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Short communication

Phenolics and terpenoids from a wild edible plant *Lactuca orientalis* (Boiss.) Boiss.: A preliminary study

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ARTICLE INFO

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Caffeic acid (PubChem CID: 689043)
Caftaric acid (PubChem CID: 6440397)
Chlorogenic acid (5-O-caffeoylquinic acid, accordingly to IUPAC guidelines from 1976) (PubChem CID: 1794427)
Cichoric acid (PubChem CID: 5281764)
Dihydroconiferin (PubChem CID 14427336)
Dihydrodehydrodiconiferyl alcohol (PubChem CID: 5274623)
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ABSTRACT

Lactuca orientalis, hitherto phytochemically unexamined plant, has been used as a food or forage plant in some countries of the Near East. Six neolignans – derivatives of dihydrodehydrodiconiferyl alcohol, one sesquiterpene lactone – leucodin, one apocarotenoide – loliolide, and two simple phenolic compounds – vanilin and methyl 4-hydroxybenzoate were isolated from roots and aerial parts of the plant. Three of the isolated neolignans, i.e. 4-*O*-methyldihydrodehydrodiconiferyl alcohol and its two glucosides, were found for the first time in the genus *Lactuca*. Moreover, presence of dihydroconiferin, nucleosides (adenosine and uridine) and caffeic acid derivatives was revealed in the plant material using combined chromatographic and spectroscopic (^{1}H NMR) techniques. Major phenolic constituents of young shoots and leaves, i.e.: caffeic acid derivatives (caftaric acid, chlorogenic acid, caffeic acid and cichoric acid), dihydroconiferin and neolignans (dihydrodehydrodiconiferyl alcohol, 4-*O*-methyldihydrodehydrodiconiferyl alcohol), were quantified. Cichoric acid (c. 0.66% dry weight) and dihydrodehydrodiconiferyl alcohol (c. 0.76% dry weight) were the most abundant phenolic compounds detected in the examined tissue.

1. Introduction

According to currently accepted taxonomic concepts, the genus *Lactuca* is considered to be a member of the family Compositae (Asteraceae), subfamily Cichorioideae, tribe Cichorieae, a subclade Lactucinae (Kilian et al., 2009). Plants of the tribe Cichorieae are mainly distributed in the temperate zone of the Northern Hemisphere. Central to Eastern Asia, the Mediterranean Basin including Southwestern Asia and, to a lesser extent, western North America are regarded as three main centers of diversity. Members of some genera (e.g., *Lactuca*, *Launaea*) are adapted to semiarid and arid environments (Kilian et al., 2009). The genus *Lactuca* comprises about 100 wild species, occurring in North America (12), Europe (17), Africa (43), and Asia (51) (Lebeda et al., 2004, 2007). In a recent taxonomical treatment of *Lactuca*, *Lactuca orientalis* (Boiss.) Boiss. (basionym: *Phaenopus orientalis* Boiss.; synonyms: *Phaenixopus orientalis* (Boiss.) Sosn., *Scariola orientalis* (Boiss.) Soják) belongs to the section Phaenixopus (Lebeda et al., 2007, and literature cited therein). *L. orientalis* is a chamaephyte

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perennial plant, 20–50 cm tall, woolly-floccose, later glabrescent. Its stems and branches are white, intricate; branchlets rigid, short, spreading, later spinescent at tip. Leaves of the plant are pinnatifid into few triangular to oblong lobes, withering soon; radical leaves tapering to a petiole; cauline leaves long-decurrent, with adnate linear appendages. Heads are mostly solitary terminal and pedunculate or lateral and sessile, with 4 (or 5) pale yellow florets. *L. orientalis* naturally inhabits rocky Mediterranean and Irano-Turanian dwarf-shrub associations. The halophytic species belongs to a tertiary gene pool of garden lettuce (*L. sativa*) which is one of the most popular leafy vegetables. The reported chromosome number (2n) of tested *L. orientalis* plants was 18 or 36 (Feinbrun-Dothan, 1978; Shi et al., 2011).

Whole young plants of *L. orientalis*, known by local communities under the name of "Khees" or "Rabhalah", are eaten raw in Jordan and neighboring countries both in normal and in food shortage times (Al-Qura'n, 2010). According to the same ethnobotanical study the plant was also used as an herbal medicine.

The aim of our work was to investigate phytochemical composition of an ethanol extract from the plant with a special interest to biologically active sesquiterpene lactones and phenolic compounds. An insight into the chemical composition of the plant may facilitate further discussion on potential beneficial effects of its consumption on human health. The results obtained in the present study could make a good starting point for further research on bioavailability of selected compounds and on optimization of the extraction and isolation steps to fulfill requirements of large-scale recovery procedure. Though "5-Stages Universal Recovery Process" (Galanakis, 2012) is a recommended procedure for recovery of high-added value plant components from plant matrix, difficulties emerged when composition of the plant material under consideration remains unknown. In the present study we followed the same laboratory procedures as those developed earlier for other species of *Lactuca* (Michaska et al., 2012; Stojakowska et al., 2013) to make comparison of the results easy.

2. Materials and methods

2.1. Chemicals and solvents

Chlorogenic acid (5-*O*-CQA, purity > 97% by HPLC), cichoric acid (DCTA, purity > 98%) and a standard sample of cynarin (1,3-DCQA, purity > 99% by HPLC) were purchased from Roth (Karlsruhe, Germany). Caftaric acid (CTA acid, purity > 97%) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). A standard sample of (±) dihydrodehydrodiconiferyl alcohol (**3**, see Fig. 1) was isolated in our laboratory from aerial parts of *L. orientalis*, and identified by comparison of its spectral (UV, ^{1}H NMR, 600.20 MHz) data with those found in the literature (Shen et al., 1998; Meng et al., 2010). The compound **3** was of purity 90.0% (by HPLC). MeOH of analytical grade was purchased from POCh S.A. (Gliwice, Poland). Water was purified by a Mili-Q system (Milipore Corp., Bedford, MA, USA). MeOH and MeCN of HPLC grade as well as formic acid and glacial acetic acid of analytical grade were purchased from Merck (Darmstadt, Germany).

