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Short communication

The reliability and limitation of UHPLC-HRMS in the sesquiterpene lactones dereplication: a case study of two Asteraceae species

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Introduction

Ultra-high-performance liquid chromatography coupled to hybrid quadrupole-Orbitrap high resolution mass spectrometry (UHPLC-HRMS) has been used as a powerful tool for metabolite profiling in phytochemistry. Sesquiterpene lactones (STLs) are specialized natural products that predominantly occur in the Asteraceae family. STLs are the bioactive principles of many medicinal plants from Asteraceae and due to their high structural variability in the family, are a very interesting class of compounds for chemosystematic/chemophenetic studies (Shulha and Zidorn, 2019). The increasing number of investigations indicate that SLs are still promising in the search for new therapeutic molecules, especially in the field of inflammation and cancer. However, the identification of known STLs in biological matrices is still a challenging work because of the large number of structures (more than 5000), isobars and isomers, and similar fragmentation pathways.

Telekia speciosa (Schreb.) Baumg and *Senecio hercynicus* Herborg are wide distributed on Vitosha Mountain, Bulgaria at 500-1600 m a.s.l. (Vladimirov, 2012). Sesquiterpene lactones of eudesmanolide, guaianolide, pseudoguaianolide and xanthanolide type together with acyclic-farnesane sesquiterpenoids and caryophyllanes have been previosusly isolated from the *T. speciosa* aerial parts. Moreover, *T. speciosa* roots are rich source of isoalantolactone and could be equivalent of *Inula helenium* L. roots (Stojakowska et al., 2015).

The genus *Senecio*, tribe Senecioneae, is the largest and most complex genus in the Asteraceae and includes more than 1500 species. *S. hercynicus* is one of the three species represented the *Senecio* nemorensis group in central Europe. Formerly, S. *hercynicus* was considered to be *Senecio nemorensis* L. var. *nemorensis* (*S. nemorensis* var. *subdecurrens* Grsb.) (Vladimirov, 2012).

This study focused on a reliability and limitation of UHPLC-HRMS in the sesquiterpene lactones dereplication of STLs composition of *T. speciosa* and *S. hercynicus* by UHPLC-HRMS. A strategy for STLs dereplication in methanol-aqueous extracts from the roots, aerial parts and flower heads of the studied species was developed.

Materials and methods

Plant material

The Plant material (roots, aerial parts and flower heads) of *T. speciosa* and *S. hercynicus* were collected on Vitosha Mt., “Zlatni mostove”, during the full flowering stage in July 2018. Voucher specimens were deposited at Herbarium of Bulgarian Academy of Sciences.

Sample extraction

Samples were extracted with 80% MeOH (1:20 w/v) by sonication (80 kHz) for 15 min (×2) at room temperature. Lyophilized crude extracts were used for further analyses.

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UHPLC-HRMS

Mass analyses were carried out on a Q Exactive Plus mass spectrometer (ThermoFisher Scientific, Inc.). Acquisition was acquired at Full-scan MS and Data Dependent-MS2 modes. Full-scan spectra over the m/z range 100 to 1 500 were acquired in negative ionization mode at a resolution of 70,000. Data acquisition and processing were carried out with Xcalibur 4.2 software and MZmine 2.51.

Chromatographic separation

Separation was achieved on a reversed phase column Kromasil EternityXT C18 (1.8 µm, 2.1 × 100 mm, AkzoNobel, Sweden) column maintained at 40°C. The separation was performed using mobile phase consisted of A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrileq and gradient elution.

Results and discussion

Dereplication represents a key step for rapidly identifying known specialized natural products in complex biological matrices. In this context, UHPLC-HRMS was used via untargeted data-dependent MS/MS experiments, and massive amounts of detailed information on the STLs composition of *T. speciosa* and *S. hercynicus* extract were generated.

The strategy for compounds recognition was based on the fragmentation rules and diagnostic ions for STLs, authentic standards and literature data. Key points in the peaks annotation and dereplication are (1) selection using chemophenetic information as a filter at the family, genus and species levels (in this case, *T. speciosa* and crosss-searched for *Senecio nemorensis*), (2) accurate masse in Full MS and dd MS2, (3) elemental composition, (4) MS/MS fragmentation patterns, (5) relative abundance of protonated and fragment ions, (6) comparison with the retention times, fragment spectra and chromatographic behavior of authentic standards. Positive ion mode was used as more informative for the analysis of STLs.

MS/MS fragmentation pathway of STLs, included characteristic ions corresponding to the neutral losses of H_2O (-18 Da), 2xH_2O (-36 Da), CO (-28 Da), CO_2 (-44 Da), CH_3COOH (-60 Da), as well as concomitant losses of H_2O+CO (-46 Da), 2H_2O+CO (-64 Da), H_2O+CO_2 (-62 Da).

Eight known sesquiterpene lactones, including 6 eudesmanolide (alantolactone/isoalantolactone; telekin/ isotelekin/ asperillin/ 3-*epi*-isotelekin), 1 guaianolides (2,3-dihydroaromaticin), and 1 heliangolide (8-*epi*-tomentosin) were tentatively annotated in *T. speciosa*. All compounds were recently described by Stojakowska et al., (2015).

A variety of STLs, including cacalolides (cacalol, dehydrocacalol), eremophilanolides (istanbulin A, B, C, D, E) and furanoeremophilanes (nemosenin A, B, C, D, senemorin) were tentatively identified in S. *hercynicus* lyophilized extracts. Above mention STLs were previously isolated from *S. nemorensis* L. (Yang et al., 2011). In addition, a fragmentation pathway of butyric/isobutiric, angelc/tiglic/senecionic and methylbutiric/valeric acid esters was established. MS/MS spectra of these type of STLs included loss of C_3H_8COOH (-88 Da), C_4H_8COOH (-100 Da) and $C_4H_{10}COOH$ (-102 Da), respectively. It is well known that pyrrolizidine alkaloids, eremophilanolides, and cacalolides are characteristic of the *Senecio* species. The presented work extended the contribution of STLs in the chemophenetic approach in *Senecio* genus.

Conclusion

The obtained strategy for the dereplication of STLs in *T. speciosa* and *S. hercynicus* extracts revealed the reliability and limitation of UHPLC-HRMS. A great number of different types of STLs were identified or tentatively elucidated in the assayed extracts.

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