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Research Article

Chemical constituents of *Centaurea dissecta* Ten. and sesquiterpenes chemotaxonomic significance

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Abstract

The phytochemical investigation of *Centaurea dissecta* Ten. led to the isolation of thirty-two compounds of diverse chemical classes including two sesquiterpene lactones (**1**, **2**), two sesquiterpenes (**3**, **4**), twelve flavonoids (**5–16**), one lignan (**17**), twelve phenols (**18–29**), one triterpene (**30**) and two phytosterols (**31**, **32**) from the EtOAc and *n*-BuOH soluble fractions of the 70% aqueous ethanol extract. Furthermore, the phenolic and flavonoid contents were measured for the EtOAc and *n*-BuOH soluble fractions. The chemical structures of these compounds were determined by using a combination of NMR spectroscopic and mass spectrometry (ESI-MS) analyses, as well as by comparison with literature values. Only apigenin (**15**) was isolated previously from *Centaurea affinis*, and all other compounds were found for the first time in this species. Noticeably, ethyl ferulate (**20**), ethyl caffeate (**21**), synapyl aldehyde (**22**), coniferyl aldehyde (**23**), 2,3-dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone (**24**), and *C*-veratroylglycol (**25**), have not been reported from the genus *Centaurea*. All compounds have been

previously isolated in various plants belonging to the Asteraceae family. Meanwhile, the chemotaxonomic significance of the isolated compounds was summarized.

Keywords

Centaurea dissecta Ten., Asteraceae, Sesquiterpene lactone, Flavonoid, NMR.

1. Subject and source

The species *Centaurea dissecta* Ten. subsp. *affinis* (Friv.) M. var. *vesceritensis* synonym of *Centaurea affinis* subsp. *affinis* (according to the [WFO Plant List database](#)), belongs to *Centaurea* genus within the Cardueae tribe and subtribe Centaureinae of the Asteraceae family. The classification of the *Centaurea* genus is highly complex and varies among authors ([Greuter, 2003](#); [Hilpold et al., 2014](#)). This genus encompasses approximately 500 species that are widely distributed across the Mediterranean region and Asia, manifesting as either annual or perennial herbs ([Mabberley, 1997](#); [Susanna and Garcia-Jacas, 2007, 2009](#)). In Algeria, they are about 42 endemic species within the *Centaurea* genus ([Quezel and Santa, 1963](#)). Furthermore, *Centaurea* species exhibit significant pharmacological interest as plants used in folk medicine for the treatment of skin cancer, rheumatism, insect bites, gastric ulcers, wounds, hemorrhoids, asthma, **malaria etc.** ([Boulos, 1983](#); [Khammar and Djeddi 2012](#); [Perrot and Paris 1971](#)). The plant *C. dissecta* which is the subject of this study is a species with solitary capitula on long branches, not enveloped by bract like leaves. Involucre bracts have an appendage with a broadly scarious margin, and lateral lobes often equal the central spine. It is growing in forested areas and rocky pastures.

In the present study, the entire plant *C. dissecta* was harvested in May 2016 in Djerma from semi-arid region of Batna, located in northeastern of Algeria. Its authenticity was confirmed by Pr. Bachir Oudjehiche of the Agronomic Institute of the University of Batna-1, Algeria. A voucher specimen bearing the reference number (991/LCCE) has been officially archived at the Faculty of Matter Sciences, University of Batna-1, Algeria, for future reference.

2. Previous work

A phytochemical study of the aerial parts of *Centaurea affinis* Friv. collected from Serbia was carried out in 2004 ([Janačković et al., 2004](#)) resulting in the isolation of two sesquiterpene lactones (cnicin and salonitenolide), two lignans (matairesinol and arctigenin) and four flavonoids (apigenin, eupatorin, salvigenin and 5-desmethylinensetin).

