Characterization of chemical compounds and antioxidant activity of *Centaurea solstitialis* ssp. *schouwii* (DC.) Q. et S. (Asteraceae)

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Abstract: ***Background*:** The antioxidant activity and the total phenolic and flavonoid contents of the derived extracts (chloroform, ethyl acetate and *n*-butanol) of the 70% hydroalcoholic extract of the aerial parts of *Centaurea solstitialis* growing in Algeria was assessed. The active extracts were selected for phytochemical investigations.

***Methods*:** The antioxidant capabilities of the extracts were assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging and cupric ion reducing antioxidant capacity (CUPRAC) assays. Butylhydroxyanisole (BHA), butylhydroxytoluene (BHT) and α –tocopherol were used as positive controls. The total phenolic content and total flavonoid content of the extracts were determined as gallic acid equivalents and quercetin equivalents respectively. Chromatographic methods were used to isolate the secondary metabolites and spectrometric and spectroscopic methods were used to determine their chemical structures.

***Results*:** The ethyl acetate extract exhibited the highest antioxidant activities followed by the *n*-butanol extract. The highest phenolic and flavonoid contents were found in the *n*-butanol extract. Phytochemical study of the ethyl acetate and n-butanol extracts led to the isolation of an undescribed guaianolide named 3-(4-hydroxybenzoyl)-cynaratriol and a known sesquiterpene lactone along with three known flavonoid glycosides. Their structures were established by spectral analyses mainly high resolution electrospray ionisation mass spectrometry (HR-ESIMS) and 1D and 2D nuclear magnetic resonance experiments.

***Conclusion*:** The extracts of aerial part of *C. solstitialis* showed significant antioxidant activities. An undescribed sesquiterpene lactone and four known secondary metabolites were isolated from the most active extracts.

Keywords: *Centaurea solstitialis*, Sesquiterpene lactones, Flavonoids, Antioxidant activity, Total phenolic and flavonoid contents.

1. INTRODUCTION

Numerous *Centaurea* species (Asteraceae) are used in folk medicine and reported various biological properties [1-7]. Moreover, several *Centaurea* plants has been recognized as excellent accumulators of bioactives molecules [8, 9]. *Centaurea solstitialis* L. and *C. solstitialis* L. ssp. *solstitialis*
have been reported to contain triterpenoids [10], flavonoids [11], sesquiterpene lactones [12-14], with sufficient biological effects [15-18].
The essential oil of the aerial parts of *C. solstitialis* ssp.

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solstitialis collected from Kovancilar province, in Turkey
[19] exhibited β-eudesmol (15.5%), bicyclogermacrene (14.2%), spathulenol (11.3%), germacrene D (6.3%), caryophyllene oxide (5.2%), hexadecanal (4.2%) and hexadecanoic acid (4.1%) as the most abundant components. Sesquiterpene lactones

have been reported from *C. solstitialis* L. ssp. schouwii [20]. From the aerial parts of *C. solstitialis* L. ssp. schouwii Dostál collected at Piano Battaglia (Palermo) Italy, lignans and sesquiterpene lactones have been described [21], while the analysis of the chemical composition of the essential oil of its flower heads showed that fatty acids and their esters (43.6%) and hydrocarbons (28.0%) were the major constituents [22]. Moreover *C. solstitialis* L. ssp. *schouwii* (DC.) Gugler is used in Sicily (Italy) for food purposes and recognized as having medicinal properties [23]. As part of our ongoing phytochemical and biological program studies on Asteraceae family [24, 25] and notably *Centaurea* species growing in Algeria [26-30], we report in this paper a detailed study on the antioxidant activity of the derived extracts (chloroform, ethyl acetate and *n*-butanol) of the 70% ethanol extract of its aerial parts, the total phenolic content (TPC) and total flavonoid content (TFC) of the three extracts, as well as the fractionation, separation and purification of secondary metabolites of the ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) extracts of *C. solstitialis* ssp. *schouwii* (DC.) Q. et S. (synonym: *C. shouwii* (DC.).

2. MATERIALS AND METHODS

2.1. Experimental

NMR spectra were obtained by Bruker AMX-500 spectrometer with standard pulse sequences, operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. MeOH-d_4, was used as solvent and TMS as internal standard. HR-ESIMS was performed with a LCT Premier XE Micromass Waters spectrometer in positive ionization mode (Waters Corporation). Column chromatography (CC) was carried out with silica gel Fluka (cat. 60737) (40-63 μm), and column fractions were monitored by thin layer chromatography (TLC) silica gel 60 F_{254}, 0.2 mm, Macherey Nagel (cat. 818-333) by detection with a spraying reagent ($CH_3COOH/H_2O/H_2SO_4$; 80:16:4) followed by heating at 100 °C. Preparative TLC was carried out on silica gel 60 $PF_{254 + 366}$ (20 × 20 cm, 1 mm thickness, Analtech cat. 02014).

