



Phytochemical analysis and antioxidative properties of *Centaurea albonitens*

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Abstract

Background and objectives: The genus *Centaurea* from the Asteraceae family is one of the most widely distributed plant genera worldwide that has been extensively used in folk medicine for hundreds of years. The present study is the first investigation about the principal constituents of *Centaurea albonitens* Turill which is native to Iran. **Methods:** The aerial parts of *C. albonitens* were extracted via maceration. Phytochemical analysis of the methanol extract was carried out via different chromatography approaches like HPLC, SPE and preparative TLC. Structures of the purified compounds were revealed through spectral analysis from 1D and 2D NMR including DQF-COSY, HSQC and DEPT in comparison with the relative data in published reports. Subsequently, the antioxidant property of the extract was evaluated via scavenging the free DPPH radicals. In addition, the total phenolic and flavonoid contents of the extract were ascertained based on Folin-Ciocalteu and colorimetric aluminum chloride methods, correspondingly. **Results:** Analysis of the extract yielded in the isolation and identification of arctiin and apigenin-4'-*O*-rhamnoside. Moreover, the antioxidant assessment determined IC₅₀ value of 389.9 µg/mL for the plant extract in DPPH assay. The total phenolics and flavonoids content of the plant extract were 2.87 g gallic acid equivalent and 0.28 g quercetin equivalent both in 100 g dried plant material. **Conclusion:** The findings of this study introduce *C. albonitens* as a suitable source for isolation of lignans (like arctiin).

Keywords: apigenin-4'-*O*-rhamnoside, arctiin, Asteraceae, *Centaurea albonitens*, lignans

Introduction

Plants of Asteraceae family have been valuable natural remedy resources for most nations. The genus *Centaurea* (Asteraceae) is one of the most widely distributed plant genera worldwide. The number of taxa included in this genus ranges from 500 to 600 globally [1]. Turkey is the most abundant habitat for *Centaurea* species [2] while Iran has been accepted as one of the major centers of diversity for the genus *Centaurea*, as

well. *Flora Iranica* [3] has reported 28 growth situ for different species of *Centaurea*. It is of note to mention that 74 species from *Centaurea* such as *C. bachtiarica*, *C. lachnopus*, *C. aziziana*, *C. ustulata*, *C. pterocaula*, *C. imperialis*, *C. pabotii*, *C. hyrcanica* are native to Iran [4]. Different species of genus *Centaurea* have been extensively used in folk medicine for hundreds of years [5] as wound healing, antidiabetic, anti-

diarrhea, antirheumatic, anti-inflammatory [6], choleric, cholagogue, digestive, stomachic, diuretic, astringent, hypotensive, antipyretic, cytotoxic [7] and antibacterial agents [8].

Phytochemical studies on various species of *Centaurea* have reported isolation of different classes of compounds such as flavonoids, sesquiterpene lactones, triterpenes and alkaloids, along with other secondary metabolites [9]. *Centaurea albonitens* Turill is one of the seventy four *Centaurea* species represented in flora of Iran. This species grows as a perennial plant in semi-arid habitats at the north-west of Iran, East Azerbaijan province. Regarding the valuable reports on chemical diversity of compounds present in different species of the genus *Centaurea* along with their valuable biological properties, we decided to study *C. albonitens* phytochemicals. It is of note to mention that the present study is the first phytochemical investigation on *C. albonitens*.

Experimental

Plant material

Aerial parts of *C. albonitens* were collected during the flowering stage from wild population growing in Mishudagh, East Azarbaijan, Iran, in June 2015. A voucher specimen (No. 6583) of the plant has been deposited at the herbarium of the East Azerbaijan research center for agriculture and natural resources Research and Education Center, Tabriz, Iran.

