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ORIGINAL ARTICLE

Antioxidant activity of crude extracts and essential oils from flower buds and leaves of *Cistus creticus* and *Cistus salviifolius*

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Abstract Volatile oils from flowers and leaves of *C. creticus* L. and *C. salviifolius* L. were extracted by two extraction methods; namely, hydrodistillation and solid-phase micro-extraction (SPME). The chemical composition of essential oils was analyzed by GC and GC–MS. The volatile extracted from leaves and flowers of *C. criticus* using SPME was dominated by monoterpenes and sesquiterpenes hydrocarbon with α-pinene, camphene and α-cubebene as major components. In hydrodistillation, the oil extracted from leaves was dominated by oxygenated diterpenes and diterpenes hydrocarbon with manoyl oxide and sclarene as major components, whereas, the oil extracted from flowers was dominated by oxygenated diterpenes and diterpenes hydrocarbon with manoyl oxide and abietatriene as major components. The volatile from flowers and leaves of *C. salviifolius* obtained by SPME were dominated by monoterpenes and sesquiterpenes with δ-3-carene, α-pinene, β-pinene, and E-caryophyllene as major constituents. On the other hand, the oils from flowers and leaves of *C. salviifolius* obtained by hydrodistillation were dominated by oxygenated diterpenes, diterpenes hydrocarbon and esters with dehydro abietol, abietol, manoyl oxide and methyl octadecenoate as major components. In the leaves, the major components of the oil were manoyl oxide, E-ethyl cinnamate, and Z-ethyl cinnamate. These oils showed weak antioxidant activity when compared to the positive controls α-tocopherol, ascorbic acid, and EDTA, while

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the crude extracts aq. MeOH, butanol, and water showed good antioxidant activity. Discriminating between the studied plants based on the extraction method was also possible upon applying Principle component analysis (PCA) to the obtained GC–MS data.

1. Introduction

Rock-rose plants and shrubs consist of 175 species of eight herbaceous genera. Genera *Cistus creticus* L. and *Cistus salvifolius* L. of the species (Cistaceae) are grown in the Mediterranean region, Europe, North America temperate areas and a limited number of them is found in South America and the northern area of Jordan (Comandini et al., 2006; Al-Eisawi, 1982; Guimarães et al., 2009). *Cistus* species are commonly used in many Mediterranean countries in traditional folk medicine as antidiarrheic and for the treatment of various skin diseases (Madaus et al., 1938; Barrajón-Catalán et al., 2010). The use of the plant in this way has multiple functions as an anti-inflammatory (Demetzos et al., 2001), antiulcerogenic, wound healing, antimicrobial (Demetzos et al., 1999), antifungal (Bayoub et al., 2010), antiviral, antitumor (Dimas et al., 2000), cytotoxic (Ben Jemia et al., 2013) and antinociceptive (Barrajón-Catalán et al., 2010). Phytochemical studies on different *Cistus* species revealed the presence of several flavonoid compounds (Pascual et al., 1977; Danne et al., 1994; Kreimyeret al., 1998; Vogt and Gulz, 1986; Petereit et al., 1991; Vogt et al., 1987; Santagati et al., 2008), labdane diterpenes (Demetzos et al., 1990; Anastasaki et al., 1999; Chinou et al., 1994; Demetzos et al., 1994) and polyphenolic glycosides (Demetzos et al., 1989). The studies on essential oil composition of *Cistus* species revealed the presence of oxygenated monoterpenes, sesquiterpenes, aromatics, oxygenated sesquiterpenes and traces of carbonyl compounds (Demetzos et al., 1997; Demetzos et al., 1995; Costa et al., 2009; Mastino et al., 2017; Maggi et al., 2016). Leaves of all *Cistus* species are covered with glands secreting resin and essential oil consisting mainly of terpenoids (Mastino et al., 2017).

In Jordanian traditional medicine *Cistus* species are used in the treatment of multiple ailments such as anti-inflammatory, gout, ulcers, gastrointestinal disorders, diabetes, as well as reduction of blood glucose (Farley and McNeilly, 2000; Al-Khalil, 1995; Yesilada et al., 1999). Previous studies on essential oil composition in Cistus *salviifolius*, and *Cistus creticus* showed that they are rich in oxygenated diterpenes such as manoyl oxide, labd-13-en-8-yl acetate, and 13-epi-manoyl oxide, as well as in oxygenated sesquiterpenes such as viridiflorol, caryophyllene oxide, vitispirane, and bulnesol and hydrocarbon sesquiterpenes such as α-cadinene and δ-cadinene (Demetzos et al., 1997; Demetzos et al., 1995; Maggi et al., 2016).

As part of our continuous effort in investigating the chemical composition of essential oil and antioxidant activity of medicinal plants (Al-Qudah, 2013, 2016; Al-Qudah et al., 2014, 2018). The objective of the present work is to investigate the chemical composition of essential oils extracted from fresh flower buds and leaves of *C. creticus* and *C. salviifolius* using hydro-distillation and solid-phase micro-extraction (SPME) followed by GC and GC–MS analysis. Also, we have examined the antioxidant activities of the oils and crude extract fractions prepared from these plants.

2. Materials and methods

2.1. Chemical reagents

Chemical reagents used in this study were: helium (high purity 99%), *n*-alkanes (C7-C30) GC grade AR., 5% diphenyl, 95% dimethyl polysiloxane (DP-5) grade AR, 2,2′-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, a-tocopherol, methanol, potassium persulfate, ferrous chloride, ferrozine, Sodium sulfate, EDTA (Ethylenediaminetetraacetic acid) and internal reference compounds. All chemicals used in this investigation were purchased from Sigma-Aldrich (Buchs, Switzerland).

2.2. Sampling and materials

The present study was carried out in April 2016. Samples of fresh flower buds and leaves of *Cistus creticus* and *C. salviifolius* were collected at maturity stage from different sites in Ajloun and Jerash areas in the northern part of the Hashemite Kingdom of Jordan. A voucher specimen of each has been deposited in the herbarium of the Department of Biological Sciences-Yarmouk University, Irbid, Jordan, *C. creticus* L. (YU/1/CC/1001) and *C. salviifolius* L. (YU/1/CC/1002).