2.2. General experimental procedures

Optical rotation was determined on a PolAAr31 polarimeter (Optical Activity Ltd., Ramsey, England). NMR spectra were recorded in $CDCl_3$ and in CD_3OD or pyridine $-d_5$ on a Bruker AVANCE III 600 (resonance frequency 600.20 MHz for ^{1}H) (Bruker Corp., Billerica, MA, USA). Analytical RP-HPLC separations were performed on a Zorbax Eclipse XDB-C18 column, 4.6 × 150 mm (Agilent Technologies, Santa Clara, CA, USA), using an Agilent 1200 Series HPLC system (Agilent Technologies) equipped with a Rheodyne manual sample injector, quaternary pump, degasser, column oven and a diode array detector. Semiprep. RP-HPLC was performed on a Waters instrument coupled to a dual wavelength UV/VIS detector operating at 210 and 260 nm, using Delta-Pak C-18 column (particle size 15 µm, 25 × 100 mm) (Waters Corp., Milford, MA, USA) eluted with H_2O-MeOH mixtures at a flow rate of 3.0 mL min^{-1}. Conventional column chromatography (CC) was carried out using Merck silica gel 60 (0.063–0.2 mm). Thin layer chromatography (TLC) was performed on Merck silica gel 60 (0.25 mm) precoated plates.

2.3. Plant material

Seeds of *L. orientalis* – used for this study – were collected in October 2011 from a single locality in Israel, in Nahal Tavia, at 31°15′19.30"'N; 35°13′13.18'E; 575 m a.s.l., located at the North-East edge of the Negev desert and the Judean desert, in a hilly desert landscape, directly in the area of the city of Arad. Seeds were collected separately from six individual plants, each referred as an "accession". For the present study seeds were combined from all of the accessions, in order to increase quantity of material for the isolation procedure. All *L. orientalis* accessions (population 447, accessions 1–6) are documented in the Institute of Evolution (or IOE, University of Haifa, Israel) *Lactuca* database (IOELDB).

Roots and shoots of *L. orientalis* plants, grown in the Garden of Medicinal Plants, Institute of Pharmacology, Polish Academy of Sciences, Kraków from the seeds collected in Israel, were harvested in August–October 2012. The plant material was dried under shade, at room temperature. A voucher specimen (3/2012) was deposited at the Garden of Medicinal Plants of the Institute of Pharmacology PAS.

2.4. Isolation and identification of secondary metabolites from L. orientalis

2.4.1. Secondary metabolites of aerial parts

The air-dried shoots (31.0 g) were powdered and extracted five times with 200 mL of 96% EtOH (v/v) at room temperature with shaking. The combined extracts were concentrated in vacuo, at 40 °C, providing c. 3.0 g of an oily residue. The residue was subjected to CC on silica (110.0 g) using gradients of EtOAc in hexane (up to 50% EtOAc) and subsequently MeOH in $CHCl_3$ (up to 50% MeOH) as elution systems. The separated fractions (50 mL each) were monitored by TLC and the relevant ones were combined. Elution with hexane-EtOAc (3:2, v/v) gave fraction 104 (2.4 mg) which contained loliolide (**1**) as the major constituent (^{1}H NMR). Fractions 178–183 (124.9 mg, eluted with $CHCl_3$–MeOH; 19:1, v/v) were further separated by CC on silica (40 g) with $CHCl_3$–MeOH gradient solvent system (up to 3% MeOH). The relevant fractions were combined, as shown by analytical RP-HPLC, to obtain (±) 4-*O*-methyldihydrodehydrodiconiferyl alcohol (**2**, 7.2 mg) and (±) dihydrodehydrodiconiferyl alcohol (**3**, 19.1 mg). Fractions 221–229 (eluted with $CHCl_3$–MeOH; 9:1) contained a mixture of adenosine, uridine, dihydroconiferin and dihydrodehydrodiconiferyl alcohol 9′-*O*-β-glucopyranoside (**4-7**) as it was shown by analytical HPLC supported by ^{1}H NMR of crude fractions.

The known compounds were identified by comparison of their ^{1}H NMR data with those previously reported (Kisiel, 1992; Pieters et al., 1990; Meng et al., 2010; Moyroud and Strazewski, 1999; Ushiyama and Furuya, 1989; Takeda et al., 1998).

2.4.2. Secondary metabolites of roots

The dried plant material (18.0 g) was powdered and extracted five times with 150 mL of 96% EtOH (v/v) by shaking at a room temp. Combined ethanol extracts were concentrated under reduced pressure, at 40 °C, and the residue (0.84 g) was subjected to fractionation on a silica gel column eluted with hexane-EtOAc (up to 100% EtOAc), followed by EtOAc-MeOH (up to 50% MeOH) gradient solvent systems.

Elution of the column with hexane-EtOAc (4:1), after purification by prep. TLC (hexane-EtOAc 4:1), allowed isolation of vanillin (8, 5.1 mg) and leucodin (9, 1.8 mg). From the fractions eluted with hexane-EtOAc (1:1), methyl-4-hydroxybenzoate (10, 2.8 mg) was isolated after prep. TLC (hexane-EtOAc 1:1). Fractions eluted with EtOAc and subsequently