3. Present study

The dried and powdered whole plant (aerial parts and roots, 1400 g) of *C. dissecta* was macerated at room temperature in 70% aqueous ethanol (14 L × 2, each 48 h). Subsequently, the mixture was concentrated under reduced pressure. The aqueous residue was sequentially extracted with petroleum ether (PE), ethyl acetate (EtOAc) and finally *n*-butanol (*n*-BuOH) (3 × 300 mL for each solvent) to give after evaporation 2.6 g of PE, 22.3 g of EtOAc, and 14.3 g of *n*-BuOH extracts. All the extracts and fractions were stored in a dark environment at room temperature prior to subsequent fractionation and purification steps.

A part of the EtOAc extract (8.0 g) was subjected to a Vacuum Liquid Chromatography (VLC) process using an RP-C₁₈ column (8 × 8 cm), sequentially eluted with 20%, 40%, 60%, 80% aqueous MeOH and finally 100% MeOH (each elution totaling 1 L) yielding five major fractions CA₁ – CA₅. The fractions CA₁–CA₄ were purified separately by flash chromatography over silica gel, employing CH₂Cl₂-MeOH-H₂O elution system (in ratios of 1:0:0 to 7:3:0 to 7:3:0.5). The purification of CA₁ (135.0 mg) led to the isolation of compounds **29** (5.0 mg) and **28** (2.0 mg). The purification of CA₂ (1.7 g) resulted in sub-fractions CA_{2-B} (49.0 mg), CA_{2-C} (110.0 mg), and CA_{2-I} (112.0 mg), which were individually purified through semi-prep. HPLC using an isocratic or a gradient system of CH₃CN in water as a mobile phase. Subfraction CA_{2-B} was subjected to an isocratic elution of 22% CH₃CN, for 20 min, to yield compounds **22** (3.7 mg, *t_R* 14.55 min) and **23** (2.1 mg, *t_R* 15.42 min). Subfraction CA_{2-C} was eluted with an isocratic 28% CH₃CN, for 20 min, to provide compound **17** (4.5 mg, *t_R* 12.50 min). Subfraction CA_{2-I} was eluted by a gradient from 20% to 25% CH₃CN in 15 min, to furnish compound **3** (2.4 mg, *t_R* 7.6 min). The purification of CA₃ (2.8 g) yielded 8 sub-fractions CA_{3-A} to CA_{3-I}. Sub-fraction CA_{3-B} (11.0 mg) was purified by semi-prep. HPLC using a gradient of 40% to 50% of CH₃CN in water as a mobile phase, in 12 min, to yield compound **20** (1.5 mg, *t_R* 11.75 min). Compounds **11** (4.7 mg) and **12** (14.7 mg) were isolated from sub-fraction CA_{3-E} (264.0 mg) by flash chromatography over an RP-C₁₈ column eluted with a gradient of 20% to 60% of MeOH in water as a mobile phase, in 25 min. The same method was applied to sub-fractions CA_{3-F} (62.0 mg) and CA_{3-H} (190.0 mg) to obtain compounds **21** (2.4 mg, from CA_{3-F}) and **13** (46.2 mg, from CA_{3-H}). Sub-fraction CA_{3-I} (13.7 mg) was subjected to semi-prep. HPLC with an isocratic elution of 26% CH₃CN for 15 min, resulting in compounds **4** (2.9 mg, *t_R* 7.35 min), **1** (1.4 mg, *t_R* 8.45 min), and **2** (3.5 mg, *t_R* 11.19 min). The purification of CA₄ (3.0 g) using the same flash chromatography process as CA₁–CA₃, led to the isolation of

compounds **14** (3.0 mg), **15** (1.3 mg), **16** (2.0 mg), **30** (13.0 mg), **31** (20.0 mg) and **32** (26.0 mg) (Figure S1).