Extraction, fractionation and isolation

The air-dried ground aerial parts of *C. albonitens* (450 g) were defatted with petroleum ether and subsequently extracted with chloroform (3×4 L) and methanol (3×4 L) respectively, for 24 h via maceration at room temperature. The crude extracts were filtered, concentrated and dried at 45 °C using a rotary evaporator (Heidolph, Germany). The greenish methanol (MeOH) extract weighing 6.07% (w/w) was selected for further evaluation which was kept in an air tight bottle in a refrigerator until use. Two g of the dried MeOH extract was fractionated by solid-

phase-extraction (SPE) on Sep-Pak C18 cartridge (10 g, Waters, Ireland) using a step-wise gradient of MeOH-H₂O mixture (10:90, 20:80, 40:60, 60:40, 80:20 and 100:0), 200 mL each affording 6 fractions. All fractions were dried under *vacuo* using a rotary evaporator at 45 °C. In order to obtain the optimum gradient time program of the SPE fractions, reversed phase analytical-HPLC (Shimadzu, Japan) equipped with a Shim-pack ODS column (250 mm ×4.6 mm I.D.) and a UV/PDA detector was applied. Accordingly, fraction 3 which was eluted with 40% MeOH-H₂O containing the main compounds was further analyzed by preparative-HPLC. Separations were carried out with a linear gradient of mobile phase, 20-50% acetonitrile in water; 50 min; flow rate: 15 mL/min. Peak fractions with retention time of 19.5 min (fraction I) and 21.5 min (fraction II) were collected according to the chromatogram (figure 1). Additional purification of the two HPLC fractions I and II were accomplished by normal phase preparative thin layer chromatography on silicagel GF₂₅₄. Mobile phase of ethyl acetate 100: formic acid 11: acetic acid 11: water 26 was employed for preparative TLC, yielding compound I (70 mg, R_f~0.5) and compound II (56 mg, R_f~0.8). Detection of the spots was fulfilled under UV-Visible light at the wavelengths of 254 and 366 nm. The spots were scrapped off the silicagel plates, extracted and eluted with methanol. Eventually, chemical structures of the purified compounds were elucidated via 1D and 2D NMR spectroscopic analyses recorded in DMSO-d₆ on a Bruker DRX 400 MHz NMR spectrometer in accordance with the obtained experimental data and authentic published data.

Determination of *in vitro* antioxidant activity

1,1-diphenyl-2-picryl-hydrazil (DPPH) was used to determine the free radical scavenging potential of the MeOH extract. Briefly, 8 mg of DPPH in 100 mL MeOH was prepared [10]. The stock concentration of MeOH extract 1 mg/mL was prepared followed by two-fold dilution series (*i.e.* 5×10^{-1} , 2.5×10^{-1} , 1.25×10^{-1} , 6.25×10^{-2} , 3.13×10^{-2} and 1.56×10^{-2} mg/mL).