2.2. Plant material

Centaurea solstitialis ssp. *schouwii* (DC.) Q. et S. was collected from El Kala in the eastern Algeria (GPS-coordinates: 36° 53' 25.644" N 8° 26' 42.275" E) in June 2006 and authenticated by Dr. D. Sarri (University of M'Sila) on the basis of Quezel and Santa (1963) [31]. A sample has been deposited in the Herbarium of the Research Unit: Valorisation des Ressources Naturelles, Molécules Bioactives et Analyses Physicochimiques et Biologiques (VARENBIOMOL), Université Frères Mentouri Constantine 1.

2.3. Extraction and isolation

Air-dried and powdered aerial parts from *C. solstitialis* ssp. *schouwii* (DC.) Q. et S. (1200 g) were extracted with EtOH-H_2O (70:30 v/v) for 24 hours, three times. The crude extract was concentrated under reduced pressure up to 35°C (500 mL), diluted with 480 mL distilled H_2O under magnetic stirring and then, kept at 4°C for one night to precipitate a maximum of chlorophylls. After filtration the remaining aqueous solution was extracted successively with petroleum ether, chloroform ($CHCl_3$), EtOAc and *n*-BuOH. The organic layers were dried with Na_2SO_4 giving after removal of solvents under reduced pressure (up to 35°C) petroleum ether (0.51 g), $CHCl_3$ (8 g), EtOAc (10.5 g) and *n*-BuOH (17 g) extracts. EtOAc extract (10 g) was fractionated by column chromatography (CC) on silica gel using chloroform with increasing percentages of methanol to yield 33 fractions (1-33) obtained by combining the eluates on the basis of TLC analysis. Fraction 10 (96:4) gave after evaporation, the pure compound (**1)** as white crystals (105 mg) identified as 4β,15- dihydro-3-dehydrosolstitialin A. Fraction 15 (54 mg) (92:8) gave after purification on preparative plates of silica gel ($CHCl_3$/MeOH, 9:1) the new sesquiterpene lactone (**2**) (32 mg). Fraction 19 (99 mg, 88:12) was subjected to successive purifications on TLC preparative to give kaempferol 3-*O*-rhamnoside (**3**) (45 mg). A part of the *n*-BuOH extract (12 g) was subjected to CC on silica gel eluted with $CHCl_3$/MeOH step gradients and then with increasing percentages of H_2O) to yield 55 fractions (1-55) obtained by combining the eluates on the basis of TLC behavior. Fraction 18 (170 mg, 90:10) was purified on TLC plates (EtOAc/MeOH/H_2O, 8:1:1) to give astragalin (**4**) (17.7 mg), and 6-methoxy kaempferol-3-*O*-glucopyranoside (**5**) (12.8 mg).

2.4. Determination of total bioactive compounds

Determination of total phenolic content (TPC)

The total phenolic contents of the three extracts from *C. solstitialis* ssp. *schouwii* (DC.) Q. et S., were determined by Folin-Ciocalteu reagent method [32]. The absorbance of the sample was read at the wavelength 765 nm. Standard solutions of gallic acid with concentrations 25 to 200 μg/mL were used to obtain the standard curve. The results were determined from the standard curve and expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/g dry extract). Analyses were done in triplicate for each extract.

Determination of total flavonoid content (TFC)

The total flavonoid contents of the three extracts were determined spectrophotometrically as previously reported [33]. The absorbance was read at the wavelength 415 nm. Standard solutions of quercetin with concentration 25 to 200 µg/mL were used to obtain the standard curve, and the results were expressed as mg quercetin equivalents per gram of dry extract (mg QE/g extract). All the experiments were carried out in triplates.

2.5. Determination of antioxidant activity

DPPH scavenging assay

The DPPH scavenging activity was determined spectrophotometrically in accordance with the method described by Blois [34]. Butylhydroxyanisole (BHA), butylhydroxytoluene (BHT) and α-tocopherol were used as antioxidant standards for comparison of the activity. The scavenging activity of DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = $[(A_{control}-A_{sample})/A_{control}]$ x100

Where $A_{Control}$ is the absorbance of the initial concentration of the DPPH and A_{Sample} is the absorbance of the remaining concentration of DPPH in the presence of the extract or positive controls. The results were given as IC_{50} value (µg/mL) corresponding to the concentration of 50% inhibition.

Cupric reducing antioxidant capacity (CUPRAC)

The cupric reducing antioxidant capacity was determined according to the method described by Apak [35]. The reducing capacity of the extracts was compared with those of BHA and BHT. The results were given as $A_{0.5}$ (µg/mL) corresponding to the concentration indicating 0.50 absorbance intensity.

3. RESULTS AND DISCUSSION

3.1. Structure elucidation of compounds 1-5

After separation and purification by chromatographic procedures, five compounds: a new guaianolide and four known secondary metabolites were isolated from the ethyl acetate and *n*-butanol soluble parts of the hydroalcoholic extract of the aerial parts of *Centaurea solstitialis* ssp. *schouwii* (DC.) Q. et S. The structures of all the compounds were established by spectral analyses, mainly UV–Vis, ESI-HRMS, ^{1}H-NMR, ^{13}C-NMR, and 2D-NMR (COSY, NOESY, HSQC and HMBC) (Fig. **1**).

