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Short Communication

C-glycosylflavones from *Linum hologynum* Rchb.

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Abstract

The current study focuses on the stem and flowers of *Linum hologynum*, an endemic species to the Balkan Peninsula, a potential source of valuable metabolites. *L. hologynum* is poorly studied in any aspects, including its phytochemistry and pharmacological activity. Applying various chromatographic techniques, two isomeric C-glycosylflavones were isolated. Structures were elucidated, by means of spectroscopic methods, including ^{1}H and ^{13}C NMR as well as LC-HRESI-MS analysis, as orientin and isoorientin. This is the first report of those metabolites from *L. hologynum*.

Keywords

Linum hologynum, *Linum*, C-glycosylflavones, orientin, isoorientin

Introduction

Linum hologynum Rchb. (Linaceae) is a 30–40 cm tall, glabrous, perennial plant with a haploid chromosome number of n = 42. The leaves are linear to filiform. The flowers are purple with a hint of violet or pinkish purple, 2–3 cm in diameter. It is endemic to the Balkan Peninsula and grows in mountain meadows in Albania, Bulgaria, Greece, Romania, and former Yugoslavia (Löve 1972; Petrova 1979). The most distinct features of this plant are its fused styles and pantoporate pollen grains. These are unusual features in the purple-flowered flaxes and a unique combination only in this taxon. Fused styles are found only in *L. tmoleum* Boiss. of Turkey, while pantoporate pollen grains are found exclusively in *L. stelleroides* Planch. of China. Therefore, *L. hologynum* is quite an interesting object for research because there is a hypothesis that this species might represent a link between European and Australasian taxa (McDill et al. 2009). At the same time, *L. hologynum* still remains poorly studied in any aspects, including its chemical compounds and pharmacological activity.

Different *Linum* species are known to produce various groups of lignans, as well as rare C-glycosylflavones (Hegnauer 1989). Orientin and isoorientin, flavonoid C-glycosides, exhibit diverse medicinal properties. Orientin demonstrates antioxidant, anti-inflammatory, vasodilatory, and cardioprotective effect, while isoorientin adds anti-nociceptive and hepatoprotective benefits, showing potential for managing oxidative stress, inflammation, and pain without significant toxicity (Küpeli et al. 2004). Isoorientin shares similar benefits, notably in combating oxidative damage and inflammation. Both compounds show radioprotective properties, reducing DNA damage and oxidative stress from radiation exposure. Neuroprotective and antidepressant-like effects have also been observed, with potential applications in neurodegenerative disorders. Additionally, they exhibit antiviral, antibacte-

rial, and antiadipogenic activities, providing promising therapeutic avenues for various conditions (Lam et al. 2016). The importance of luteolin glycosides is also linked to their role in reducing the risk of neoplasms. It acts as a topoisomerase I poison by stabilizing the DNA-topoisomerase I cleavable complex, impairing DNA relaxation, and potentially inducing apoptosis through DNA damage and inhibition of replication and repair processes (Chowdhury et al. 2002). Xu et al. 2020 highlight isoorientin's potential as a lung cancer therapeutic agent. Isoorientin induces apoptosis in A549 lung cancer cells through mitochondrial dysfunction and ROS-mediated signaling. By activating the MAPK/STAT3/NF-κB pathway, isoorientin promotes mitochondrial-dependent apoptosis, G2/M cell cycle arrest, and oxidative stress. Isoorientin is also shown to inhibit monocarboxylate transporter (MCT) activity and expression, as well as cell migration in A549 lung cancer cells, without affecting viability. By downregulating MCTs1/4, CD147, and MMPs2/9, isoorientin disrupts tumor glycolysis and migration pathways, suggesting its potential as a therapeutic agent for lung cancer (Huang et al. 2020). The 6-C-glucoside of luteolin also exhibits potent anti-cancer effects against pancreatic cancer by inducing apoptosis, reducing malignancy, and downregulating markers like epithelial–mesenchymal transition, MMPs, and VEGF. This activity is mediated through the activation of the AMPK signaling pathway, with PRKAA1 identified as essential for its apoptotic and malignancy-reducing effects, highlighting its therapeutic potential for pancreatic cancer (Ye et al. 2016).