The *n*-BuOH extract (10.0 g) was subjected to a VLC process over an RP-C₁₈ column (8 × 8 cm), eluted by MeOH-H₂O (0:1, 2:8, 4:6, 6:4, 8:2 and 1:0, 1 L, each) to give fractions CB₁–CB₅, respectively. The fractionation of CB₁ (2.8 g) by flash chromatography over silica gel, using a CH₂Cl₂-MeOH-H₂O elution system (1:0:0 to 7:3:0, to 7:3:0.5), resulted in three significant sub-fractions: CB_{1-D} (41.0 mg), CB_{1-G} (42.0 mg), and CB_{1-T} (360.0 mg). Sub-fraction CB_{1-D} was purified by semi-prep. HPLC with CH₃CN-H₂O elution gradient (10% to 18% CH₃CN, in 17 min), yielding compounds **25** (3.1 mg, *t_R* 7.59 min), **24** (1.6 mg, *t_R* 8.53 min) and **26** (1.5 mg, *t_R* 14.55 min). A similar purification method was employed for sub-fraction CB_{1-G}, employing an elution gradient of 10% to 40% CH₃CN in water, over 20 min, allowing the isolation of compound **27** (3.1 mg, *t_R* 6.35 min). Sub-fraction CB_{1-T} was subjected to preparative HPLC with CH₃CN-H₂O elution gradient (10% to 20% CH₃CN, in 40 min), to give compounds **19** (18.0 mg, *t_R* 4.83 min) and **18** (46.7 mg, *t_R* 5.81 min). Fraction CB₂ (2.7 g) from the VLC was subjected to flash chromatography over silica gel, utilizing CH₂Cl₂-MeOH-H₂O elution system (1:0:0 to 7:3:0, and to 7:3:0.5), resulting in two sub-fractions: CB_{2-R} (124.0 mg) and CB_{2-S} (516.0 mg). Sub-fraction CB_{2-R} was further purified by semi-prep. HPLC, eluting with CH₃CN-H₂O (15% to 20% CH₃CN, in 20 min), to isolate compounds **7** (3.1 mg, *t_R* 9.27 min), **9** (8.9 mg, *t_R* 10.03 min), and **5** (5.3 mg, *t_R* 12.28 min). Compounds **8** (5.5 mg, *t_R* 13.71 min), **6** (35.6 mg, *t_R* 16.57 min), and **10** (23.0 mg, *t_R* 31.43 min) were obtained from sub-fraction CB_{2-S} by preparative HPLC (10% to 40% CH₃CN, in 40 min) (Figure S2).

After careful analysis of ¹H and ¹³C NMR, COSY, HMBC, HSQC spectra and ESI-MS data, as well as comparison with published data in the relevant literature, the structures of the isolated compounds were elucidated as 11,13-dehydromelitensin (**1**) (Demir et al., 2017), 8 α -*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin (**2**) (Tuzun et al., 2017), 8 α -*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-6 α 15-dihydroxyelema-1.3.11(13)-trien-12-oic acid (**3**) (Skaltsa et al., 2000), methyl 8 α -*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-6 α ,15-dihydroxyelema-1,3,11(13)-trien-12-oate (**4**) (Cardona et al., 1997), vicenin-1 (**5**) (Sato and Koide, 2010), vicenin-2 (**6**) (Lu and Foo, 2000), vicenin-3 (**7**) (Sato and Koide, 2010), lucenin-2 (**8**) (Erel et al., 2011), isoschaftoside (**9**) (Hooper et al., 2010), apigenin-7-*O*-glucuronopyranosyl (**10**) (Ozgen et al., 2010), eupatilin (**11**) (Lee et al., 2018), joaceosidin (**12**) (Yuan et al., 2016),