Compound **2** gave a molecular formula $C_{22}H_{26}O_7$ deduced from its TOF-HR-ESIMS *m/z* 421.1483 $[M+H_3O]^+$ indicating ten degrees of unsaturation in it. Its NMR spectra were very similar to those of cynaratriol, a sesquiterpene lactone with a guaianolide skeleton type isolated in particular from *Cynara cardunculus* L. [36] and from *Centaurea musimomum (musimonum)* [37, 38]. The difference found in the ^{1}H NMR spectrum of this compound compared to that of cynaratriol was the appearance of two additional resonances corresponding to two doublets of 2H each at δ_H 7.72 and δ_H 6.63 (J = 8.7 Hz). These signals were characteristic of a *para*-substituted aromatic ring. The ^{13}C NMR and DEPT experiment spectra when compared to those of cynaratriol, confirmed this hypothesis by exhibiting additional resonances consisting in two signals of aromatic CH (δ_C 132.33 and 115.26 ppm and two quaternary carbon atoms at δ_C 128.90 and δ_C 160.91 ppm (Table **1**) **.** The values of the chemical shifts of these two last carbons corresponded to a nonoxygenated and an oxygenated aromatic quaternary carbons. These assumptions were consolidated by the observed correlations between the protons of the aromatic CH groups in the COSY spectrum and between the quaternary carbon at δ_C 128.90 and the protons of the doublet at δ_H 6.63 and between the quaternary carbon at δ_C 160.91 and the protons of the doublet at δ_H 7.72 in the HMBC experiment spectrum. These observations permitted the assignment of these quaternary carbons to C-1' and C-4', respectively and the assignment of the two 2H doublets to H-3', H-5' and H-2', H-6', respectively of this aromatic cycle (Table 1)**.** In fact, the combination of the molecular formula ($C_{22}H_{26}O_7$) and the NMR spectral data revealed the presence in this molecule of a *para*-hydroxybenzoate group instead a hydroxyl group in cynaratriol. The carbon of the carbonyl of this group (C-7') was deduced at δ_C 171.54 from the correlation with H-2' and H-6' found in the HMBC spectrum. The analyses of all the NMR spectra led in particular, to the assignment of H_3-15, H-2α, H-2β, H-4β, H-3, H-14a and H-14b of the guaianolide skeleton, to the signals at δ_H 1.10 (d, J = 6.3 Hz, δ_C 18.72), δ_H 1.97 (m, δ_C 39.33), δ_H 1.60 (m, δ_C 39.33), δ_H 1.65 (m, δ_C 47.76), δ_H 3.53 (m, δ_C 78.92), δ_H 4.83 (s, δ_C 112.53) and δ_H 4.80 (s, δ_C 112.53) respectively. In the NOESY experiment spectrum, pertinent correlations were observed between protons of the guaianolide skeleton and the protons of the aromatic ring of the *p*- hydroxybenzoyl group notably: H_3-15/ H-2', H-6'; H-2β/H-3', H-5', H-2', H-6'; H-4β/H-3', H-5'; H-3/H-3', H-5' and H-14a, H-14b/H-2', H-6', H-3', H-5'. From the presence of these correlations, we conclude that the *p*-hydroxybenzoate group is connected in C-3 of the guaianolide skeleton and has a β-orientation. Thus compound **2** was identified as a new derivative of cynaratriol named 3-(4-hydroxybenzoyl)-cynaratriol. This result agreed with literature data where guaianolides bearing 4-hydroxybenzoate group have been reported from *Acroptilon repens* [39] and *Centaurea glastifolia* [40] of Asteraceae family. The known compounds were characterized by comparison of their spectral data with those reported in literature.

Table 1. NMR data (MeOH-d_4), ^{1}H (500 MHz, J/Hz), ^{13}C (125 MHz) of compound 2

Position	δ_C(ppm)	δ_H(ppm)
1	43.04	H-1α 2.70 *m*
2	39.33	H-2α 1.97 *m* H-2β 1.60 *m*
3	78.92	H-3α 3.53 *m**
4	47.76	H-4β 1.65 *m*
5	53.12	H-5α 1.78 *m*
6	86.15	H-6β 4.08 (*t*, 10.2)
7	56.13	H-7α 2.10 (*ddd*, 12.5, 9.8, 3.6))
8	28.13	H-8α 1.94 *m* H-8β 1.57 *m*
9	38.01	H-9β 2.55 (*dt*, 12.6, 3.9) H-9α 1.75 *m*
10	151.37	/
11	78.64	/
12	180.93	/
13	64.43	3.55 *brs*
14	112.53	H-14a 4.83 *brs* H-14b 4.80 *brs*
15	18.72	1.10 (*d*, 6.3)
2',6'	132.33	7.72 (*dd*, 8.7)
3',5'	115.26	6.63 (*dd*, 8.7)
1'	128.90	/
4'	160.91	/
7'	171.54**	/

* : Partialy obscured by the signal of H_2-13. **: Not detected, deduced from HMBC spectrum.