Thus, this study aims to investigate the chemical composition of *L. hologynum*, focusing on its potential production of bioactive compounds like flavonoid C-glycosides, known for their diverse medicinal benefits.

Materials and methods

General experimental procedures

Solvents EtOAc, MeOH, CH_2Cl_2, and ACN were obtained from Fischer Chemicals (Loughborough, UK) and were at least of analytical grade. The water used for assays was obtained from a Millipore Milli-Q system (Bedford, MA, USA) dispenser and filtered through a 0.22 μm membrane before utilization. A semi-preparative HPLC system (KNAUER Wissenschaftliche Geräte GmbH, Berlin, Germany) consisting of a Knauer Azura P 6.1L pump, a variable wavelength UV-Vis detector (Knauer UVD 2.1L), a hand injector, a fraction collector (FOXY R1), and an AZURA column thermostat (CT 2.1) was used for the isolation. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVII+ 600 spectrometer (Bruker, Karlsruhe, Germany), operating at a proton NMR frequency of 600.13 MHz in DMSO (99.5%, Deutero GmbH) and carbon NMR frequency of 150.90 MHz with TMS as the internal standard. LC-HRESI-MS analysis of pure compounds was performed on the Thermo Scientific Q Exactive Plus quadrupole-Orbitrap mass spectrometer used in ultra-high-resolution mode (70 000, at *m/z* 200) coupled with a UPLC Dionex Ultimate 3 000 RSLC system equipped with an RP-18 Kinetex column (2.10 mm × 100 mm, 2.6 μm, Phenomenex (Corporation, Torrence, CA, USA)). A gradient elution was performed using filtered and degassed MS-grade solvents A (0.1% FA in H_2O) and B (0.1% FA in ACN) as follows: 0' 5% B; 3' 10% B; 16' 20% B; 17' 20% B; 19' 40%; 20' 50% B; and 22.5' 95% B. The mobile phase flow rate was 0.3 mL/min, and the column temperature was set at 40.0 °C. The injection volume was 2.5 μL. Sample recording was performed under negative ionization mode. The full MS scan lasted 25 min, with a run time of 0.62 to 23.99 minutes, resolution set at 70,000; AGC target at 3e6, max. IT at 100 ms, and a scan range of 100 to 1500 *m/z*. The MS/MS scan was set to a resolution of 17 500 and an AGC target of 1e5, a maximum IT of 50 ms, a scan range of 200 to 2000 *m/z*, an isolation window of 2.0 *m/z*, and a step (N)CE of 20, 40, or 70. The following parameters were used: dry gas flow (N_2 8.0 L/min), capillary temperature 320 °C, source temperature 320 °C, sheath gas flow – 36 AU, auxiliary flow – 11 AU, source voltage – 3.5 kV, and capillary voltage – 320 V. Data acquisition and processing were performed using Thermo Xcalibur 2.2 software (Thermo Fischer Scientific Inc., Waltham, MA, USA). All the other parameters were set to obtain the most intense signal for $[M-H]^-$. All data were recorded and processed using Xcalibur software, version 2.0 (Thermo Fisher).

Plant material

The stem and flowers of *L. hologynum* Rchb. (Linaceae) was collected in Pirin mountain, Banderiska Polyana region in July 2023 and deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOM) (No. 178667).