2.3. Preparation of essential oils

The essential oils from fresh leave and flower buds of *C. creticus* and *C. salviifolius* were isolated as described (Al-Qudah, 2013; Al-Qudah et al., 2014, 2018). Fresh leaves and flower buds of *C. creticus* and *C. salviifolius* (200 g), were chopped into small parts and hydrodistillation for 4 h using a Clevenger-type apparatus. Subsequently, oils were dried over anhydrous sodium sulfate and immediately stored in GC-grade *n*-hexane at 4 °C until the analysis by gas chromatography/mass spectrometry (GC/MS) is carried out.

2.4. Solid phase micro-extraction (SPME) of volatile oils

The SPME experiments were performed using the fiber assemblies (PDMS/DVB; df 65 μm, length 1 cm) for manual sampling (Supelco, USA). About 0.1 g of freshly leave and flower buds of *C. creticus* and *C. salviifolius* were put into 5.0 mL amber glass vials, tightly capped with PTFE-coated septa, and SPME extraction was performed for 2.0 min at RT. Desorption of the analytes was carried out at 240 °C for 60 s. Each sample was repeated twice.

2.5. GC and GC–MS analysis

For chemical identification, a small portion of 1 μL extracted oils were diluted to 10.0 μL with GC grade *n*-hexane, then analyzed by GC–MS (Model Varian Chrompack CP-3800 GC/MS, Saturn, Netherlands) system, equipped with a DB-5 GC capillary column (5% diphenyl, 95% dimethyl polysiloxane, 30 m × 0.2 5 mm i.d., 0.25 μm film thicknesses). For mass spectroscopy detection, an electron ionization mode of 70 eV energy was used with a specific mass range. The flame ionization detector (FID) and injector temperature in the MS source were set at 180 °C. The temperature column was also programmed from 60 °C for 1 min (isothermal) to 246 °C at a constant rate of 3 °C/min, with the lower and upper temperatures being held for 3 min. The carrier gas was helium and was set at a flow rate of 0.9 mL/min. Quantitative analysis was performed using the Hewlett-Packard HP-8590 gas chromatography (Hewlett-Packard Co., Palo Alto, CA, USA) equipped with a split-splitless injector (split ratio 1:50) and a flame ionization detector (FID) was used. The device was connected to a 5% diphenyl, 95% dimethyl polysiloxane (optima-5) fused silica capillary column (30 m × 0.25 mm, 0.25 μm film thickness) (Varian Capillary Column) and under the same conditions described for the GC/MS analysis part.

2.6. Identification of the chemical constituents

A hydrocarbon mixture of *n*-alkanes (C_7–C_{30}) was analyzed separately under similar chromatographic conditions using the same DP-5 column. The identification of separated volatile components was achieved by matching their recorded mass spectra with the built-in library spectra (NIST, Gaithersburg, MD, USA, and Wiley Co., Hoboken, NJ, USA) and by comparing their calculated Kovats retention index (KI) relative to (C_7–C_{30}) n-alkanes values measured with the column of identical polarity. Further identification of major components of the extracts was confirmed by injecting authentic standard reference compounds on the same chromatography column and comparing their retention times with those of their counterparts from the oil samples.

2.7. Preparation of crude fractions

The fresh plants material from the flowers and leaves of *C. salviifolius* and *C. creticus* were air-dried in the shade for 1 month as previously described (Al-Qudah et al., 2018). Afterward, they were ground to fine powders and defatted with petroleum ether in Soxhlet extractor. After this, the plant residue was extracted in the same apparatus in methanol. The obtained alcoholic gummy residue was then partitioned between $CHCl_3$ and H_2O (1:1). The dried chloroform residue was then subjected to partitioning between 10% aqueous methanol (aq.MeOH) and hexane. The polar organic compounds were extracted from water by *n*-butanol. The different fractions obtained were assayed for their total phenol contents (TPC), total flavonoid contents (TFC) and in vitro and antioxidant activities.

2.8. Determination of total flavonoid (TFC) and phenol contents (TPC)

The total flavonoids contents of the crudes (Aq. MeOH, Butanol and water extracts) from the flowers and leaves of *C. salviifolius* and *C. creticus* were determined by the Folin-Ciocalteu method (Al-Qudah et al., 2018). 1.0 mL aliquot from the stock solution (1 mg/mL) of each extract, diluted in 4.0 mL distilled water, were introduced into a 10.0 mL volumetric flask, to which 0.30 mL of sodium nitrite solution (5% $NaNO_2$, w/v) were added. The resulting mixture was allowed to stand for 5 min and then, 0.30 mL of aluminum chloride solution (10% $AlCl_3$, w/v) was added. The resulting solution was incubated for another 6 min after which, 2.0 mL of 1.0 M NaOH solution was added and the final volume was adjusted to 10.0 mL with distilled water. After 15 min, the absorbance was measured at the wavelength, λ, of 510 nm. Methanol was used as blank. The total flavonoids content is expressed, in mg/g, as mass of quercetin with respect to the mass of the dry extract.

The phenols' contents of the crudes from the flowers and leaves of *C. salviifolius* and *C. creticus* was determined by aluminum chloride assay (Al-Qudah et al., 2018) 0.5 mL aliquot from the stock solution (1 mg/mL) of each extract was treated with 2.5 mL of Folin–Ciocalteu reagent (2 N) (diluted ten folds) and 2 mL of Na_2CO_3 (75 g/L). The mixture was allowed to stand at room temperature for 15 min and the absorbance was then recorded at the wavelength, λ, of 765 nm. Methanol was used as a blank solution. The total phenol content in the different extracts of both plants was expressed as mg gallic/g dry extract.

2.9. Determination of antioxidant activity

Also, the antioxidant activity of the crude and essential oils was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical scavenging, hydrogen peroxide scavenging and ferrous ion chelating activity assay (Al-Qudah, 2013, 2016; Al-Qudah et al., 2014, 2018).

2.9.1. DPPH free radical scavenging activity

Briefly, to 1.0 mL of 0.1 mM DPPH• solution (dissolved in MeOH), 2.0 mL of various concentrations (0.005–0.5) mg/mL of each methanolic extract solutions were added. The solutions were allowed to stand at room temperature in dark for 30 min and then the absorbance of the solutions was measured at the wavelength, λ, of 517 nm against blank samples using UV–VIS spectrophotometer.

