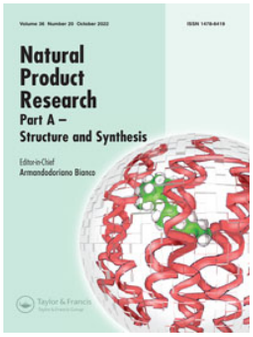

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SHORT COMMUNICATION

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Phytochemical investigation of *Volutaria lippii* and evaluation of the antioxidant activity

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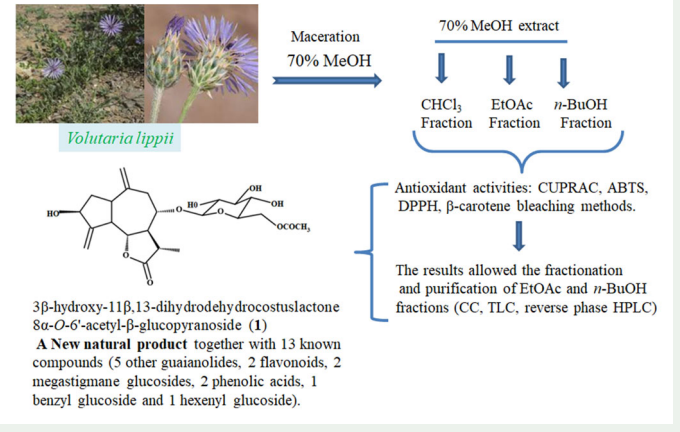
ABSTRACT

Volutaria lippii (L.) Cass. ex Maire, syn. *Centaurea lippii* (L.), (Asteraceae) is a plant from the central region of Algeria, considerably distributed in all Mediterranean areas. Herein, the antioxidant activity of the three derived fractions [chloroform ($CHCl_3$), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH)] of the 70% methanol extract of the aerial parts (leaves and flowers), was assessed by using CUPRAC, ABTS, DPPH free radical scavenging, and β-carotene bleaching methods. The results obtained allowed to guide the fractionation of EtOAc and *n*-BuOH fractions by CC followed by purification by TLC and reverse phase HPLC. A guaianolide glucoside, 3β-hydroxy-11β,13-dihydrodehydrocostuslactone 8α-*O*-(6′-acetyl-β-glucopyranoside) (**1**), never reported in the literature, was isolated together with other known compounds (**2**–**14**). Their structures were elucidated by the extensive use of 1 D- and 2 D-NMR experiments along with ESI-MS analyses and with comparison with literature data.

Graphic Abstract



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1. Introduction

The genus *Centaurea* of the family Asteraceae includes more than 500 species widespread worldwide, among which 45 are found in Algeria (Quezel and Santa 1963, Labed et al. 2019).

Previous studies revealed the richness of this genus in sesquiterpene lactones and flavonoids (Fernandez et al. 1989; Marco et al. 1992). *Centaurea* species are known for their important activities like antidiabetic, antirheumatic, anti-inflammatory, colagog, choleretic, digestive, diuretic, antipyretic and antibacterial (Aktumsek et al. 2013, Ugur et al. 2009, Shakeri et al. 2019). Quite close to the *Centaurea* genus, the genus *Volutaria* Cass., tribe Cardueae, subtribe Centaureinae of the Asteraceae family, comprises eighteen species growing in semiarid to arid zones in the Mediterranean and Irano-Turanian region from Arabia and Iran to Morocco (Kadereit and Jeffrey 2007). In Algeria there are five species of *Volutaria* distributed in the south region, two of them are endemic to the Sahara (Quezel and Santa 1963). *Volutaria lippii* (L.) Cass. ex Maire (Asteraceae), synonyms; *Centaurea lippii* (L.); *Amberboa lippii* (L.) DC., is considerably distributed in all Mediterranean areas. Previous phytochemical investigations of this species have led to the isolation of sesquiterpene lactones and flavonoids (Mezache et al. 2010; Rafrafi et al. 2021). In the present work, the antioxidant activity of the three derived fractions of this plant was evaluated. The obtained results prompted us to investigate the chemistry of the EtOAc and *n*-BuOH fractions. So, the extracts were purified by different chromatographic steps, affording the sesquiterpene lactone glucoside (**1**), never reported in the literature, together with 13 known compounds (**2-14**), among which compounds **2**, **4**, **6-9**, **11-14** were identified for the first time in the aerial parts of this species. Their structures were established by a combination of one- and two-dimensional NMR techniques, and mass spectrometry.