eupafolin (**13**) (Ko et al., 2014), quercetin (**14**) (Yuan et al., 2016), apigenin (**15**) (Ersöz et al., 2002), chrysin (**16**) (Liu et al., 2010), syringaresinol (**17**) (Son et al., 2005), chlorogenic acid (**18**), 3,5-*O*-dicoffeoylquinic acid (**19**) (Satake et al., 2007), ethyl ferulate (**20**) (Chen et al., 2022), ethyl caffeate (**21**) (Chen et al., 2010), synapyl aldehyde (**22**), coniferyl aldehyde (**23**) (Lim et al., 2005), 2,3-dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone (**24**) (Lee et al., 2002), *C*-veratroylglycol (**25**) (Li and Seeram, 2010), syringic acid (**26**) (Świsłocka, 2013), protocatechuic acid (**27**), 2,4-dihydroxybenzoic acid (**28**) (Flamini et al., 2001), 4-hydroxybenzoic acid (**29**) (Dhakal et al., 2009), lupeol (**30**) (Silva et al., 2017), β -sitosterol (**31**) (Chaturvedula and Prakash, 2012), β -sitosterol-3-*O*-glucopyranosyl (**32**) (Flamini et al., 2001). Their chemical structures were displayed in Figure 1.

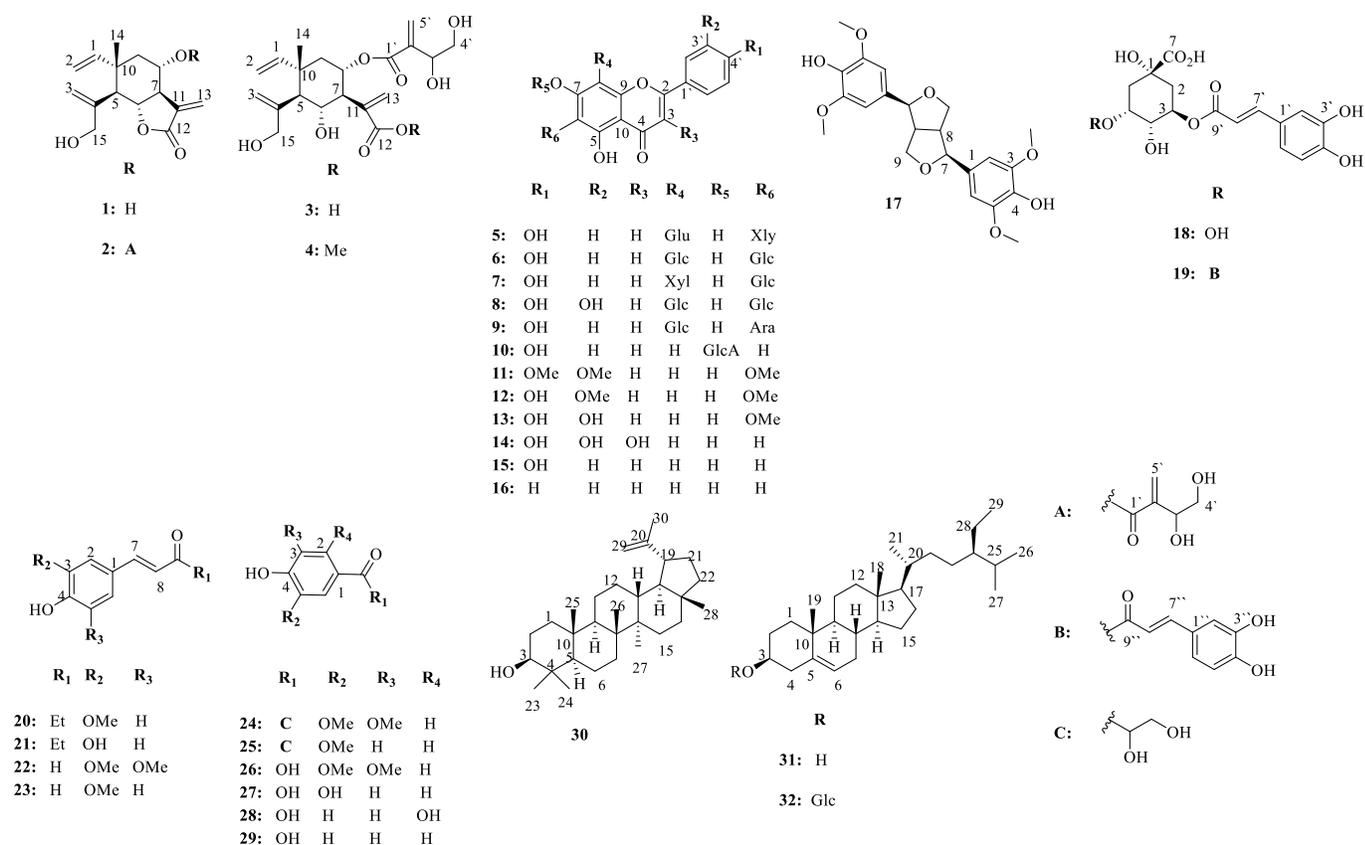


Figure 1. Chemical structures of compounds 1–32 isolated from *C. dissecta*.

The obtained soluble fractions i.e. PE, EtOAc and *n*-BuOH from the 70% EtOH extract of *C. dissecta* were investigated for their total phenolic and flavonoid contents. Total phenolics content (TPC) was measured using the Folin-Ciocalteu method (Müller et al., 2010). A gallic acid

solution at various concentrations was used for the calibration, and the result was expressed in gallic acid equivalents (mg GAE/g extract). Total flavonoids content (TFC) was measured using the trinitroaluminium method (Topçu et al., 2007). The TFC of extracts was given in quercetin equivalents (μg QE/mg extract), with quercetin for calibration (Table 1). As a result, the determination of total phenolic contents indicated that the EtOAc extract had the highest phenolic content (37.97 ± 2.21 mg GAE/g) even more than the *n*-BuOH extract (28.14 ± 1.57 mg GAE/g). However, the PE extract gave low phenolic contents (7.45 ± 0.91 mg GAE/g). In addition, the extract richest in flavonoids was the EtOAc extract (46.57 ± 1.285 mg QE/g), followed by the *n*-BuOH extract (27.48 ± 1.06 mg