2.9.2. ABTS radical scavenging assay

The $ABTS^{\bullet+}$ cation radical solution was prepared by reaction of similar quantities of 7 mM of ABTS and 2.4 mM of potassium persulfate ($K_2S_2O_8$) solution and allowed to react for 16 h at R.T in the dark. Before use; this solution was diluted with methanol to get an absorbance of 0.75 ± 0.02 at 734 nm. The reaction mixture comprised 3.0 mL of ABTS•+ solution and 1 mL of the extracts at various concentration (0.005–0.50) mg/mL. The absorbance of the mixture was measured at the wavelength, λ, of 734 nm by using a UV–Vis spectrophotometer. The blank was run in each assay and all measurements were done after at least 5 min.

2.9.3. Hydroxyl radical assay

1 mL of different concentrations of the extract solution in methanol (0.005–0.50) mg/mL were added to a 0.5 mL $FeSO_4$

solution (6 mM). Then, 0.5 mL of 6 mM H_2O_2 was added to the mixture. Subsequently, after shaking and incubation of the reaction mixture for 10 min at room temperature, a 1 mL of 6 mM salicylic acid was added and further incubated for 30 min at room temperature. The absorbance was at the wavelength, λ, of 510 nm.

2.9.4. Ferrous ion chelating (FIC) effect

3.0 mL of methanol solution containing the different concentrations of crudes and essential oils (0.005–0.50) mg/mL was added to a 0.25 mL of 2 mM ferrous chloride ($FeCl_2$) reagent. Subsequently, a 0.2 mL of 5 mM ferrozine solution was added to the mixture and allowed to stand at r.t. for 10 min after vigorous shaking. Then, the reduction in the absorbance of the visible radiation of red color was measured spectrophotometrically at 562 nm. The scavenging activity of the tested extracts and essential oils was compared to those of the positive control's ascorbic acid, α- tocopherol, and EDTA under similar conditions.

The percentage of scavenging activity of the tested extracts and essential oils was calculated using the equation: Scavenging activity (%) = $(A_c - A_s/A_c) \times 100$; where A_c is the absorbance of the control and A_s is the absorbance in the presence of either extracts or control substance.

Non-linear regression analysis of GraphPad Prism 6 (GraphPad Software, San Diego, California, USA) was applied for the determination of IC_{50} in all of the antioxidant assays from the sigmoidal curve which was obtained by plotting the percentages of scavenging relative to the control *versus* logarithmic concentration of test compound. Each concentration was tested three times in 3 independent experiments.

2.10. Data analysis

MAT LAB 7.0.4 (Math Works, MA, USA) with PLS Toolbox 4.0 (Eigenvector Research, Inc, WA, and the USA) software were used for data processing and PCA analysis.

3. Results and discussion

3.1. Essential oil composition of flowers and leaves from ***Cistus salviifolius***

The essential oils yield of leaves and flowers from *C. salviifolius* was 0.05% w/w and 0.03% w/w, respectively. Analysis of extracted oils from leaves and flowers of genus *salviifolius* was achieved by GC and GC–MS. Also, the 48 and 17 constituents which represent 99.9% and 99.8% of the oils obtained by hydrodistillation were identified, respectively (Table 1).

The total high percentage of principal oxygenated diterpenes oil in flowers (81.0%), associated with dehydro abietol (59.0%) and abietol (12.7%), was detected. Esters were found in a percentage of (9.9%) associated with methyl octadecanoate (9.2%) and E-ethyl cinnamate (0.7%). The analysis of the components of hydrodistilled oil from flowers contained two diterpene hydrocarbons (4.9%) and two sesquiterpenes (1.4%) as seen in Table 1. The essential oil of the leaves has E-ethyl cinnamate (17.5%), manoyl oxide (13.2%), abietatriene (12.3%), Z-ethyl cinnamate (11.3%) and carvenone (11.2%), which seem to be the major components of the essentail oil from leaves of *C. salviifolius*.

The volatiles from the flowers and leaves of *C. salviifolius* were extracted and collected by the SPME method for analysis using the GC–MS technique, as mentioned in the literature (Saleh et al., 2017). The analysis of volatiles from were identified 53 components from flowers and 50 components from leaves, with a percentage of 85.1% up to 92.1% of the total composition, respectively. The results of the analysis are shown in Table 1. Table 1 shows flower oil consisting of 13 monoterpene hydrocarbons (48.8%), associated with δ-3-carene (15.7%), α-pinene (12.8%) and β-pinene (7.0%) as major constituents. The identification reveals 17 sesquiterpene hydrocarbons (16.5%), 10 other compounds (9.3%), two oxygenate diterpenes (4.9%), four oxygenated monoterpenes (1.8%), four oxygenated sesquiterpenes (1.9%), two ester compounds (1.5%) and one diterpene hydrocarbon (0.4) from flower oil of *C. salviifolius*, which indicates that there is a high similarity in active essential oil components with *C. creticus* L. A different set of extracted oils was detected from leaves, which gave different components including 13 monoterpene hydrocarbons (48.9%), associated with δ-3-carene (15.5%) and α-pinene (11.9%) along with 19 sesquiterpene hydrocarbons (31.6%), associated with E-caryophyllene (10.4%), three oxygenated monoterpenes (3.2%), 8 other compounds (4.0%) and four oxygenated sesquiterpenes (1.8%) all of which may be considered as major compounds.

3.2. Essential oil composition of flowers and leaves from C. creticus

The distilled essential oils from leaves and flower buds are characterized to range from yellowish to yellow color and the yields were 0.02% w/w and 0.01% w/w, respectively. In terms of chemical structure, volatiles and essential oil components are classified into (8) classes associated with the calculated Kovats indices and mass spectra compared to those stored in the GC–MS built in libraries. Table 1 represents the GC and GC–MS analysis of the *C. creticus* oils from leaves and flower buds, where the results led to the identification of 21 and 47 constituents (100%) and (96.7%), respectively.

Experimentally, flower oil contents are manoyl oxide and 3-α-acetoxy-manool with a percentage of (19.5%) and (5.9%), respectively, whereas oxygenated diterpenes (27.9%) represent the major content of the total oil weight. Diterpenes (24.7%), abietatriene (12.0%) and sclarene (7.2%) were found to be the major components of the oils. The percentage composition of flower oil constituents obtained by the hydrodistillation method are summarizes in Table 1. It contains eight categories of volatiles with six oxygenated sesquiterpenes (8.1%), twelve sesquiterpene hydrocarbons (11.1%), ten aliphatic compounds (19.9%), two oxygenated monoterpenes (3.0%) and two monoterpene hydrocarbons (0.2%).