2. Results and discussion

The antioxidant activity of the $CHCl_3$, EtOAc and *n*-BuOH fractions was evaluated using CUPRAC, ABTS, DPPH free radical scavenging, and $\beta-$carotene bleaching methods (Apak et al. 2004, Montoro et al. 2013, Blois 1958, Marco 1968). Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and α-tocopherol were used as positive controls. The tests were performed at different concentrations to calculate the IC_{50} and $A_{0.50}$ values. The EtOAc fraction showed the highest activities followed by *n*-BuOH fraction. The results were statistically significant ($p < 0.05$) compared to the controls in each test (Table S1).

Considering the tested activities, the EtOAc and *n*-BuOH fractions were investigated from a chemical point of view. Therefore, the EtOAc and *n*-BuOH fractions of the air-dried and powdered aerial parts of *Volutaria lippii* were fractionned by CC on silica gel and purified by different chromatographic steps to yield a new sesquiterpene lactone glucoside, 3β-hydroxy-11β,13-dihydrodehydrocostuslactone 8α-*O*-(6′-acetyl-β-glucopyranoside (**1**), associated to thirteen known compounds (**2-14**) (Figure 1) identified as 3β-hydroxy-11β,13-dihydrodehydrocostuslactone 8α-*O*-β-glucopyranoside (**2**) (Shimizu et al. 1988), 11β,13-dihydrodeacylcynaropicrin (**3**) (Rafrafi et al. 2021), desacylcynaropicrin (**4**), cynaropicrin (**5**) (Choi et al. 2005), deacylcynaropicrin 8-*O*-[(S)-3-hydroxy-2-