Chemistry

Spectral data for orientin: ^{13}C NMR (DMSO) δ, ppm: 61.67 ($C^{6''}$, $-CH_2-$), 70.71 ($C^{4''}$, −CH=), 70.83 ($C^{2''}$, −CH=), 73.53 ($C^{1''}$, −CH=), 78.78 ($C^{3''}$, −CH=), 81.97 ($C^{5''}$, −CH=), 102.12 (C^{6}_{arom}), 102.21 (C^{3}, −CH=), 103.63 (C^{4a}_{arom}), 104.56 (C^{8}, −CH=), 113.78 ($C^{2'}$, −CH=), 115.72 ($C^{5'}$, −CH=), 119.35 ($C^{6'}$, −CH=), 121.65 ($C^{1'}_{arom}$), 145.95 ($C^{3'}_{arom}$), 150.17 ($C^{4'}_{arom}$), 156.04 (C^{8a}_{arom}), 160.44 (C^{5}_{arom}), 160.44 (C^{7}_{arom}), 164.00 (C^{2}_{arom}), 181.83 (C^{4}, =C=O). HR-ESI-MS deprotonated molecular ion at *m/z* 447.0930 $[M-H]^-$ (calcd for $C_{21}H_{19}O_{11}$, 447.0922).

Spectral data for isoorientin: ^{13}C NMR (DMSO) δ, ppm: 61.51 ($C^{6''}$, $-CH_2-$), 70.18 ($C^{4''}$, −CH=), 70.63 ($C^{2''}$, −CH=), 73.97 ($C^{1''}$, −CH=), 78.97 ($C^{3''}$, −CH=), 81.57 ($C^{5''}$, −CH=), 93.57 (C^{8}, −CH=), 102.68 (C^{3}, −CH=), 103.20 (C^{4a}_{arom}), 108.92 (C^{6}_{arom}), 113.17 ($C^{2'}$, −CH=), 116.05 ($C^{5'}$, −CH=), 118.97 ($C^{6'}$, −CH=), 121.24 ($C^{1'}_{arom}$), 145.84 ($C^{3'}_{arom}$), 150.00 ($C^{4'}_{arom}$), 156.27 (C^{8a}_{arom}), 160.74 (C^{5}_{arom}), 163.62 (C^{7}_{arom}), 163.80 (C^{2}_{arom}), 181.80 (C^{4}, =C=O). HR-ESI-MS deprotonated molecular ion at *m/z* 447.0931 $[M-H]^-$ (calcd for $C_{21}H_{19}O_{11}$, 447.0922).

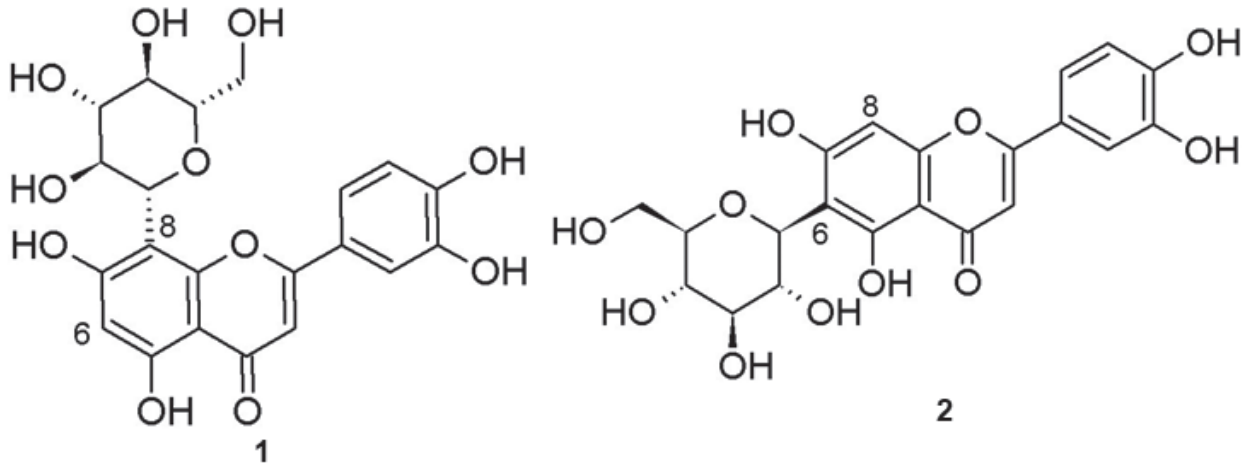

Figure 1. Structures of isolated orientin (**1**) and isoorientin (**2**) from the stem and flowers of *L. hologynum*.