In comparison, the essential oil of the leaves has manoyl oxide (27.0%), sclarene (13.3%), γ-cadinene (10.8%), and 3-α-acetoxy-manool (5.6%) as major components. Organic active components of volatile oils extracted from flowers and leaves of *C. creticus* L. were obtained by Solid Phase Micro-Extraction (SPME) method and GC–MS technique using the procedure mentioned in the literature (Saleh et al., 2017). Table 1 shows the results of volatile principle active

Table 1 Chemical composition of essential oils from flowers and leaves of *C. salviifolius* L. and *C. creticus* L. obtained by SPME and hydrodistillation.

No.	KI (reported)	KI (Exp.)	Compound	% Area *C. salviifolius* L.				% Area *C. creticus* L.				Method of identification
				SPME		Hydrodistillation		SPME		Hydrodistillation		
				Flowers	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers	Leaves	
1	821	826[a]	2E-Octene	1.1	–	–	–	0.1	–	0.1	–	MS[b], RI,
2	830	835	2E-Hexenal	3.4	0.4	–	0.9	–	–	2.8	1.3	MS, RI
3	856	863	E-Salvene	–	2.3	–	–	–	–	0.1	–	MS, RI
4	888	895	santene	0.7	3.9	–	–	–	–	0.1	–	MS, RI
5	901	903	Ethyl pentanoate	1.0	–	–	–	–	–	–	–	MS, RI
6	926	926	tricyclene	0.3	0.2	–	–	0.8	0.7	–	–	MS, RI
7	939	934	α-pinene	12.8	11.9	–	0.3	25.9	19.4	–	–	MS, RI, RC[c]
8	952	951	α-fenchene	3.1	1.4	–	–		0.8	–	–	MS, RI
9	954	952	camphene	–	–	–		12.0	9.3	–	–	MS, RI
10	975	973	sabinene	0.9	0.8	–	–	0.1	–	–	–	MS, RI
11	979	984	β-pinene	7.0	5.2	–	–	8.5	6.3	–	–	MS, RI, RC
12	990	990	myrcene	–	–	–		3.0	7.5	–	–	MS, RI
13	1004	1007	p-mentha-1(7),8-diene	–	–	–		0.8	0.8	–	–	MS, RI
14	1017	1023	α-terpinene	0.3	0.4	–	–	0.7	0.6	–	–	MS, RI
15	1024	1029	p-cymene	4.8	5.7	–	–	0.6	0.9	–	–	MS, RI
16	1031	1034	δ-3-carene	15.7	15.5	–	–	6.4	5.9	–	–	MS, RI, RC
17	1050	1046	E-β-ocimene	0.7	0.5	–	–	0.1	0.3	–	–	MS, RI
18	1059	1061	γ-terpinene	1.7	0.6	–	–	1.6	1.2	–	–	MS, RI
19	1070	1064	*cis*-sabinene hydrate	0.6	0.4	–	–			–	–	MS, RI
20	1088	1087	terpinolene	0.2	0.3	–	–	0.7	0.7	–	–	MS, RI
21	1091	1091	p-cymenene	0.7	–	–	–	–	–	–	–	MS, RI
22	1096	1101	linalool	0.7	0.8	–	–	–	–	–	–	MS, RI
23	1100	1106	n-Undecane	–	0.7	–	–	–	–	0.6		MS, RI,
24	1100	1107	n-Nonanal	0.3	0.5	0.4	–	–	–	–	–	MS, RI
25	1102	1108	*cis*-thujone	0.3	–	–	–	–	–	–	–	MS, RI
26	1146	1147	camphor	0.2	–	–	3.0	–	–	–	–	MS, RI
27	1177	1180	terpinen-4-ol	–	–	–	–	0.1	0.4	0.8		MS, RI
28	1188	1194	α-terpineol	–	–	–	–	–	0.1	2.2		MS, RI
29	1200	1200	n-Dodecane	0.5	0.4	–	–	–	–	–	–	MS, RI
30	1201	1206	n-Decanal	0.4	0.7	–	–	–	–	–	–	MS, RI
31	1225	1232	citronellol	–	2.0	–	0.4	–	0.1	–	–	MS, RI
32	1248	1249	Benzeneacetic acid, ethyl ester	–	–	–	0.4	–	–	–	–	MS, RI
33	1258	1258	carvenone	–	–	–	11.2	–	–	–	–	MS, RI, RC
34	1265	1264	*cis*-chrysanthenyl acetate	–	–	–	0.2	–	–	–	–	MS, RI
35	1285	1288	bornyl acetate	–	–	–	0.7	–	–	–	–	MS, RI
36	1299	1298	Z-Methyl cinnamate	–	–	–	0.3	–	–	–	–	MS, RI
37	1300	1305	n-tridecane	0.2	0.5	–	–	–	–	–	–	MS, RI
38	1346	1346	Benzyl butanoate	–	–	–	0.5	–	–	–	–	MS, RI
39	1351	1353	α-cubebene	1.4	1.0	–	–	10.4	12.0	0.8	4.2	MS, RI, RC
40	1369	1356	eugenol	–	–	–	–	–	–	10.2	–	MS, RI
41	1370	1360	silphiperfol-5,7(14)-diene	–	–	–	–	0.3	0.2			MS, RI
42	1371	1367	cyclosativene	0.4	1.3	–	0.3	–	–	–	–	MS, RI
43	1376	1374	α-copaene	1.1	3.3	–	–	–	–	–	–	MS, RI
44	1381	1375	daucene	–	–	–		5.6	4.0	0.3	–	MS, RI
45	1377	1383	Z-Ethyl cinnamate	–	–	–	11.3	0.7	0.5	–	–	MS, RI, RC
46	1378	1386	E-Methyl cinnamate	–	–	–	1.3	–	–	–	–	MS, RI
47	1388	1388	β-cubebene	0.3	0.3	–	–	1.2	1.6	0.6	2.8	MS, RI
48	1398	1398	methyl eugenol	–	–	–	–	–	–	0.5	–	MS, RI
49	1400	1400	n-Tetradecane	0.2	0.4	–	–	–	–	–	–	MS, RI
50	1409	1404	α-gurjunene	–	0.4	–	–	0.3	0.3	–	–	MS, RI
51	1419	1417	E-caryophyllene	2.9	10.4	0.58	1.6	4.4	8.4	0.6	2.7	MS, RI, RC