QE/g), then PE extract (14.68 ± 1.14 mg QE/g). The four sesquiterpene (1-4), lignan (17), flavonoids (11–16), phenols (20–23), triterpene (30) and phytosterols (31, 32) were identified in the EtOAc extract, while the six glycosylated flavonoids (5–10), and phenols (18–19, 24–27), were identified in *n*-BuOH extract, justifying these results.

Table 1. Phenolic and flavonoid contents in *C. dissecta* extracts

Extracts	TPC (mg GAE ^x /g)	TFC (mg QE ^y /g)
PE	7.45±0.91	14.68±1.14
EtOAc	37.97±2.21	46.57±1.28
<i>n</i> -BuOH	28.14±1.57	27.48±1.06

^x Gallic acid equivalent. ^y Quercetin equivalent

4. Chemotaxonomic significance

The genus *Centaurea* L. constitutes an important member of tribe Cardueae Cass. of the family Asteraceae (Compositae). This genus is divided into three well defined subgenera: *Lopholoma*, *Cyanus* and *Centaurea*. These subgenera, in turn, consist of numerous sections and subsections. According to the literature, the section *Centaurea* within the subgenus *Centaurea*, is recognized in three subsections: *Phalolepis*, *Willkommia* and *Centaurea* (formerly known as section *Acrolophus*) (Hilpold et al., 2014). *Centaurea dissecta* Ten subsp. *affinis* (Friv.) M. var. *vesceritensis* is a wild species that grows in Algeria (Quezel and Santa, 1963) and belongs to the subsection *Acrolophus*.

The *Centaurea* genus is characterized by its ability to biosynthesize a wide range of specialized metabolites including phenolics, flavonoids, alkaloids, lignans and sesquiterpene lactones (Grienke et al., 2018; Sarker et al., 2001; Shoeb et al., 2004). Significant variations can be observed in the presence and distribution of these metabolites among various species within the *Centaurea* genus. Sesquiterpene lactones (SLs) are the major secondary metabolites in *Centaurea* species (Grafakou et al., 2018) and notably occur in four main chemical classes by order of their abundance: guaianolides, germacranolides, eudesmanolides and elemanolides. They obviously play a crucial role in the identification and classification of different species within the *Centaurea* genus, which is why they are important chemotaxonomic markers (Bruno et al., 2013).

