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Short communication

New insights into sesquiterpene lactones composition of Western Balkan's genus *Amphoricarpos* revealed by rapid resolution liquid chromatography coupled with quadrupole time-of-flight mass spectrometry

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Introduction

Our previous phytochemical studies revealed that the plant species of the genus *Amphoricarpos* from Montenegro are characterized with an appreciable content (>0.3–2.3% per dry weight of aerial plant material) of γ-sesquiterpene lactones with guaianolide skeleton, so-called amphoricarpolides that are mostly deposited on the leaf surface (>1.0–3.4% per dry weight of leaves) (Djordjević et al., 2021; Jadranin et al., 2013). These compounds showed *in vitro* protective effect on chromosome aberrations in peripheral human lymphocytes (Cvetković et al., 2021) and *in vitro* cytotoxic (Djordjević et al., 2021), antifungal (Jadranin et al., 2013) and antibacterial (Gavrilović et al., 2016) activities. The amphoricarpolides are significant as chemotaxonomic markers as they gave insights about the taxonomic status of the genus (Djordjević et al., 2021).

This study revealed results from reinvestigation of sesquiterpene lactones composition in the crude and leaf surface extracts of *Amphoricarpos* plants by rapid resolution liquid chromatography (RRLC) coupled with quadrupole time-of-flight mass spectrometry (QToF MS) and the tentative identification of undescribed sesquiterpene lactones is presented.

Materials and methods

Plant material

The aerial plant parts of *Amphoricarpos* sp. were collected during the flowering phase over several years at different localities in Montenegro, as described by Djordjević et al., (2021).

Extraction of the plant material

The crude extracts (BEM: Pet ether/Et_2O/MeOH and DCM: CH_2Cl_2) were prepared from the ground air-dried aerial parts and the intact air-dried leaves, respectively, using the procedure described by Djordjević et al., (2021). After filtration and evaporation of the solvent, the residues were stored frozen at −4 °C until RRLC-QToF MS analysis.

Prior to the RRLC-QToF MS analysis, the frozen residues were thawed, dissolved in methanol (c = 1.00 mg/mL) and filtered (0.45 μm RC filter).

Solvents and reagents

Methanol (LC-MS, Chromasolv®, Fluka Analytical), acetonitrile (LiChrosolv®, hypergrade for LC-MS,

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Merck, Germany) and deionized water (18.2 MΩ cm^{-1}, Barnstead™ Smart2Pure™ Water Purification System, Thermo Scientific) were used for dissolution and preparation of the mobile phases for the LC-HRMS analyses. Ammonium formate (puriss. p.a., eluent additive for LC-MS, Fluka, USA) was used for the preparation of eluent additive for RRLC-QToF MS.

RRLC-QToF MS measurements

For untargeted analysis, the prepared samples were injected into LC/MS system including liquid chromatograph (1290 Infinity LC system; Agilent Technologies, Waldbronn, Germany), with a quaternary pump, a column oven, and an autosampler, connected to the Quadrupole Time-of-Flight mass detector (6550 iFunnel Q-TOF MS, Agilent Technologies; Santa Clara, CA, USA) equipped with a dual spray Agilent Jet Stream (AJS) electrospray ion source. The separation of compounds was performed using an Zorbax Eclipse XDB-C18 column RRHT (100 mm × 4.6 mm; 1.8 μm, Agilent Technologies). The mobile phase was composed of a solvents A (water) and B (ACN), both containing 5 mM ammonium formate under following gradient program: 0–2 min 10% B, 2–6 min 10–35% B, 6–10 min 35% B, 10–14 min 35–50% B, 14–26 min 50 – 60% B, 26–26.1 min 60–10% B, 26.1–30 min 10% B. The flow rate was 0.40 $mLmin^{-1}$, column temperature was 40 °C and injection volume of samples was 1 μL. The compounds were analysed using a mass detector operated in an accurate TOF/MS scanning (positive) ion mode in the *m/z* range of 100–1,000, under following conditions: capillary voltage, 3,500 V, fragmentor voltage, 70 V, nozzle voltage, 1,000 V, skimmer 1, 65 V, octopole RF peak, 750 V, desolvatation gas (nitrogen) temperature, 150 °C, desolvatation gas (nitrogen) flow, 14 $Lmin^{-1}$, nebulizer, 40 psig, sheat gas (nitrogen) temperature, 300 °C, sheat gas (nitrogen) flow, 11 $Lmin^{-1}$. Ions *m/z* 121.05087300 and 922.00979800 were used as lock masses for accurate mass measurements. A personal computer system running Agilent MassHunter software (revisions B.06.01 and B.07.00) was used for data acquisition and processing, respectively.

Results and discussion

In our previous study for secondary metabolites composition of *Amphoricarpos* sp. from Montenegro, 34 amphoricarpolides have been isolated and fully characterized (Cvetković et al., 2021; Djordjević et al., 2021). Five additional components have been detected and tentatively identified as sesquiterpene lactones in the BEM and DCM extracts of the plant species from genus *Amphoricarpos* from Montenegro by high performance liquid chromatography (HPLC) coupled with time-of-flight mass spectrometry (ToF MS) (Djordjević et al., 2021). However, this time, extraction of the raw data (d) using both, the find-by-molecular-feature (MFE) and find-by-formula algorithms (FBF) in Agilent MassHunter Qual. software (revision B.07.00) RRLC-QToF MS analysis, as more sensitive, allowed tentative identification of additional 18 and 24 sesquiterpene lactones in the same BEM and DCM extracts of the *Amphoricarpos* species, respectively. It can be assumed that these compounds were not isolated previously due to a low abundance and/or chemical instability.

Conclusion

RRLC-QToF MS analysis revealed the presence of undescribed sesquiterpene lactones in the BEM and DCM extracts of the plant species of genus *Amphoricarpos* from Montenegro. It should be examined whether and how this knowledge affects the metabonomic model based on the previous results of HPLC ToF MS analysis.

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