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Antiproliferative metabolites from the Northern African endemic plant *Daucus virgatus* (Apiaceae)

Alì Snene [a, 1], Carmina Sirignano [b, 1], Daniela Rigano [b], Carmen Formisano [b], Ridha El Mokni [c], Giuseppe Ercolano [b], Hatem Dhaouadi [a], Angela Ianaro [b], Saoussen Hammami [a], Orazio Taglialatela-Scafati [b, *]

[a] Research Unit Applied Chemistry and Environment 13ES63, Monastir University, Faculty of Sciences of Monastir, 5000 Monastir, Tunisia
[b] Department of Pharmacy, University of Naples Federico II, Via D. Montesano, 49, I-80131 Naples, Italy
[c] Laboratory of Pharmaceutic Botany, Monastir University, Monastir Faculty of Pharmacy, BP. 80, Avenue Avicenne, 5000 Monastir, Tunisia

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ABSTRACT

Chemical analysis of the dichloromethane fraction obtained from aerial parts of the Northern African endemic plant *Daucus virgatus* led to the isolation of three previously undescribed sesquiterpenoids, namely the daucane vaginatin B, a eudesmane and the elemane elemavirgolide, along with five known metabolites. The structures of these compounds were determined by a detailed MS and NMR analysis and they were evaluated for antiproliferative activity against three human cell lines, A375 (melanoma), MCF-7 (breast adenocarcinoma), and HACAT (keratinocyte). The phytoalexin 6-methoxymellein revealed a previously unreported antiproliferative activity, while the eudesmane and the elemane derivatives exhibited a selective activity (SI = 11.1 and 3.3, respectively) against melanoma tumor cell lines.

1. Introduction

Plants of the African flora are well known for their great, and still largely unexplored, biodiversity and chemodiversity and, not surprisingly, in recent years they have been attracting growing scientific interest (Hostettmann et al., 2000). Ethnobotanical and ethnopharmacological applications of these plants in the rich and complex African traditional medicine can constitute a valid guide for chemists looking for new bio-pharmaceutical leads in order to select specimens worthy of detailed investigation (Hostettmann et al., 2000). In spite of an alarming rate of deforestation, the African continent continues to hold the highest rate of plant endemism, with the Mediterranean regions occupying the first positions in this ranking (Linder, 2001). Within this area, Tunisia occupies a privileged place since it harbors nearly 2100 vascular plant species, many of which endemic. In particular, Tunisian steppes are endowed with a variety of aromatic and medicinal plants, widely used for traditional health care (Neffati et al., 2017). Considering that the majority of these plants has been only superficially investigated, the Tunisian flora can be a promising source for drug discovery.

The genus *Daucus* (family Apiaceae) includes more than 80 accepted species, distributed mostly in Europe, North Africa, West Asia and only few in North America and Australia. Eleven *Daucus* species and seven subspecies are widespread in Tunisia, and they have been included in the traditional Tunisian medicine for their diuretic properties or as a remedy for the treatment of cutaneous infections (Rokbeni et al., 2013). *Daucus virgatus* (Poir.) Maire (Apiaceae), syn. *Caucalis virgata* Poir., *Ctenodaucus virgatus* (Poir.) Pomel, grows in Tunisia as a herbaceous annual or biennial species. It has slender, erect, decumbent and rigid stems of 30–70 cm; umbels are short and pedunculated with small pink or white colored flowers. Interestingly, no data appear to be available in the literature about the phytochemical composition of *D. virgatus*.

Thus, in continuation of our contribution to the biological and chemical profile of medicinal plants growing in Tunisia (Hammami et al., 2013, 2016; Faidi et al., 2014), we report here on the results of the phytochemical analysis of the aerial parts of *D. virgatus*. Our analysis led to the isolation of eight specialized metabolites in the

* Corresponding author.
E-mail address: scatagli@unina.it (O. Taglialatela-Scafati).
[1] These two authors contributed equally.

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pure form, including the previously undescribed natural products **6**–**8**, along with the known metabolites **1**–**5** (Fig. 1). The chemical structures of these compounds were determined through extensive analysis of HR-ESIMS, 1D and 2D NMR data. The specialized metabolites isolated from *D. virgatus* were evaluated for their antiproliferative activity against human tumor (melanoma and breast cancer) and non-tumor cell lines and interesting selective activities were unveiled.

2. Results and discussion

Aerial parts of *D. virgatus* specimens, collected during the flowering stage in Biserta, Northwest Tunisia, were dried in the shade for 4–5 days, pulverized, and exhaustively extracted by maceration in methanol (3 × 6L) to afford about 130 g of crude extract, after removal of the solvent in vacuum. The methanol extract was dissolved in water and then partitioned against *n*-hexane, CH_2Cl_2, and butanol. The CH_2Cl_2 fraction, showing a moderate antiproliferative activity (IC_{50} ab. 300 μg/mL) on A375 (melanoma) cells, was selected for further investigation. Thus, repeated purifications of this fraction by column chromatography and HPLC afforded pure compounds **1**–**8**, including the previously undescribed natural products vaginatin B (**6**), eudesmane **7**, and elemavirgolide (**8**). Structures of the known compounds were established by comparison of their spectroscopic data with those available in the literature. Thus, compound **1** was identified as 6-methoxymellein (Sondheimer, 1957). This dihydroisocoumarin had been indicated as the main bitter component of wild carrot (*D. carota*); however, based on sensory and quantitative studies, this role has been recently questioned (Scmiech et al., 2008). Compound **2** was identified as matairesinol, a widespread plant lignan (Fischer et al., 2004), compound **3** as the phenylpropanoid diester laserine oxide (Pinar et al., 1982), compound **4** as 6-hydroxycamphor (Funk et al., 1992) and compound **5** as the daucane sesquiterpene vaginatin, first isolated from the roots of *Selinum vaginatum* (Mesta et al., 1968), but later found also as antimicrobial metabolite in *Ferula hermonis* (Ibraheim et al., 2012).