Regarding the *Acrolophus* (Cass.) DC. subsection within *Centaurea* genus, cnicin sesquiterpene lactone is considered the main phytochemical constituent of this subsection. This compound has been found in the following studied taxa: *C. affinis* Friv. (Janačković et al., 2004), *C. affinis* subsp. *Pallidior* (Halacsy) Hayek (Nowak et al., 1984), *C. derventana* Vis. & Pancic (Tesevic et al., 1998), *C. orphanidea* Heldr. & Sart. ex Boiss (Gousiadou and Skaltsa, 2003) and *C. grisebachii* subsp. *grisebachii* (Djeddi et al., 2008). However, the chemistry of this subsection is characterized by the absence of guaianolide sesquiterpene lactones (Bruno et al., 2013), which are common metabolites in other *Centaurea* species (Bruno et al., 2013).

In this study thirty-two compounds, comprising four sesquiterpenoids (1–4) among them two are sesquiterpene lactones, twelve flavonoids (5–16), one lignan (17), twelve phenols (18–29), one triterpenoid (30) and two phytosterols (31, 32), were isolated from the EtOAc and *n*-BuOH soluble fractions of the 70% aqueous ethanol extract of the whole plant of *C. dissecta*.

Our investigation demonstrated that elemene-type metabolites were the only sesquiterpenes (1–4) isolated from *C. dissecta*. dehydromelitensin (1) was previously isolated from numerous *Centaurea* species such as *C. grisebachii* ssp. *grisebachii* (Djeddi et al., 2008), *C. castellana* (Gonzalez et al., 1984), *C. cineraria* ssp. *busambarensis* (Bruno et al., 1998) and *C. kilaea* Boiss. (Sen et al., 2017), while 8 α -*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin (2) has been found in *C. cineraria* ssp. *busambarensis* (Bruno et al., 1998), *C. grisebachii* ssp. *grisebachii* (Djeddi et al., 2008), *C. attica* Nyman (Skaltsa et al., 1999), *C. cuneifolia* subsp. *cuneifolia* (Aslan and Oksüz, 1999), and *C. spinosa* L. (Saroglou et al., 2005). The two other elemanolides were identified as 8 α -*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-6a15-dihydroylema-1,3,11(13)-trien-12-oic acid (3), methyl 8 α -*O*-(3,4-dihydroxy-2-

methylenebutanoyloxy)-6 α ,15-dihydroxyelema-1,3,11(13)-trien-12-oate (**4**) (Cardona et al., 1997). The sesquiterpene (**3**) was only described once in *C. attica* subsp. *attica* (Skaltsa et al., 1999), whereas (**4**) was reported previously from *Centaurea* species such as *C. spinosa* L. (Saroglou et al., 2005) and *C. virgata* Lam. (Tuzun et al., 2017). Therefore, our results are consistent with earlier studies.

The species of the section *Acrolophus* are characterized by the abundance of flavones such as eupatilin (**11**), jaceosidin (**12**) and apigenin (**15**) (Csapi et al., 2010; Formisano et al., 2012; Janackovic et al., 2004; Sen et al., 2017; Tuzun et al., 2017). Eupafolin (**13**), the major flavone of this extract, have been previously isolated in *Centaurea collina* L. with apigenin (**15**) (Fernandez et al., 1989). However, C-glycosylflavones are not very common in *Centaurea* species. Of the five C-glycosylflavones isolated from *C. dissecta*, three ones namely vicenin-2 (**6**), lucenin 2 (**8**), and isoschaftoside (**9**) were obtained previously from *Centaurea* species within the section *Acrolophus* like *C. calolepis* Boiss. (Erel et al., 2011) and *C. virgata* Lam. (Oksuz et al., 1984). Compounds vicenin-1 (**5**) and vicenin-3 (**7**) were isolated from *Centaurea* genus for the first time. Moreover, these C-glycosylflavones are found in various species of other tribes and subtribes of the Asteraceae family such as *Atractylis cancellata* (Badaoui et al., 2019), *Atractylis flava* (Chabani et al., 2013), and *Flourensia cernua* (Dillon et al., 1976).