4β,15-dihydro-3-dehydrosolstitialin A (**1**): TOF-HRESIMS (+): *m/z* 303.1203 $[M+Na]^+$ (calculated for $C_{15}H_{20}O_5Na$: 303.1208, formula $C_{15}H_{20}O_5$. ^{1}H-NMR (400 MHz, CD_3OD) δ_H (ppm): 1.20 (3H, d, *J* = 6.7 Hz, H3-15), 1.81 (1H, qd, *J* = 12.5, 5.0 Hz, H-8β), 2.13 (1H, m, H-9α), 2.17 (1H, m, H-8α), 2.25 (1H, brt, *J* = 8.5 Hz, H-5α), 2.33 (1H, m, H-4β), 2.34 (2H, brs,11-OH and 13-OH), 2.45 (1H, dd, *J* = 19.0, 3.6 Hz, H-2β), 2.54 (1H, m, H-7α), 2.61 (1H, dd, *J* = 19.0, 7.8 Hz, H-2α), 2.67 (1H, m, H-9β), 3.18 (1H, ddd, *J* = 8.5, 7.8, 3.6 Hz, H-1α), 3.69 (1H, d, *J* = 10.5 Hz, H-13b), 3.72 (1H, d, *J* = 10.5Hz, H-13a), 4.23 (1H, dd, *J* = 9.9, 8.5 Hz, H-6β), 4.65 (1H, s, H-14b), 5.00 (1H, s, H-14a). ^{13}C-NMR (100 MHz, CD_3OD) δ_C (ppm): 14.48 (C-15), 28.00 (C-8), 39.95 (C-9), 41.00 (C-1), 44.92 (C-2), 48.49 (C-4), 51.23 (C-7), 52.84 (C-5), 64.59 (C-13), 88.69 (C-6), 78,64 (C-11), 112.43 (C-14), 151.54 (C-10), 180.77 (C-12), 222.05 (C-3). All these data were in good agreement with those reported for the same molecule isolated from *Centaurea musimomum* [41].

Kaempferol 3-*O*-rhamnoside (**3**): $C_{21}H_{20}O_{10}$, UV (MeOH, λ_{max}, nm): 266, 350; +NaOH: 275, 324, 401 (with hyperchromic effect); +$AlCl_3$: 272, 305, 352, 398; +$AlCl_3$+HCl:274, 302, 344, 397; +NaOAc: 273, 306, 374; +NaOAc+H_3BO_3: 267, 356. ^{1}H-NMR (300 MHz, CD_3OD) δ_H (ppm) aglycone: 7.79 (2H, d, *J* = 7.9 Hz, H-2' and H-6'), 6.96 (2H, d, *J* = 7.9 Hz, H-3'and H-5'), 6.39 (1H, d, *J* = 2.0 Hz, H-8), 6.22 (1H, d, *J* = 2.0 Hz, H-6), sugar moiety: 5.39 (1H, d, *J* = 1.2 Hz, H-1"), 0.94 (3H, d, *J* = 6.5 Hz, H_3-6''), the complete identity of the sugar was established by acidic hydrolysis and co-chromatography with authentic samples of various sugar molecules. All the obtained spectroscopic data were in accordance with those reported in the literature for the same compound isolated from the leaves of *Schima wallichii* Korth. [42].

Astragalin (**4**): $C_{21}H_{20}O_{11}$, ^{1}H-NMR (500 MHz, CD_3OD) δ_H(ppm) aglycone: 8.07 (2H, d, *J* = 8.8 Hz, H-2'and H-6'), 6.90 (2H, d, *J* = 8.8 Hz, H-3'and H-5'), 6.37 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6), *O*-β-glucopyranoside moiety: 5.18

(1H, d, *J* = 7.3 Hz, H-1”), 4.60 (1H, dd, *J* = 12.0, 2.3 Hz, H-6”a), 4.46 (1H, dd, *J* = 12.0, 5.4 Hz, H-6”b), 3.35 (m,1H, H-2”), 3.34 (m, 1H, H-3”), 3.24 (H-4”, obscured by the signal of the solvent, deduced from COSY and HSQC spectra), 3.12 (1H, m, H-5”).^{13}C-NMR (125 MHz, CD_3OD) δ_C (ppm) aglycone: 178.08 (C-4), 164.68 (C-7), 161.53 (C-5), 160.29 (C-4’), 157.70 (C-2), 157.09 (C-9), 134.20 (C-3), 131.19 (C-2’ and C-6’), 121.36 (C-1’), 114.98 (C-3’ and C-5’), 104.39 (C-10), 98.78 (C-6), 93.66 (C-8), *O*-β-glucopyranoside moiety: 102.86 (C-1”), 77.11 (C-5”), 76.54 (C-3”), 74.36 (C-2”), 69.98 (C-4”), 61.25 (C-6”). These data were in perfect agreement with those reported for astragalin isolated from *Helianthemum ruficomum* [43].

