 mm, 2.7 μm d.p. (ESI, negative ionization mode).

Peak	Compound	t_R (min)	Molecular formula	$[M-H]^-$	PDA (nm)	Leaves (mg/g ± %RSD)	Roots (mg/g ± %RSD)
*	Procyanidin B1	10.1	$C_{30}H_{26}O_{12}$	577	277	–	1.73 ± 1.25
**	Catechin	12.7	$C_{15}H_{14}O_6$	289	278	–	1.87 ± 1.63
1	Caffeic acid-*O*-hexoside	14.0	–	341, 179	325	2.82 ± 1.56	–
2	Caffeic acid	15.5	$C_9H_8O_4$	179	325	0.44 ± 2.43	–
3	Vanillic acid hexoside	19.7	$C_{16}H_{14}O_5$	329	291	0.19 ± 2.87	–
4	Vanillic acid hexosylpentoside	20.6	–	461	292	0.46 ± 2.53	–
5	Glucosyringic acid	23.4	$C_{16}H_{14}O_5$	359	292	1.02 ± 1.98	–
6	Quercetin hexoside	27.1	$C_{22}H_{22}O_{12}$	477, 301	355	1.91 ± 1.21	–
7	*p*-coumaric acid derivative	29.8	–	1057	314	10.9 ± 0.98	–
8	*p*-coumaric acid derivative	30.0	–	925	314	4.66 ± 1.01	–
9	*p*-coumaric acid derivative	32.1	–	925	314	4.10 ± 0.99	–
10	*p*-coumaric acid derivative	38.2	–	1057	314	1.06 ± 0.97	–
11	*p*-coumaric acid derivative	39.0	–	925	314	2.64 ± 1.64	–
12	*p*-coumaric acid derivative	42.1	–	–	314	2.64 ± 1.71	–
13	*p*-coumaric acid derivative	48.9	–	–	314	2.65 ± 1.68	–

lower activity for caffeic acid than *p*-coumaric acid, with IC_{50} values of 11.63 ± 0.34 μg/mL and 9.07 ± 0.27 μg/mL, respectively. In another study an IC_{50} value of 2.39 μg/mL was found for caffeic acid in the DPPH test (Spagnol et al. 2019). Based on all the above, it can be supposed that the effects observed for *S. vulgaris* subsp. *macrocarpa* leaf Mac-MeOH extract could depend mainly on caffeic and *p*-coumaric derivatives contained in the phytocomplex.

The Mac-MeOH root extract contains two polyphenolic compounds only, namely catechin and procyanidin B1 that are a flavan-3-ol and a proanthocyanidin respectively; by contrast it doesn't contain phenolic acids. Flavan-3-ols have been shown to behave as antioxidants *via* several mechanisms including the scavenging of free radicals as well as chelation of transition metals (Aron and Kennedy 2008). In particular, the ability of flavan-3-ols as well as of their polymeric condensation products, the proanthocyanidins, to bind such divalent transition metals was demonstrated (Maffei Facino et al. 1996; Beecher 2004). Thus, the chelating ability of the Mac-MeOH root extract could be related, at least in part, to these compounds.

Conclusions

The results of this investigation indicated that methanol represents a suitable solvent for the effective recovery of antioxidants from leaves and roots of *S. vulgaris* subsp. *macrocarpa*, and that maceration is the most efficient extraction technique. By the *A. salina* lethality bioassay the potential safety of the extracts was shown. Besides, the polyphenolic fingerprint of *S. vulgaris* subsp. *macrocarpa* leaves and roots was characterized for the first time. The noticeable radical scavenging and chelating activities of the leaf Mac-MeOH extract could be related to the phenolic acids, whereas the good chelating properties of the root Mac-MeOH extract could be associated to the flavan-3-ols. Altogether, our findings enrich the knowledge about the phytochemical composition and the biological properties of *S. vulgaris* subsp. *macrocarpa,* and highlight that this edible plant species represents a safe source of antioxidants.

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Disclosure statement

The authors declare no potential conflict of interest, including any financial interest.

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