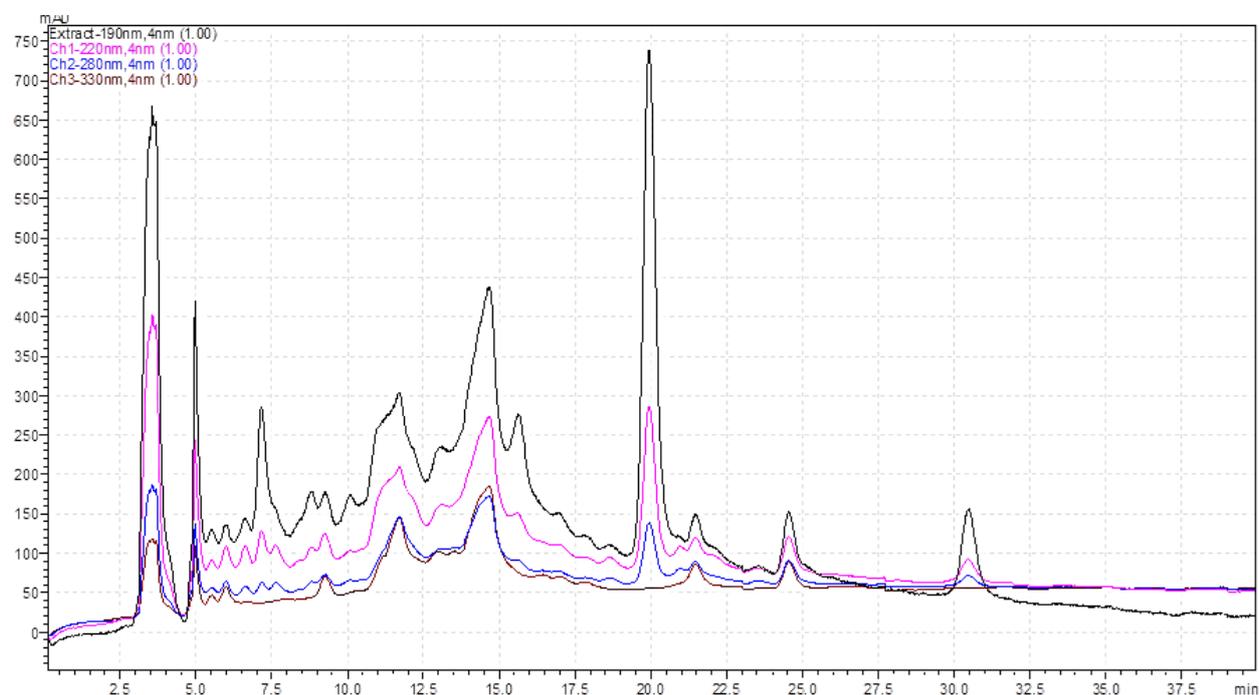


Figure 1. HPLC chromatogram of of fractions I and II from Sep-Pak 40% with retention times (tR 19.5 and 21.5 min) depicted at different wavelengths of 220, 280 and 330 nm

Correspondingly, 5 mL of DPPH solution was added to 5 mL of each concentration. The mixture was shaken and allowed to stand at room temperature for 30 min. Absorbance of the samples were recorded against a blank (methanol) at 517 nm using a UV spectrophotometer (Shimadzu, Japan). The experiments were performed in triplicate and the average absorption was recorded for each concentration. The free radical scavenging percentage (I %) was calculated by following formula:

$$I(\%) = 100 \times [(A \text{ blank} - A \text{ sample}) / A \text{ blank}]$$

IC₅₀ (50% inhibitory concentration), concentration of the MeOH extract reducing 50% of the DPPH free radicals, was calculated from the graph of inhibition percentages against different concentration of *C. albonitens* MeOH extract in mg/mL. Besides, the same procedure was practiced with quercetin as the positive control [11].

Assay for total phenolics content

The total phenolics content of the MeOH extract was determined by Folin-Ciocalteu method [12]. Briefly, 0.5 mL of MeOH extract, 5 mL of Folin-Ciocalteu 10% (v/v) in distilled water and 4 mL Na₂CO₃ 1 M were all mixed. Following the incubation at room temperature for 15 min, the absorbance was measured by UV-VIS spectrophotometer at 765 nm. The standard calibration curve of gallic acid was achieved by 25, 50, 100, 200 and 400 µg/mL in MeOH: water (50:50, v/v). The total phenolics content was calculated from the calibration curve of gallic acid. Total phenolics content of MeOH extract was mentioned as g gallic acid equivalent per 100 g of dried plant material [13].

Assay for total flavonoid content

The flavonoid content of MeOH extract was determined using colorimetric aluminum chloride method [14]. Briefly, 0.5 mL of the extract, 1.5 mL MeOH, 0.1 mL aluminum chloride (10%), 0.1 mL potassium acetate (1M) and 2.8 mL distilled water were mixed. Following the

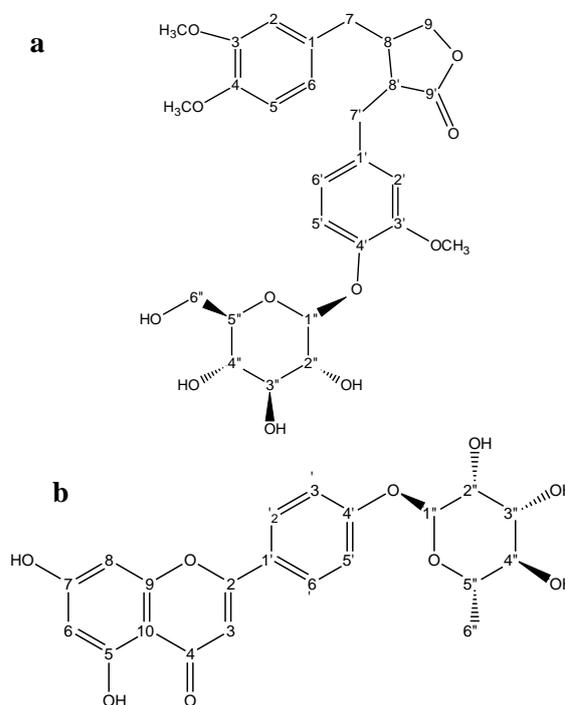
mixture incubation at room temperature for 30 min, the absorbance was measured by UV-VIS spectrophotometer at 415 nm. The standard calibration curve of quercetin was achieved by 25, 50, 100, 200 and 400 $\mu\text{g/mL}$ in MeOH. The total flavonoid content was calculated from the calibration curve of quercetin. Total flavonoid content of MeOH extract was mentioned as g quercetin equivalent per 100 g of dried plant material [15].