6-Methoxy kaempferol 3-*O*-β-glucopyranoside (**5)**: $C_{22}H_{22}O_{12}$, ^{1}H-NMR (400 MHz, CD_3OD) δ_H (ppm) aglycone: 8.04 (2H, d, *J* = 8.8 Hz, H-2’ and H-6’), 6.88 (2H, d, *J* = 8.8
Hz, H-3’ and H-5’), 6.48 (1H, s, H-8), 3.87 (3H, s, OCH_3), *O*-β- glucopyranoside moiety: 5.23 (1H, d, *J* = 7.4 Hz, H-1”), 3.68 (1H, dd, *J* = 11.9, 2.2 Hz, H-6”a), 3.53 (1H, dd, *J* = 11.9, 5.4 Hz, H-6”b), 3.45 (1H, m, H-2”), 3.43 (1H, m, H-3”), 3.30 (H-4”, obscured by the signal of the solvent, deduced from the HMQC and HMBC spectra), 3.20 (1H, m, H-5”).^{13}C-NMR (100 MHz, CD_3OD) δ_C (ppm) aglycone: 179.72 (C-4), 161.64 (C-4’), 160.07 (C-7), 159.13 (C-2), 154.09 (C-5), 153.69 (C-9), 135.17 (C-3), 133.08 (C-6), 132.32 (C-2’and C-6’), 122.91 (C-1’), 116.13 (C-3’and C-5’), 105.88 (C-10), 95.34 (C-8), 60.94 (OCH_3), *O*-β-glucopyranoside moiety: 104.21 (C-1”), 78.48 (C-5”), 78.10 (C-3”), 75.78 (C-2”), 71.41 (C-4”), 62.63 (C-6”). All these data were in good agreement with those reported by Wei *et al.* (2004) [44] for the same compound isolated from *Mikania micrantha.*

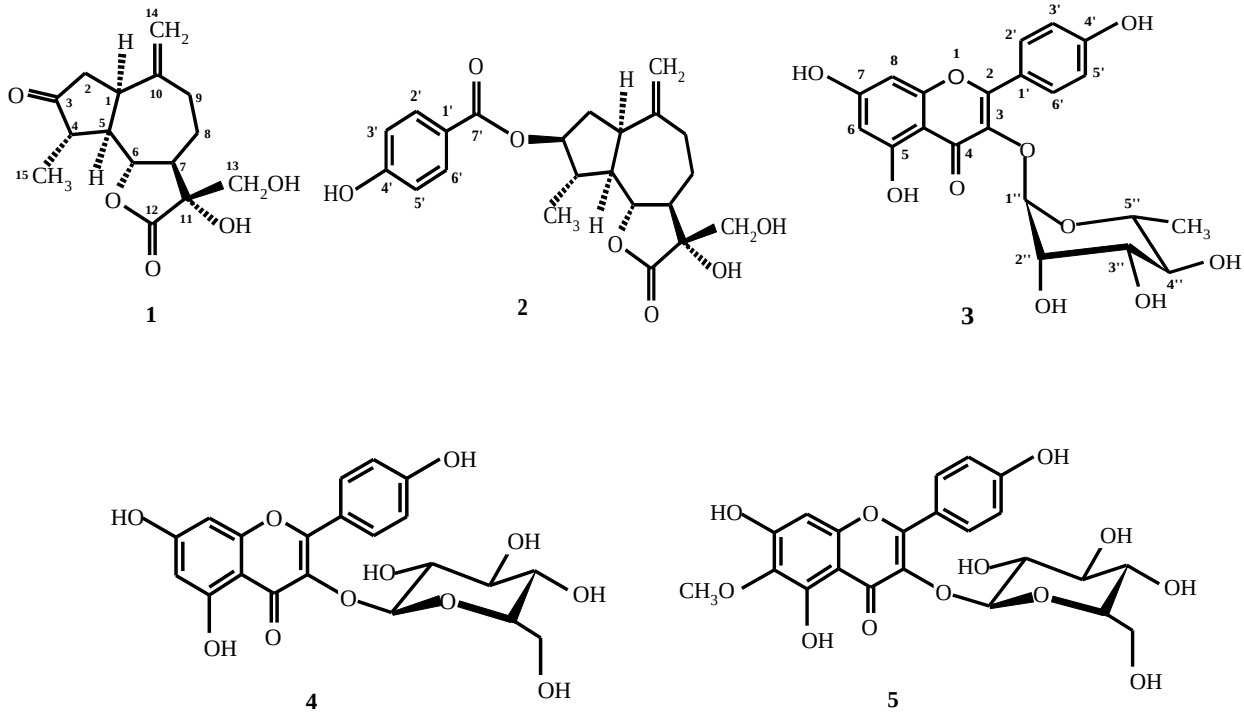


Fig. (1). Structures of compounds 1-5 isolated from *C. solstitialis* ssp. *schouwii* (DC.) Q. et S.