Vaginatin B (**6**) was isolated as an optically active colorless oil. Its molecular formula, $C_{25}H_{36}O_6$, determined by HR-ESIMS, was indicative of eight unsaturation degrees. In spite of this formula, spectral data of **6** readily suggested that, more than being a sesterterpene, vaginatin B (**6**) actually was a sesquiterpene decorated by two C_5 acyl groups. Indeed, NMR data of vaginatin B closely paralleled those of vaginatin (**5**) (Ibraheim et al., 2012), but some significant differences could be detected: i) the deshielded methyl at δ_H 1.84 (H_3-14) was absent, replaced by a pair of doublets resonating at δ_H 4.57 and 4.51 (J = 13.2 Hz); ii); the resonances of an additional angeloyl group were present, flanking those attributable to the acyl group attached at C-10. Inspection of 2D NMR COSY and HSQC spectra of **6** confirmed this assignment, disclosing the presence of the same spin systems (with parallel resonances) present in vaginatin. The HSQC spectrum associated the oxymethylene proton signals resonating in the midfield region of the ^{1}H NMR spectrum to the carbon at δ_C 69.1. The HMBC spectrum provided the final clues to confirm the structure of vaginatin B, fully confirming the structure of the daucane sesquiterpenoid core and allowing the unambiguous linkage of the two angelate groups at C-10 (cross-peak H-10/C-1′) and C-14 (cross-peaks H_2-14/C-1″), respectively. Since the ^{13}C NMR resonances at the four stereogenic carbons of vaginatin B (**6**) were almost completely superimposable to those reported for **5** (δ_C values of **5** in ppm: C-1 = 60.1, C-4 = 50.8, C-5 = 82.3, C-10 = 75.5; δ_C values of **6** in ppm: C-1 = 60.2, C-4 = 51.0, C-5 = 82.6,

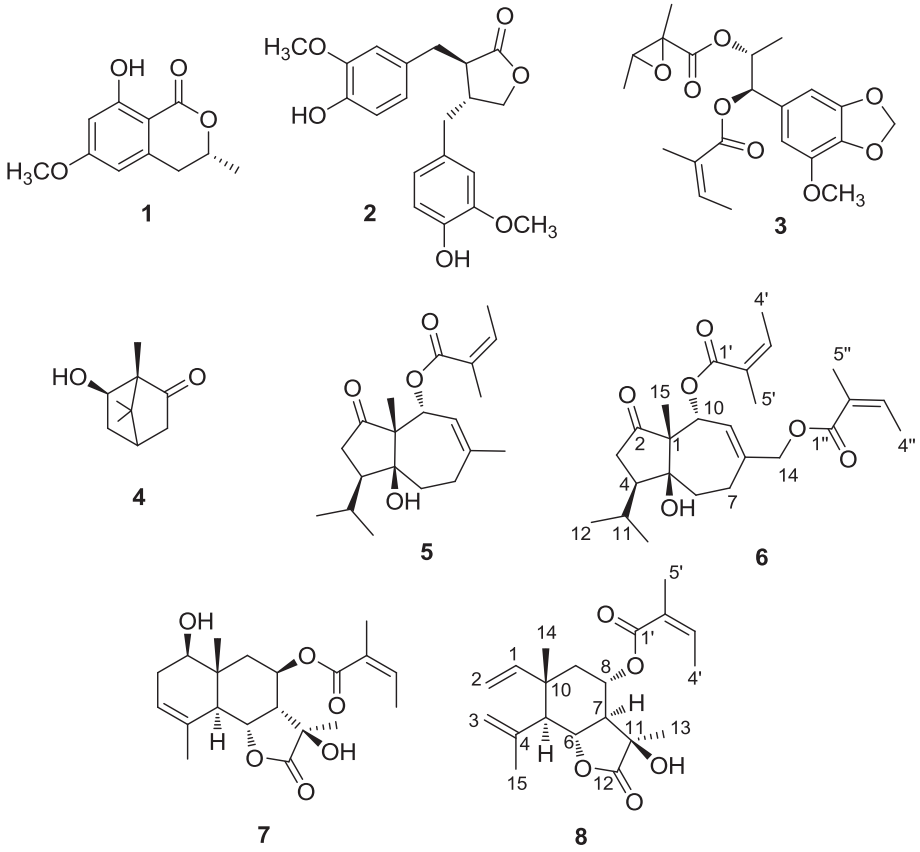

Fig. 1. Chemical structures of metabolites isolated from *Daucus virgatus*.