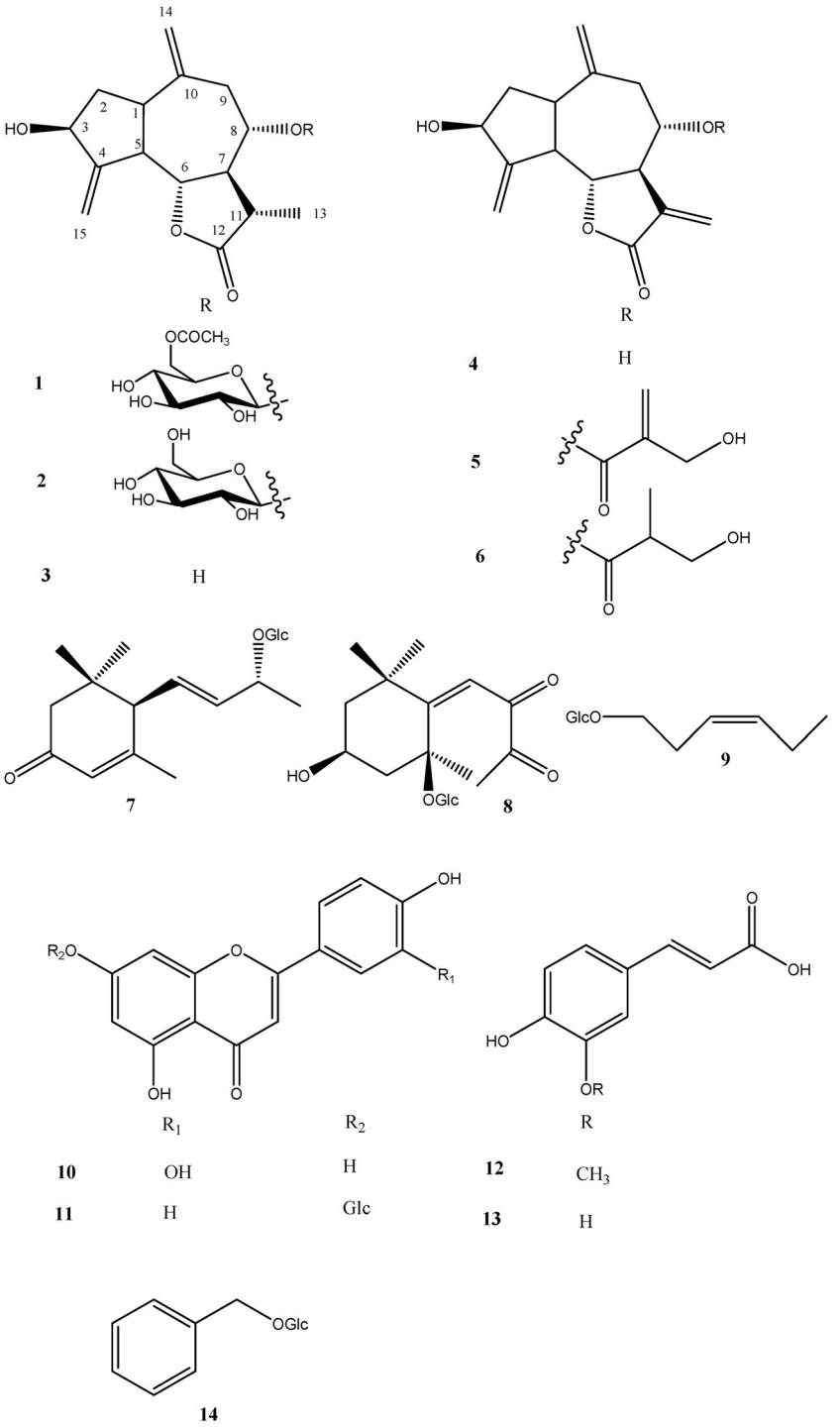

Figure 1. Compounds (1 – 14) isolated from *Volutaria lippii*.

methylpropionate] (**6**) (Marco et al. 1992), (6*R*,9*R*)-3-oxo-α-ionol-β-D-glucopyranoside (**7**) (Pabst et al. 1992), lippianoside E (**8**) (Zhang et al. 2015), (Z)-3-hexenyl β-D-glucopyranoside (**9**) (Moon et al. 1996), luteolin (**10**), apigenin-7-*O*-glucoside (**11**) (Lee et al. 2013), ferulic acid (**12**) (Sajjadi et al. 2012), caffeic acid (**13**) (Lemoui et al. 2018), β-D-glucopyranoside, phenyl-methyl (**14**) (Ghosh et al. 2015). The structures of these compounds were assigned using the ^{1}H NMR, HSQC, HMBC and COSY spectra and by comparison with the literature data. Compounds **11** and **13** were previously detected in *V. lippii* by LC-ESI-MS/MS (Ben Salah et al. 2019), but never isolated before.

The negative ESI-MS spectrum of compound **1**, isolated as a white solid, showed a chlorinated adduct ion $[M_{(1)} + Cl]^-$ at *m/z* 503.17, supporting the molecular formula $C_{23}H_{32}O_{10}$. The presence of chlorine was deduced from the natural isotope distribution pattern of chlorine $^{35}Cl/^{37}Cl$ ~3/1. This spectrum also showed another adduct ion at *m/z* 513.20 corresponding to $[M_{(1)} + HCOO]^-$ which confirmed the molecular formula of compound **1**.

The ^{1}H and ^{13}C NMR analysis suggested the presence of a sesquiterpene derivative of a guaianolide class (Shakeri et al. 2019).

The ^{1}H NMR spectrum of **1** showed signals for two exomethylene at δ 5.33 (brs, H-15a), and 5.29 (brs, H-15b), and δ 5.07 (2H, s, H_2-14). In addition, the proton spectrum showed three oxygenated methine protons at δ 4.51 (brt, $J = 9.8$ Hz, H-3), δ 4.15 (t, $J = 9.8$ Hz, H-6), and δ 3.80 (m, H-8), an acetyl group at δ 2.07 (3H, s), and a signal for a secondary methyl group at δ 1.43 (3H, d, $J = 7.0$ Hz, Me-13). In the ^{1}H NMR spectrum, a signal corresponding to one anomeric proton at δ 4.49 (d, $J = 7.4$ Hz) was present. The chemical shifts of all the individual protons of the sugar unit were ascertained from a combination of 1 D-TOCSY and DQF-COSY spectral analysis, and the ^{13}C NMR chemical shifts of their attached carbons could be unambiguously assigned by the analysis of HSQC spectrum experiment. These data demonstrated the presence of a β-glucopyranosyl unit. The chemical downfield shifts of C-6′ at δ 64.4 and H_2-6′ at δ 4.22 and 4.47 were indicative of an acylation at this position, further confirmed by the HMBC correlations between the proton signal at 2.07 (CH_3 of the acetyl group) with the carbon resonance at δ 172.0. Based on these data, and in particular the absence of other carbonyls other than that of the lactone function at δ 180.0, an acetate function was located at C-6′ of the glucopyranosyl unit. A detailed analysis of 2 D-NMR HSQC, HMBC and COSY) experiments revealed that the structure of compound **1** was almost comparable with those of the molecule 11β,13-dihydrodehydrocostuslactone 8α-*O*-(6′-acetyl-β-D-glucopyranoside) (Li et al. 2007) except for a further hydroxy function evident for compound **1** at δ_H 4.51. This additional hydroxy function was located at C-3 of the guaianolide skeleton based on the HMBC correlations between the proton resonances of exomethylene group (C-15) at δ 5.33 (H-15a), 5.29 (H-15b) and the carbon resonance at δ 73.4 (C-3). A β − orientation for the hydroxy group at C-3 was established in according with the carbon resonance at δ 73.4 (Shimizu et al. 1988; Yang et al. 2008). In fact, the value of the C-3 chemical shift is reported higher for the same class of molecules with a group 3α-hydroxy oriented (Li and Jia 1989). So, compound **1** was identified as 3β-hydroxy-11β,13-dihydrodehydrocostuslactone 8α-*O*-(6′-acetyl-β-D-glucopyranoside) never reported before in literature.