Table 1 (*continued*)

No.	KI (reported)	KI (Exp.)	Compound	% Area *C. salviifolius* L.				% Area *C. creticus* L.				Method of identification
				SPME		Hydrodistillation		SPME		Hydrodistillation		
				Flowers	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers	Leaves	
52	1420	1427	β-copaene	–	–	–	–	0.2	0.1			MS, RI
53	1450	1446	*cis*-muurola-3,5-diene	0.2	0.5	–	–	0.6	0.6	0.4	1.4	MS, RI
54	1454	1453	α-humulene	–	1.0	–	0.4	0.3	0.5	–	–	MS, RI
55	1460	1457	Allo-aromadendrene	0.6	1.4	–	0.4	0.1	–	–	–	MS, RI
56	1467	1462	E-Ethyl cinnamate	–	–	0.66	17.5	–	–	–	–	MS, RI
57	1473	1467	drima-7,9(11)-diene	–	–	–	–	–	0.3	0.8	3.1	MS, RI
58	1477	1469	*trans*-cadina-1(6),4-diene	0.4	0.6	–	–	1.7	1.5	0.8	2.5	MS, RI
59	1480	1472	γ-muurolene	0.3	0.8	–	–	0.3	0.3	–	–	MS, RI
60	1485	1478	germacrene D	0.4	–	–	0.1	0.1	0.1	–	–	MS, RI
61	1484	1488	γ-amorphene	–	0.4	–	–	0.1	–	0.5	1.5	MS, RI
62	1492	1489	selinene	0.4	–	–	0.5	1.7	2.2	–	–	MS, RI
63	1493	1489	*trans*-muurola-4 (14),5 diene	–	–	–	–	0.5	0.6	0.8	2.8	MS, RI
64	1500	1495	n-Pentadecane	0.3	0.5	–	–	0.7	0.9	–	–	MS, RI
65	1500	1498	bicyclogermacrene	–	–	–	2.39	–	–	–	–	MS, RI
66	1500	1500	α-muurolene	0.4	1.5	–	–	0.4	0.3	–	–	MS, RI
67	1505	1503	(E,E)-α-farnesene	4.8	1.2	0.8	–	0.1	–	–	–	MS, RI,
68	1511	1508	δ-amorphene	–	–	–	–	0.2	0.1	–	–	MS, RI
69	1513	1510	γ-cadinene	0.4	1.8	–	–	0.1	–	3.8	10.8	MS, RI
70	1523	1515	δ-cadinene	1.8	4.5	–	–	4.2	3.8	0.9	2.1	MS, RI
71	1529	1520	zonarene	0.3	0.4	–	–	1.5	1.3	–	–	MS, RI
72	1534	1529	*trans*-cadina-1(2),4-diene	0.4	0.4	–	–	1.2	1.5	0.5	1.5	MS, RI
73	1532	1534	Z-nerolidol	–	–	–	–	0.1	0.2	0.4	–	MS, RI
74	1538	1536	α-cadinene	–	0.4	–	–	–	–	–	–	MS, RI
75	1566	1569	3Z-hexenyl benzoate	0.3	1.1	–	0.5	–	–	–	–	MS, RI
76	1571	1572	dendrolasin	0.8	0.5	–	–	–	–	–	–	MS, RI
77	1583	1582	caryophyllene oxide	–	0.3	–	0.9	0.2	0.5	0.4	–	MS, RI
78	1587	1593	davanone	–	–	–	4.77	–	–	–	–	MS, RI
79	1619	1605	1,10-di-epi-cubenol	0.4	0.7	–	–	0.2	–	3.9	5.3	MS, RI
80	1625	1613	citronellyl pentanoate	0.2	–	–	–	–	–	–	–	MS, RI
81	1640	1634	caryophylla-4(14),8 (15)-diene-5,α-ol	–	–	–	–	–	0.2	–	0.4	MS, RI
82	1642	1640	α-muurolol (Torreyol)	–	–	–	–	0.2	–	1.9	3.1	MS, RI
83	1650	1652	7-epi-α-eudesmol	–	0.4	–	–	0.1	0.3	–	–	MS, RI
84	1668	1663	E-citronellyl tiglate	0.4	–	–	–	–	–	–	–	MS, RI
85	1672	1677	n-Tetradecanol	2.5	–	–	–	–	–	–	–	MS, RI
86	1845	1855	Z-ternine	0.3	–	2.0	0.4	–	–	–	–	MS, RI
87	1878	1879	cubitene	–	–	–	–	0.1	–	1.2	2.5	MS, RI
88	1894	1896	catalponone	–	–	–	–	–	–	2.7	–	MS, RI
89	1900	1900	n-Nonadecane	–	–	0.4	–	–	–	–	–	MS, RI,
90	1905	1907	isopimara-9(11),15-diene	–	–	–	0.3	–	–	–	–	MS, RI
91	1922	1916	totarene	–	–	–	0.3	–	–	0.2	–	MS, RI
92	1921	1922	methyl hexadecanoate					–	–	0.6	–	MS, RI
93	1934	1929	isohibaene	–	–	–	0.3	–	–	–	–	MS, RI
94	1938	1953	cembrene	–	–	–	1.5	–	–	–	–	MS, RI
95	1948	1957	3E-cembrene A	–	–	0.5	0.1	–	–	0.2	–	MS, RI
96	1960	1959	nootkatin	–	–	–	–	0.2	–	0.2	–	MS, RI
97	1974	1979	dolabradiene	–	–	–	–	–	–	0.5	–	MS, RI
98	1974	1987	sclarene	–	–	–	0.5	–	–	7.2	13.3	MS, RI, RC

(*continued on next page*)

Table 1 (*continued*)