Results and Discussion

Phytochemical analysis of the MeOH extract from the aerial parts of *C. albonitens* resulted in isolation of a lignan and a flavonoid. The structures of the isolated compounds were determined using 1D and 2D NMR spectral analyses, and also by comparing the obtained findings with published data in the literature. The structures of the isolated compounds have been shown in figure 2. The relative $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ data were as follows:

Compound (1): arctiin; $^1\text{H-NMR}$ (400 MHz, DMSO d_6): δH 6.79 (1H, *d*, $J=1.6$ Hz, H-6 H-2), 6.99 (1H, *d*, $J=8.4$ Hz, H-5), 6.67 (1H, *dd*, $J=8.4$ Hz, 1.6 Hz, H-6), 2.5 (2H, *m*, H-7) 2.75 (1H, *m*, H-8), 4.10 (1H, *m*, H-9a), 3.89 (1H, *m*, H-9b), 6.67 (1H, *d*, $J=2$ Hz, H-2'), 6.84 (1H, *d*, $J=8$ Hz, H-5'), 6.62 (1H, *dd*, $J=8$ Hz, 2 Hz, H-6'), 2.82 (2H, *m*, H-7'), 2.5 (2H, *m*, H-8'), 4.84 (1H, *d*, $J=7.6$ Hz, Glu H-1"), 3.1-3.3 (4H, *m*, sugar protons), 3.45 (2H, *d*, $J=5.2$ Hz, H-6"a) and signals for three protons of methoxy groups (3-OCH₃, 3'-OCH₃, 4'-OCH₃) appeared in the range of 3.6-3.8.

$^{13}\text{C-NMR}$ (125 MHz, DMSO d_6): δC 132.20 (C-1), 114.22 (C-2), 147.74 (C-3), 56.07 (C-3, OCH₃), 115.48 (C-5), 121.78 (C-6), 37.29 (C-7), 46.01 (C-8), 71.18 (C-9), 131.66 (C-1'), 112.80 (C-2'), 149.09 (C-3'), 55.92 (C-3', OCH₃), 145.73 (C-4'), 55.84 (C-4', OCH₃), 112.27 (C-5'), 120.89 (C-6'), 33.95 (C-7'), 41.20 (C-8'), 178.95 (C-9, C=O), and sugar: 100.63 (C-1"), 73.67 (C-2"), 77.27 (C-3"), 70.08 (C-4"), 77.47 (C-5"), 61.07 (C-6").



[18], *C. sphaerocephala* [19] and *C. pamphylica* [17].

Compound (2): apigenin-4'-*O*-rhamnoside; ¹H-NMR (400 MHz, DMSO-d₆): δH 6.77 (1H, *s*, H-3), 6.07 (1H, *brs*, H-6), 6.36 (1H, *brs*, H-8), 8.00 (2H, *d*, J=9.2 Hz, H-2', 6'), 7.19 (2H, *d*, J=9.2 Hz, H-3', 5'), 5.27 (1H, *d*, J=7.6 Hz, Rhm H-1"), 3.1-3.3 (*m*, sugar protons), 1.02 (3H, *d*, J=6.0 Hz, H-6").

According to the spectral data analysis which were characteristics of the flavonoid structures and also in comparison with published data, the structure of compound 2 was suggested to be apigenin-4'-*O*-rhamnoside in agreement with literature [20]. To the best of our knowledge, the lignan named arctiin (compound 1) and apigenin-4'-*O*-rhamnoside (compound 2) are being reported here for the first time from *C. albonitens*.