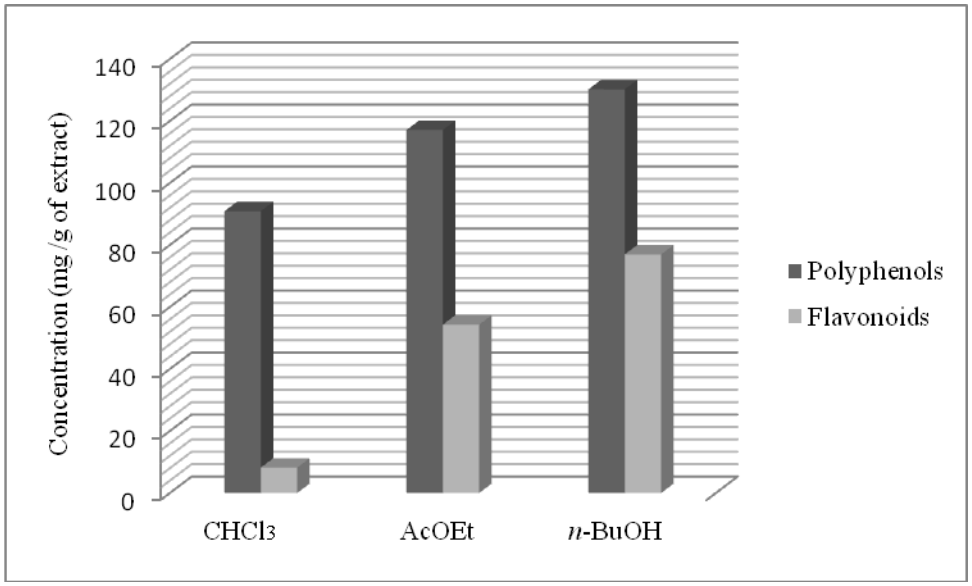
3.2. Determination of total bioactive compounds

The TPC in various extracts were reported in term of gallic acid equivalents using the standard curve equation y = 0.002x + 0.045, R^2 = 0.997. TPC in various extracts of *C. solstitialis* ssp. *schouwii* (DC.) Q. et S. showed different results ranged from 90.90 ± 4.37 to 130.20 ± 4.71 mg GAE/g of extract. The *n*-BuOH extract had the highest phenolic content (130.20 ± 4.71 mg GAE/g) (Table **2**, Fig. **2**). TFC in various extracts were reported in term of quercetin equivalents using the standard curve equation y = 0.006x + 0.017, R^2 = 0.976. TFC in different extracts showed different results ranged from 8.2 ± 0.57 to 76.97 ± 6.19 mgQE/g of extract (Table **2**, Fig. **2**). The *n*-BuOH extract had the highest TFC (76.97 ± 6.19 mgQE/g of extract); the lowest (8.2 ± 0.57 mgQE/g of extract) was given by the chloroform extract.

Table 2. Total phenolic and flavonoid contents of the three extracts of *C. solstitialis* ssp. *schouwii* (DC.) Q. et S.

Extracts	Total phenolic content (mgGAE/g extract)	Total flavonoid content (mgQE/g extract)
Chloroform extract	90.90 ± 4.37	8.2 ± 0.57
Ethyl acetate extract	117.08 ± 8.54	54.31 ± 2.87
n-Butanol extract	130.20 ± 4.71	76.97 ± 6.19

Fig. (2). Total phenolic and total flavonoid contents in the three extracts of *C. solstitialis* ssp. *schouwii* (DC.) Q. et S.



Antioxidant activity

There was no previous study regarding antioxidant activity of the three different polarity extracts (chloroform, ethyl acetate and *n*-butanol) from *C. solstitialis* ssp. *schouwii* (DC.) Q. et S. Two methods were selected to determine the antioxidant capacity of the extracts, DPPH free radical scavenging activity and the CUPRAC. The free radical scavenging activity of the extracts is illustrated in Fig. (**3)** and reported in Table **3**. The values of three standard compounds BHA (IC_{50} value: 6.14±0.41 µg/mL), BHT (IC_{50} value: 12.99±0.41 µg/mL) and α –tocopherol (IC_{50} value: 13.02±5.17 µg/mL), were obtained and compared to those of the studied extracts. The examination of antioxidant activities of the extracts from *C. solstitialis* ssp. *schouwii* (DC.) Q. et S.. showed different values, the ethyl acetate extract exhibited the highest activity (IC_{50} value: 44.01 ± 1.06 µg/mL). A moderate activity was found for *n*- BuOH extract (65.46 ± 3.07 µg/mL). In comparison to IC_{50} values of BHT and α –Tocopherol, EtOAc extract exhibited the strongest capacity for neutralization of DPPH radicals (Table **3**).

Table 3. Antioxidant activity by the DPPH assay[a].