No.	KI (reported)	KI (Exp.)	Compound	% Area *C. salviifolius* L.				% Area *C. creticus* L.				Method of identification
				SPME		Hydrodistillation		SPME		Hydrodistillation		
				Flowers	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers	Leaves	
99	1978	1991	bifloratriene	–	–	–	0.4	–	–	–	–	MS, RI
100	1988	1996	1-Eicosene	–	–	–	–	–	–	0.3	–	MS, RI
101	2003	2007	manoyl oxide	4.5	0.5	4.1	13.2	0.9	2.7	19.5	27.0	MS, RI, RC
102	2010	2017	epi-13-manoyl oxide	0.4	–	–	–	–	–	–	–	MS, RI
103	2056	2053	abietatriene	0.4	–	4.4	12.3	–	–	12.0	–	MS, RI
104	2057	2060	manool	–	–	–	–	–	0.1	1.1	2.1	MS, RI
105	2077	2082	octadecanol	–	–	–	–	–	–	1.2	4.0	MS, RI
106	2087	2088	abietadiene	–	1.0	–	0.9	–	–	3.3	–	MS, RI
107	2100	2098	n-heneicosane	–	–	–	–	–	–	1.2	–	MS, RI
108	2116	2110	laurenan-2-one	–	–	–	0.7	–	–	–	–	MS, RI
109	2125	2118	methyl octadecanoate	–	–	9.2	–	–	–	–	–	MS, RI
110	2133	2125	nezukol	–	–	0.3	0.9	–	–	–	–	MS, RI
111	2141	2136	osthole				1.1	–	–	0.6	–	MS, RI,
112	2184	2175	incensole acetate	–	–	2.6	–	–	–	0.3	–	MS, RI
113	2184	2180	sandaracopimarinal	–	–	0.5	0.5	–	–	–	–	MS, RI
114	2200	2198	n-docosane	–	–	–		–	–	0.1	–	MS, RI
115	2203	2204	α-santonine	–	–	–		–	–	1.2	–	MS, RI
116	2210	2212	phyllocladanol	–	–	–	0.6	–	–	–	–	MS, RI
117	2237	2235	7-α-hydroxy-manool	–	–	–	0.9	–	–		–	MS, RI
118	2241	2242	Z-isoeugenyl phenylacetate	–	–	–		–	–	1.2	–	MS, RI
119	2275	2270	dehydroAbietal	–	–	0.4	0.4	–	–		–	MS, RI
120	2310	2305	isopimarol	–	–	–	0.6	–	–	0.4	–	MS, RI
121	2313	2310	abietal	–	–	–	0.3	–	–	–	–	MS, RI
122	2314	2317	*trans*-totarol	–	–	–	0.7	–	–	–	–	MS, RI
123	2332	2328	*trans*-ferruginol	–	–	–	1.4	–	–	–	–	MS, RI
124	2360	2360	3-α-acetoxy-manool	–	–	–	1.0	–	–	5.9	5.6	MS, RI
125	2368	2371	dehydro abietol	–	–	59.0	0.9	–	–	–	–	MS, RI
126	2401	2408	abietol	–	–	12.7	0.5	–	–	–	–	MS, RI
127	2422	2420	labd-13E-8,15-diol	–	–	1.4	–	–	–	–	–	MS, RI
		Total		85.1%	92.1%	99.9%	99.8%	98.9%	98.0%	96.7%	100%	
			Monoterpenes Hydrocarbon	13[1] (48.8)[2]	13 (48.9)	0	1 (0.3)	13 (61.2)	13 (53.5)	2 (0.2)	0	
			Oxygenated monoterpenes	4 (1.8)	3 (3.2)	0	5 (15.5)	1 (0.1)	3 (0.6)	2 (3.0)	0	
			Sesquiterpenes Hydrocarbon	17 (16.5)	19 (31.6)	2 (1.4)	7 (5.7)	23 (34.2)	20 (38.6)	12 (11.1)	11 (35.2)	
			Oxygenated Sesquiterpenes	4 (1.9)	4 (1.8)	0	2 (5.6)	6 (0.9)	4 (1.0)	6 (8.1)	3 (8.7)	
			Diterpenes Hydrocarbon	(1) 0.4	1 (1.0)	2 (4.9)	9 (16.6)	1 (0.1)	0	7 (24.7)	2 (16.0)	
			Oxygenated Diterpenes	4 (4.9)	1 (0.5)	7 (81.0)	15 (23.0)	1 (0.9)	2 (2.8)	6 (27.9)	3 (34.7)	
			Esters	2 (1.5)	1 (1.1)	2 (9.8)	7 (31.8)	1 (0.7)	1 (0.5)	2 (1.8)		
			Others	10 (9.3)	8 (4.0)	3 (2.8)	2 (1.3)	2 (0.8)	1 (0.9)	10 (19.9)	2 (5.3)	

[a] KI (Exp.) refers to the Kovats retention index experimentally calculated using C7 – C30 n-alkanes on HP-5MS capillary column.
[b] MS, identification by mass spectrum (NIST and our local generated libraries were used for all MS comparisons).
[c] RC, the identity of the major components was confirmed by injecting authentic reference compounds on the same chromatography column.
[1] Number of compounds.
[2] % area of compounds.

Table 2 Total flavonoids and total phenolic content of crude extract fractions from flowers and leaves of *C. salviifolius* L. and *C. creticus* L.

Crude	*C. salviifolius* L.				*C. creticus* L.			
	Flowers		Leaves		Flowers		Leaves	
	Total flavonoids (mg quercetin/g of extract)	Total phenol mg gallic/g dry extract	Total flavonoids (mg quercetin/g of extract)	Total phenol mg gallic/g dry extract	Total flavonoids (mg quercetin/g of extract)	Total phenol mg gallic/g dry extract	Total flavonoids (mg quercetin/ g of extract)	Total phenol mg gallic/g dry extract
aq. MeOH	393 ± 8	183.8 ± 0.1	313 ± 4	199 ± 2	288 ± 9	221 ± 5	261 ± 8	172 ± 3
BuOH	173 ± 6	149.5 ± 0.3	284 ± 5	187.0 ± 0.2	369 ± 9	283 ± 4	273 ± 8	182 ± 5
Water	125 ± 1	110.9 ± 0.4	223 ± 5	161.0 ± 0.6	217 ± 9	196 ± 2	208 ± 1	112.4 ± 0.6

components of *C. creticus* L., which was analyzed by using GC–MS techniques. The results led to the identification of forty-eight flower and forty-four leave constituents, amounting up to 98.9% and 98.0% of the total composition, respectively. There have been 13 monoterpene hydrocarbons (61.2%) in flower oil, associated with α-pinene (25.9%) and camphene (12.0%) as major constituents. Furthermore, the experimental analysis indicated twenty-three sesquiterpene hydrocarbons (34.2%), associated with α-cubebene (10.4%). Sex oxygenated sesquiterpenes (0.9%), one oxygenated diterpene (0.9%), one diterpene (0.1%), and two aliphatic compound (0.8%) are the main active compounds. Oil extracted from leaves contains 13 monoterpene hydrocarbons (53.5%), associated with α-pinene (19.4%), camphene (9.3%), 20 sesquiterpene hydrocarbons (38.6%), α-cubebene (12.0%) E-caryophyllene (8.4%), in addition to two oxygenated diterpenes (2.8%), four oxygenated sesquiterpenes (1.0%) and three oxygenated monoterpenes (0.6%) as major contents as seen in Table 1.