3. Experimental

The aerial parts of *Volutaria lippii* (leaves and flowers, 3300 g) were air-dried, powdered with a light temperature controlled grinding (up to 35 °C) and then macerated at room temperature with MeOH/H_2O (70:30, v/v, 1500 mL) for 48 hours, the operation was repeated three times. The filtrates were combined and concentrated under reduced pressure (up to 35 °C) to reach a volume for around 1450 mL. The remaining solution was diluted with distilled water (1200 mL) under magnetic stirring and then kept at 4 °C for one night to precipitate a maximum of chlorophylls and waxes. After filtration using common filter paper, the resulting aqueous solution was successively extracted with solvents of increasing polarity: chloroform ($CHCl_3$, 700 mL, three times), ethyl acetate (EtOAc, 700 mL, three times), and *n*-butanol (*n*-BuOH, 700 mL, three times). The organic phases were dried with anhydrous sodium sulfate (Na_2SO_4), filtered and concentrated in vacuum (up to 35 °C) to obtain the following partition fractions: 9.2 g, 6.5 g and 41.0 g, respectively (Esseid et al. 2021), see details in supplementary materials.

3.1. 3β-Hydroxy-11β,13-dihydrodehydrocostuslactone 8α-O-(6′-acetyl-β-glucopyranoside) (1)

^{1}H-NMR (400 MHz, MeOH-d_4, δ_{ppm}, J_{Hz}): aglycon moiety: δ_H 1.43 (d, $J = 7.0$, H-13), 1.71 (m, H-2b), 2.23 (m, H-2a), 2.24 (m, H-7), 2.45 (dd, $J = 13.4$, 6.9, H-9b), 2.74 (m, H-11), 2.83 (m, H-5), 2.84 (m, H-9a), 2.95 (q, $J = 8.6$, H-1), 3.80 (m, H-8), 4.15 (t, $J = 9.8$, H-6), 4.51 (brt, $J = 9.8$, H-3), 5.07 (s, H-14b and H-14a), 5.29 (brs, H-15b), 5.33 (brs, H-15a). Sugar moiety: 2.07 (s, $COCH_3$), 4.49 (d, $J = 7.4$, H-1′), 3.24 (dd, $J = 9.0$, 7.4, H-2′), 3.38 (t, $J = 9.0$, H-3′), 3.31 (t, $J = 9.0$, H-4′), 3,53 (m, H-5′), 4.22 (dd, $J = 11.8$, 5.0, H-6′b), 4.47 (dd, $J = 11.8$, 2.5, H-6′a); ^{13}C-NMR (MeOH-d_4, δ_{ppm}, from HSQC and HMBC experiment spectra): aglycon moiety: δ_C 16.1 (C-13), 39.0 (C-2), 42.0 (C-11), 42.6 (C-9), 44.4 (C-1), 51.0 (C-5), 55.1 (C-7), 73.4 (C-3), 80.7 (C-6), 85.2 (C-8), 110.2 (C-15), 115.8 (C-14), 145.2 (C-10), 154.0 (C-4), 180.0 (C-12). Sugar moiety: 104.7 (C-1′), 75.0 (C-2′), 78.0 (C-3′), 71.4 (C-4′), 74.8 (C-5′), 64.4 (C-6′), 172.0 (C = O), 20.3 ($COCH_3$).

4. Conclusion

The results presented in this study were the first report on the evaluation of the antioxidant activity of *Volutaria lippii* (L.). The phytochemical investigation of the ethyl acetate and *n*-butanol fractions of the 70% methanol extract of aerial parts from *V. lippii*, led to the isolation of fourteen compounds among which eleven (**1**, **2**, **4**, **6**-**9**, **11**-**14**) were described for the first time from this species. It is important to note that compound 3β-hydroxy-11β,13-dihydrodehydrocostuslactone 8α-*O*-(6′-acetyl-β-glucopyranoside) (**1**) was a natural compound for the first time described in the literature. The nature of the isolated and described components was in good agreement with the results of studies carried out on *Centaurea* and *Volutaria* species.

Disclosure statement

The authors declare no conflict of interest.

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