The *in vitro* free radical scavenging activity of MeOH extract of *C. albonitens* was determined by DPPH assay. According to the results of the antioxidant assay, the IC₅₀ value for free radical scavenging ability of MeOH extract and quercetin were determined as 389.9 µg/mL and 4.7 µg/mL, respectively (table 1). Besides, the total phenolic content according to the Folin-Ciocalteu method was determined from the standard curve which was prepared by (50- 250 µg/mL) solutions of gallic acid against the absorbance and via the equation below:

Absorbance = 0.0063 × Gallic acid (µg) + 0.0372, (R² = 0.999)

The total phenolic content of the plant aqueous extract was equivalent to 2.87 g of gallic acid per 100 g powdered dry plant material (table 1). The assay for flavonoid content was done by colorimetric aluminum chloride method and the absorbance of the plant extract was calculated from the equation of the standard curve below:

Absorbance = 0.0088 × Quercetin (µg) - 0.0625, (R² = 0.9965)

The total flavonoids content of the plant aqueous extract was equivalent to 0.28 g of quercetin per 100 g powdered dry plant material (table 1).

The results of antioxidant activity indicated that

the antioxidant properties present in the aerial parts of *C. albonitens* could be assigned to the polar compounds, flavonoid and phenolic contents of the MeOH extract. Apparently, an increase in the phenolics and flavonoids content of the plant extract in higher concentrations were in association with the superior radical scavenging activity of the extract.

Table 1. Antioxidant activity, total phenolics and flavonoids contents of *Centaurea albonitens* MeOH extract.

	DPPH Scavenging activity IC ₅₀ (µg/mL)	Total phenolics content g gallic acid/g	Total flavonoids content g quercetin/g
MeOH extract	389.9	2.87	0.28
Quercetin	4.7	-	-

Literature review on different species of genus *Centaurea* revealed valuable various compounds for instance in a study conducted by Hodaj *et al.*, phytochemical investigation on the seeds of *C. vlachorum* led to the isolation and identification of four compounds including two indole alkaloids N-(*p*-coumaroyl) serotonin (1) moschamine (2) and two dibenzylbutyrolactone lignans: matairesinol (3) and arctiin (4) [21]. Elsewhere, investigation on aerial parts of *C. triumfetti* revealed the presence of various types of plant secondary metabolites like apigenin, luteolin and chrysoeriol [22]. Flamini *et al.*, confirmed that aerial parts of *C. bracteata* contained nineteen compounds, a sterol glucoside, two phenolic acids, three quinic acid derivatives, and 13 flavonoids, two of which were new compounds; centabracein and bracteoside [23]. In another study, different sesquiterpene lactone derivatives (repin, solstitialin A, janerin, cynaropicrin) were isolated from the aerial parts of *C. Solstitialis* L. subsp. *solstitialis* which were evaluated and verified for their antinociceptive and antipyretic activities [24]. In a report on essential oil of *C. kilaia* flowers, nineteen compounds were identified which represented 59.5% of the essential oil. The oil contained hexadecanoic acid 26.2% and tetradecanoic acid 18.1% as the main components accompanied by β- eudesmol 3.3%, decanoic

acid 3.1% and dodecanoic acid 2.0%. It was reported that *C. kilaea* essential oil contained high amounts of fatty acids and minor amounts of sesquiterpene alcohols [25]. Additionally, fatty acids were the major compounds of the essential oils of *C. aladaghensis*, *C. luschaniana* and *C. saligna* [26].

Generally, the taxa in the subfamily Carduoideae (tribe Cynareae) are rich in sesquiterpene lactones as well as lignans. Earlier phenolic surveys on *Centaurea* sp. have revealed presence of flavonoids like vitexin, apigenin, luteolin and chrysoeriol with their derivatives, alongside a nearly inclusive dominance of lignin compounds such as arctiin, arctigenin, matairesinol, matairesinoside and lappaol. According to the recently published papers on the potential biological effects of arctiin we may draw readers' attention to the various reported pharmacological properties of arctiin [27]; anti-inflammatory [28], anti-proliferative [23,29,30], antioxidative [31], anti-tumor [32,33], anti-diabetic [34,35], anti-adipogenic [36], and anti-bacterial [37] activities together with being influenza therapeutic agent [38]. In view of the distinguished biological activities of arctiin and apigenin-4'-*O*-rhamnoside, the findings of this study underline *C. albonitens* as a resource of the bioactive compounds which could be subjected to the isolation of arctiin and also other lignans, for supplementary evaluation on their toxicological and further pharmacological properties.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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