In comparison to the previously published data on the oil composition of *C. criticus* and *C. salviifolius* (Mastino et al., 2017; Morales-Soto et al., 2015), our results show significant differences in the concentrations of the reported main components. The volatile components of the aerial parts of *C. salviifolius* from Spain were reported camphor (43.86%), E-caryophyllene (19.26%), eucalyptol (19.14%) and (β-bourbonene) (13.27%) as mainly compounds (Morales-Soto et al., 2015). But the oil from the aerial parts of *C. salviifolius* from Sardinia showed a high quantity of manoyl oxide (Mastino et al., 2017). The chemical composition from the aerial parts of *C. creticus* from Torre Beregna showed a high quantity of linolenic (39.2%), hexadecanoic (14.7%) and linoleic (12.9%) (Maggi et al., 2016). Whereas from the essential oils of the leaves of *C. creticus* subsp. from Greece were rich in sesquiterpenes such as δ-cadinene (5.6%), a-cadinene (6.5%), bulnesol (6.3%), and viridiflorol (5.4%) (Demetzos et al., 1997). These changes in the essential oil compositions might arise from several factors such as geological, geographical, seasonal, and climatic (Perry et al., 1999).

3.3. Screening of the total phenolic and flavonoid contents and evaluation of antioxidant activity

Table 2 summarizes the total phenols in mg/g gallic acid for aq. MeOH, butanol and aqueous extracts, which varied among

Table 3 IC_{50} ((mg/mL))values of essential oils and crude extracts from flowers and leaves of *C. salviifolius* L., *C. criticus* L. and standards by using DPPH, ABTS, hydroxyl, *Ferrous Ion Chelating (FIC)* methods.

C. salviifolius L.	Parts	Crude	DPPH	ABTS	Hydroxyl	FIC
	Flowers	Essentail Oil	0.15 ± 0.06	0.09 ± 0.02	0.36 ± 0.02	0.37 ± 0.02
		aq.MeOH	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.04 ± 0.00
		BuOH	$(1.6 \pm 0.06) * 10^{-2}$	$(1.5 \pm 0.06) * 10^{-2}$	0.12 ± 0.04	0.07 ± 0.00
		Water	$(1.5 \pm 0.06) * 10^{-2}$	$(1.9 \pm 0.06) * 10^{-2}$	0.05 ± 0.00	0.04 ± 0.00
	Leaves	Essentail Oil	0.16 ± 0.01	0.13 ± 0.01	0.28 ± 0.05	0.29 ± 0.03
		aq.MeOH	$(1.8 \pm 0.06) * 10^{-2}$	0.01 ± 0.00	0.01 ± 0.00	0.11 ± 0.02
		BuOH	$(2.4 \pm 0.06) * 10^{-2}$	0.01 ± 0.00	0.11 ± 0.00	0.12 ± 0.00
		Water	$(2.5 \pm 0.06) * 10^{-2}$	0.01 ± 0.00	0.09 ± 0.01	0.14 ± 0.00
C. criticus L.	Flowers	Essentail Oil	0.36 ± 0.06	0.31 ± 0.01	0.61 ± 0.10	0.58 ± 0.07
		aq.MeOH	$(1.5 \pm 0.06) * 10^{-2}$	$(1.2 \pm 0.06) * 10^{-2}$	0.14 ± 0.01	0.21 ± 0.01
		BuOH	$(2.3 \pm 0.16) * 10^{-3}$	$(6.0 \pm 0.24) * 10^{-3}$	0.10 ± 0.00	0.10 ± 0.00
		Water	$(6.3 \pm 0.01) * 10^{-3}$	0.01 ± 0.00	0.15 ± 0.00	0.19 ± 0.00
	Leaves	Essentail Oil	0.33 ± 0.01	0.31 ± 0.11	0.57 ± 0.02	0.55 ± 0.07
		aq.MeOH	$(2.4 \pm 0.06) * 10^{-2}$	0.02 ± 0.00	0.06 ± 0.00	0.16 ± 0.01
		BuOH	$(1.7 \pm 0.06) * 10^{-2}$	$(1.4 \pm 0.06) * 10^{-2}$	0.03 ± 0.00	0.11 ± 0.00
		Water	$(1.7 \pm 0.06) * 10^{-2}$	$(1.6 \pm 0.06) * 10^{-2}$	0.04 ± 0.00	0.19 ± 0.00
Ascorbic acid			$(1.8 \pm 0.06) * 10^{-3}$	$(1.9 \pm 0.06) * 10^{-3}$	$(2.6 \pm 0.03) * 10^{-3}$	$(1.9 \pm 0.02) * 10^{-3}$
α-tocopherol			$(2.3 \pm 0.04) * 10^{-3}$	$(1.8 \pm 0.01) * 10^{-3}$	$(2.8 \pm 0.05) * 10^{-3}$	$(2.9 \pm 0.02) * 10^{-3}$
EDTA					$(1.3 \pm 0.02) * 10^{-3}$	$(2.2 \pm 0.01) * 10^{-3}$

the different extracts of plant ranging from 111 to 183.8 mg/g gallic acid in flowers of *C. salviifolius* and from 196 to 283 mg gallic/g dry extract in flowers of *C. creticus*, as well as from 126 to 393 mg gallic/g dry extract in leaves of *C. salviifolius*. The screening of aq. MeOH fraction from flowers and leaves of *C. salviifolius* showed the highest phenolic and flavonoid compound concentrations, compared with aqueous methanol and water extracts. Whereas, the butanol extract from flowers and leaves of *C. creticus*. contained the highest phenolic and flavonoid concentrations concerning the two different extracts (mg quercetin/g of a plant).