Extraction and isolation

The pre-dried and ground plant material, the stem and flowers of *L. hologynum* (148.20 g), was subjected to extraction by percolation using 80% MeOH (6 L) until complete exhaustion. The collected extracts were concentrated using a vacuum evaporator, yielding a total extract, which was further defatted using CH_2Cl_2 (3 × 1L). The defatted extract was exhaustively extracted with EtoAc (3 × 1L). Dissolved in acidified water (0.1% formic acid), EtoAc fraction is subjected to further purification with column chromatography (CC) against Diaion HP-20. The gradient elution was applied with the following solvent mixture (v/v): MeOH/H_2O (30/70), MeOH/H_2O (50/50), MeOH (90/10), MeOH (100). The 50% MeOH fraction (500 mg) is subjected to further purification by a semi-preparative system using an Ascentis® C18 column (250 mm x 10 mm, 5 µm). A gradient elution was performed using filtered and degassed HPLC-grade solvents A (0.1% FA in H_2O) and B (0.1% FA in ACN) as follows: 0' 10% B; 5' 10% B; 20' 45% B; 32' 65% B; 33' 100%.

Results and discussion

Two major flavone C-glycosides – orientin (**1**) and isoorientin (**2**) – were isolated for the first time from the stem and flowers of *L. hologynum* (Fig. 1). Both compounds showed deprotonated molecular ions at *m/z* 447 (Suppl. material 1: figs S1, S2), which corresponds to the structure of isomeric compounds. The typical fragmentation of *O*-glycosyl flavonoids [M-H-hexose]$^-$ was substituted by internal cleavage in the sugar moiety, which is favored over breaking C-C bonds with C-glycosyl flavonoids (Geng et al. 2016). Onother specific precursor to product ion transion *m/z* 447→327 characteristic of the isomeric pair orientin-isoorintin was also observed (Zhang et al. 2018). Comparing the chemical shifts extracted from ^{13}C NMR spectra, both compounds differ only by a mean of only two aromatic carbons – d 102.12 (C-6) and d 104.56 (C-8) for compound **1** and d 108.92 (C-6) and d 93.57 (C-8) for compound 2, respectively (Suppl. material 1: figs S3, S4). Those observations and data reviews led to the identification of compound **1** as luteolin 8-C-glucoside (orientin) and compound **2** as luteolin 6-C-glucoside (isoorientin) (Kato and Morita 1990).

Conclusion

Our results have led to the identification of C-glycosides – orientin and isoorientin – which are not typical for the Linaceae family, known for lignan production. The biogenesis of lignans is linked to that of flavonoids due to common precursors (p-coumaric, ferulic, and caffeic acids) and their synergistic action within the biosynthetic pathway of polyphenolic compounds. This first-time isolation of these compounds from *L. hologynum* broadens our understanding of the plant phytochemical profile. The obtained findings would be valuable for further elucidating fundamental mechanisms in the biosynthesis of these two groups of compounds – flavonoids and lignans – in the genus *Linum*. Moreover, this highlights the need for further investigations to identify the full spectrum of bioactive compounds present in various *Linum* species, along with their pharmacological potential.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

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Author contributions

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary data

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Data type:docx

Explanation note: **figure S1.** LC-HRESI-MS of orientin. **figure S2.** LC-HRESI-MS of isoorientin. **figure S3.** ^{13}C NMR spectrum (DMSO) of orientin, frequency of 150.90 MHz with TMS as the internal standard. **figure S4.** ^{13}C NMR spectrum (DMSO) of isoorientin, frequency of 150.90 MHz with TMS as the internal standard.

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