On the other hand, this study reports the presence of lignan, syringaresinol (**17**). It has been previously isolated from other *Centaurea* species, namely *Centaurea arenaria* (Csapi et al., 2010) and *Centaurea aegyptiaca* (Mostafa, 2021).

Concerning phenolic compounds, prior chemical investigations have indicated that the genus *Centaurea* is notably abundant in these substances, with a notable prevalence of phenolic acids, which are commonly encountered within the plants of this genus (Alper et al., 2021; Ayad and Akkal, 2019; Bouafia et al., 2021; Yildirim et al., 2022). For example, chlorogenic acid (**18**) was the most abundant in our study, which has also been isolated from other *Centaurea* species such as *C. cyanus* L. (Pirvu et al., 2015); *C. balsamita* Lam., *C. depressa* Bieb. and *C. lycopifolia* Boiss. & Kotschy (Boğa et al., 2016); *C. pichleri* subsp. *pichleri* (Polat et al., 2022); *C. calcitrapa* (Dimkić et al., 2020); *C. aksoyi* Hamzaoglu & Budak and *C. amaena* Boiss. & Balansa (Albayrak et al., 2017). As well, protocatechuic acid (**27**) has been identified from *C. pseudoscabiosa* subsp. *pseudoscabiosa* (Flamini et al., 2002); *C. choulettiana* Pomel (Azzouzi et al., 2016); and *C. calcitrapa* (Dimkić et al., 2020).

Furthermore, this work reports for the first time the identification in this genus of simple phenols like ethyl ferulate (**20**), ethyl caffeate (**21**), synapyl aldehyde (**22**), coniferyl aldehyde (**23**), 2,3-dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone (**24**), and *C*-veratroylglycol (**25**). These compounds have been previously isolated in various plants belonging to the Asteraceae family for instance *Lactuca altaica* (Abdel Bar et al., 2023), *Pluchea indica* Less. (Ruan et al., 2018), and *Xanthium strumarium* L. (Van Kiem et al., 2020).

This investigation also showed the presence of phytosterols (**31**, **32**) and triterpene (**30**). These compounds are minor in *Centaurea* species (Ayad and Akkal, 2019). They were isolated previously from *Centaurea omphalotricha* (Mouffok et al., 2012) and *C. behen* (Mosaddegh et al., 2018). Here the phytosterols were in abundant amounts.

The diversity of compounds isolated from *C. dissecta* growing in Algeria reveals its interesting chemodiversity. They differ notably from those previously reported from the same plant species growing in Serbia. This indicates that the chemical composition of *C. dissecta* can vary based on its geographical location and environmental conditions. However, this study suggests that the chemical composition of *C. dissecta* is comparable to that of *Centaurea* species previously investigated and classified within the same taxonomic section. In summary, chemotaxonomic studies have proven to be of great significance in providing essential insights into the classification and relationships among various taxa within the *Centaurea* genus.

CRedit authorship contribution statement

Mohamed Ibrahim Badaoui: Investigation, Writing - Original Draft, Visualization, **Sonia Chabani:** Investigation, Writing - Original Draft, **Abdulmagid Alabdul Magid:** Validation, Writing - Review & Editing, Supervision, **Laurence Voutquenne-Nazabadioko:** Validation, Resources, Supervision, **Charlotte Sayagh:** Data curation, Software, **Dominique Harakat:** Resources, **Hamada Haba:** Conceptualization, Writing - Review & Editing, Supervision.

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Supplementary material

The general experimental conditions, NMR and MS data of isolated compounds are available online at

Disclosure statement

No conflict of interest reported by authors.

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