Table 3 represents the evaluation results of antioxidant activity of essential oils and crude extracts from flowers and leaves of *C. salviifolius* and *C. creticus* using different methods described in (Al-Qudah, 2013, 2016; Al-Qudah et al., 2014, 2018). Weak scavenging effects are shown in all the different antioxidant assays. Generally, the oils from leaves of *C. salvifolius* and *C. creticus* exhibited a higher scavenging effect than the ones obtained from the flower parts. The antioxidant activities for the different extract fractions from flowers and leaves of *C. salviifolius* and *C. creticus* showed strong scavenging effects in all the different antioxidant assays. The aq. MeOH crude from flowers and leaves of *C. salviifolius* exhibited a higher scavenging effect than the other crudes. Butanol extracted from flowers and leaves of *C. creticus* showed a higher scavenging effect than the other crudes.

Principle component analysis (PCA), is a multivariate data analysis method that can be used for finding differences or similarities among a given dataset. In PCA, the score plot or model contains information about samples (objects), while the PCA loading plot involves variable information (Obeidat et al., 2014).

PCA has been applied to the data obtained from the mass spectrometry (Table 1) to investigate chemical composition differences between the two studied plants, or among the different organs within the plant using different extraction procedures. The first few PCs were studied; the best result was obtained upon having a two-dimensional PCA model that accounts for more than 45% of the total variation in the data set using the first and the fifth PCs. Fig. 1 represents the resulted score PCA model, as it appears in this figure the leaves and flowers from each plant were almost overlapped when

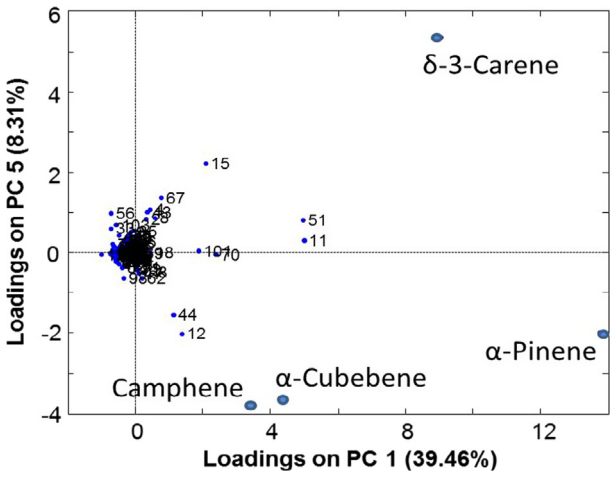

Fig. 2 The PCA score plot for chemical composition of the extracts obtained by hydrodistillation HD and solid phase micro-extraction (SPME), from flowers and leaves of *C. creticus* and *C. salviIfolius*.

extracted with the SPME method. In predicting the PCA score model, the greater the distance between the points in the model the more different they are (Al-Qudah et al., 2018). Therefore, it can be concluded that flowers and leaves of the same plant produced almost similar chemical compounds when extracted with the SPME method. On the other hand, the large distance in the PCA model between the points that represent SPME extracts of *C. salviifolius*. (leaves and flowers) and that of *C. creticus*. (leaves and flowers), reflects the variance in chemical composition between the two plants when extracted via the SPME method. Discriminating among plants based on chemical composition extracted via the hydrodistillation method was not possible using the score PCA model (Fig. 1) indicating that almost similar compounds were extracted from the leaves and flowers in the two studied plants when extracted with the hydro-distillation method.

Further investigations of the PCA loading model (Fig. 2) showed that the responsible compound for distinguishing between *C. saliifolius*. and *C. creticus*. on the first PC was α-Pinene, while the compound δ-3-Carene had the greatest

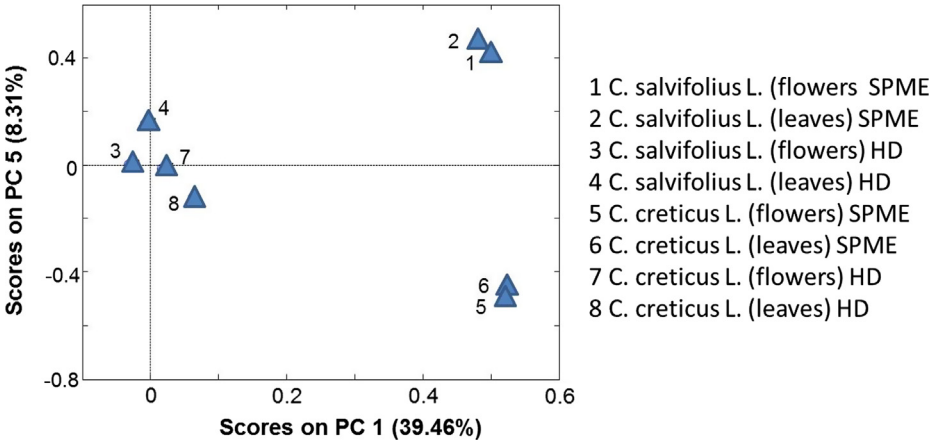

Fig. 1 The PCA score plot for chemical composition of the extracts obtained by hydrodistillation HD and solid phase micro-extraction (SPME), from flowers and leaves of *C. creticus* and *C. salviIfolius*.

impact on the variation in the fifth PC, and for a less extent come to the compounds Camphene and α-Cubebene, respectively.

4. Conclusions

The extraction methods used, such as solid-phase micro-extraction method, showed that monoterpenes and sesquiterpenes are the most volatile constituents. However, hydrodistillation method indicated that diterpenes and oxygenated diterpenes are the principal classes represented in the volatile constituents of the essential oils. There are differences in chemical composition between the two organs, leaves, and flower buds in both plants. These oils showed weak antioxidant activity when compared to the positive controls, α-tocopherol, ascorbic acid, and EDTA, while crude extracts (aq. MeOH, butanol, water) showed good antioxidant activity, which could advocate their use as a source of natural antioxidants. PCA score plot showed that compounds extracted from the leaves and flowers of each plant were different upon extracted with SPME, while almost no significant difference between the two plants (*C. salviifolius* and *C. creticus*) was detected when extracted using hydrodistillation method.

Declaration of Competing Interest

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

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