Extacts / Concentrations	% Inhibition in DPPH assay							
	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL	800 µg/mL	IC_{50} µg/mL
$CHCl_3$ extract	NA	NA	NA	5.08±1.71	16.63±2.93	27.80±0.59	46.29±0.74	< 800
EtOAc extract	7.22±0.22	11.07±0.22	37.99±4.04	86.39±0.56	88.10±0.53	88.27±0.71	88.19±0.00	44.01±1.06
n-BuOH extract	3.32±0.77	14.36±3.24	34.61±3.92	81.60±1.97	88.06±0.64	87.50±3.08	88.70±0.00	65.46±3.07
BHA[b]	76.55±0.48	79.89±0.26	81.73±0.10	84.18±0.10	87.13±0.17	89.36±0.19	90.14±0.00	6.14±0.41
BHT[b]	49.09±0.76	72.63±2.06	88.73±0.89	94.00±0.31	94.97±0.08	95.38±0.41	95.02±0.23	12.99±0.41
α-Tocopherol[b]	37.21±1.82	81.53±1.51	89.23±0.12	89.38±0.19	89.45±0.22	89.99±0.23	89.52±0.33	13.02±5.17

[a]Values expressed as means ± S.D. of three parallel measurements. (p<0.05).
[b]Reference compounds. NA: no absorbance

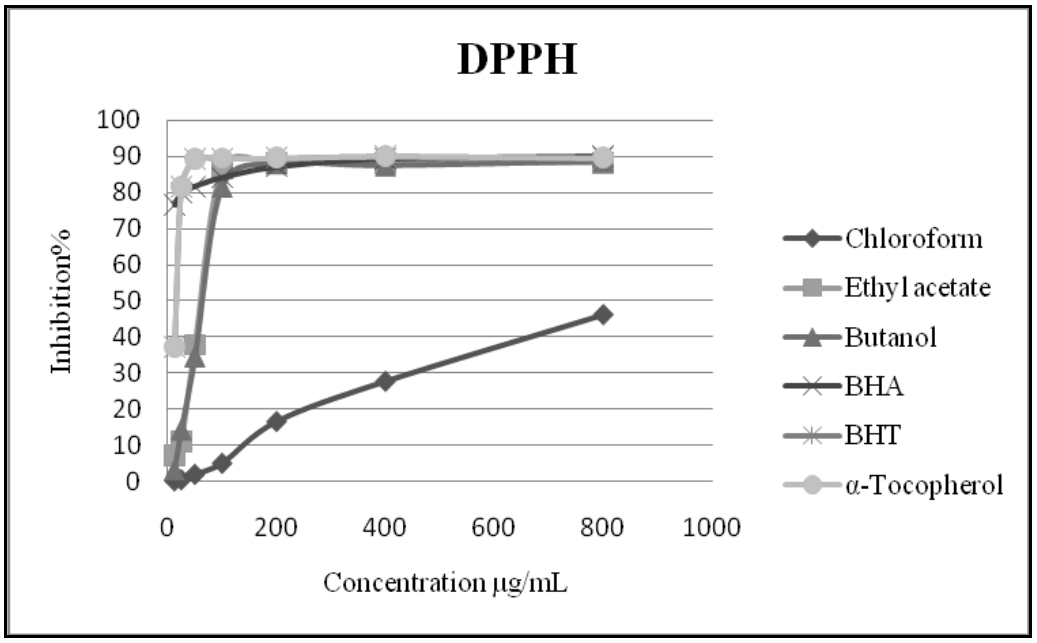


Fig. (3). DPPH radical scavenging activity of the three extracts, BHA, BHT and α-Tocopherol at different concentrations (mean±SD, n=3).

The results of CUPRAC of the extracts are compared with those of BHA and BHT (Fig. **4** and Table **4**), the activity (absorbance) increased linearly with the increasing amount of extracts. The EtOAc extract exhibited the highest activity ($A_{0.50}$ value: 30.93±2.50 µg/mL) among the extracts, followed by *n*-butanol extract ($A_{0.50}$ value: 44.80±3.74 µg/mL) and chloroform

extract ($A_{0.50}$ value: 515.33±39.56 µg/mL). However, none of the extracts exhibited higher activity than those of the antioxidant standards.

Table 4. Antioxidant activity by the CUPRAC assay[a].

Extracts / Concentrations	% Inhibition in CUPRAC assay							
	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL	800 µg/mL	$A_{0.50}$ µg/mL
$CHCl_3$ extract	0.09±0.01	0.09±0.01	0.12±0.01	0.17±0.02	0.25±0.01	0.43±0.04	0.74±0.04	515.33±39.56
EtOAc extract	0.27±0.04	0.39±0.09	0.84±0.12	1.24±0.28	2.09±0.44	2.73±0.72	3.64±0.39	30.93±2.50
n-BuOH extract	0.22±0.02	0.29±0.01	0.56±0.04	0.98±0.20	1.58±0.12	2.45±0.33	3.49±0.32	44.80±3.74
BHA[b]	1.12±0.05	1.95±0.31	3.14±0.46	3.58±0.42	3.35±0.20	3.77±0.19	3.92±0.13	5.35±0.71
BHT[b]	1.41±0.03	2.22±0.05	2.42±0.02	2.50±0.01	2.56±0.05	2.86±0.07	3.38±0.13	8.97±3.94

[a]Values expressed as means ± S.D. of three parallel measurements. (p<0.05).
[b]Reference compounds. .

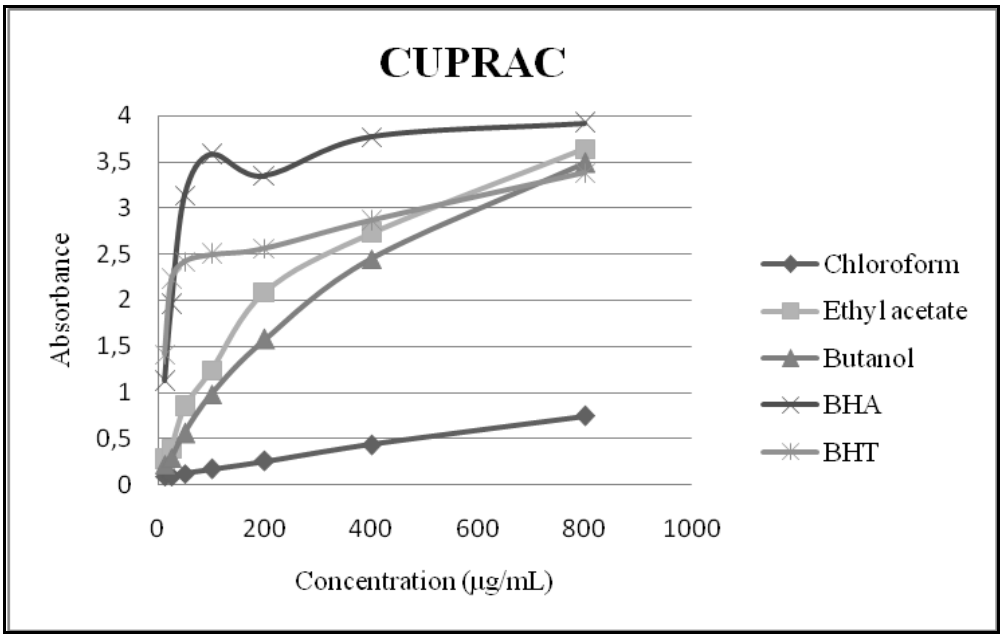


Fig. (4). CUPRAC activity of the three extracts, BHA and BHT at different concentrations (mean±SD, n=3).

CONCLUSION

The Algerian *Centaurea solstitialis* ssp. *schouwii* (DC.) Q. et S. was studied phytochemically for the first time. The antioxidant activities of the $CHCl_3$, EtOAc and *n*-BuOH soluble parts of the aqueous-EtOH (70%) extract of the aerial parts were evaluated using two methods, DPPH radical scavenging and CUPRAC. The total phenolic and total flavonoid contents of these extracts were also determined. In comparison to IC_{50} values of BHA (6.14 ± 0.41 µg/mL), BHT(12.99± 0.41 µg/mL) and α –Tocopherol (13.02 ± 5.17 µg/mL), the ethyl acetate extract exhibited the highest activity (IC_{50} value: 44.01± 1.06 µg/mL) among the studied extracts, followed by *n*- butanol extract (IC_{50} value: 65.46 ±3.07 µg/mL), in the DPPH assay. The results of CUPRAC assay of the three extracts compared with those of BHA ($A_{0.50}$ value: 5.35 ± 0.71) and BHT ($A_{0.50}$ value: 8.97 ± 0.71) showed that among the three extracts, the EtOAc extract exhibited the highest activity ($A_{0.50}$ value: 30.93 ± 2.50 µg/mL) followed by *n*-butanol extract ($A_{0.50}$ value: 44.80 ± 3.74 µg/mL). In addition, the *n*-butanol extract had the highest phenolic and flavonoid contents (130.20 ± 4.71 mg GAE/g extract and 76.97 ± 6.19 mg QE/g extract, respectively, followed by the ethyl acetate extract, 117.08 ± 8.54 mg GAE/g extract and 54.31 ± 2.87 mg QE/g extract, respectively. Phytochemical investigation of ethyl acetate and *n*-butanol extracts led to the isolation of an undescribed guaianolide named 3-(4-hydroxybenzoyl)-

cynaratriol (**2**) together with four known compounds: 4β,15-dihydro-3-dehydrosolstitialin A (**1**), kaempferol 3-*O*- rhamnoside (**3**), astragalin (**4**) and 6-methoxy kaempferol 3-*O*-β-glucopyranoside (6-methoxy astragalin) (**5**).

CONFLICT OF INTEREST

There are no conflicts of interest.

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