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Short communication

Insight into *C, O* – glycosyl flavones of two Bulgarian *Gypsophila* species

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Introduction

The species of genus *Gypsophila* L. (Caryophyllaceae) are a very rich source of triterpenoid saponins (roots) and flavonoids (aerial parts), responsible for their cytotoxic, enzyme inhibitory and antioxidant effect (Gevrenova et al., 2014; 2018; Jakimiuk et al., 2021). However, some Bulgarian species of the genus needs further research. In Bulgarian flora, *Gypsophila trichotoma* Wend. and *G. tekirae* Stef. are presented as two separate species, and their conservation status is endangered and critically endangered species, respectively. In the European flora (Barkoudah et al., 1993) and The Plant List (www.theplantlist.org), taxa refer to *G. perfoliata* L. *sensu lato*. According to Flora Bulgaricae, *G. tekirae* was registered in one locality (the Rhodope foothills of Tekira, Pazardzhik region) (Valev, 1966). Although there are studies on the chemical composition of *G. trichotoma* and *G. tekirae* (Gevrenova et al., 2018, 2021; Zheleva-Dimitrova et al., 2018), the taxonomic position of both species is indistinct. The object of this study is an insight into the *C, O* – glycosyl flavones of *G. trichotoma* and *G. tekirae*. Thus, the chemotaxonomic examination will clarify and help into their taxonomic categorization and relationship.

Materials and methods

Plant material and sample extraction

Aerial parts of *G. tekirae* were collected at Ognyanovo village, Pazardzhik region, Bulgaria in July 2020. *G. trichotoma* aerial parts were collected at the Black Sea coast (Kavarna region), Bulgaria in August 2004.

Air-dried aerial parts were extracted with 80% MeOH (1:20 w/v) by sonication (100 kHz) for 15 min (×2) at room temperature. Subsequently, the extracts were concentrated in vacuo and lyophilized.

UHPLC-HRMS and spectrophotometric analyses

Mass analyses were carried out on a Q Exactive Plus mass spectrometer (ThermoFisher Scientific, Inc.) equipped with a heated electrospray ionization (HESI-II) probe (ThermoScientific). 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. For DD-MS2 mode, resolution was set to 17,500, isolation window 2.0 *m/z*, stepped collision energy (NCE) 20, 40, 70 eV. Data acquisition and processing were carried out with Xcalibur 4.2 software (ThermoScientific).

Chromatographic separation

Separation was achieved on a reversed phase column Kromasil EternityXT C18 (1.8 µm, 2.1 × 100 mm, AkzoNobel, Sweden) maintained at 40°C. The binary mobile phase consisted of A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile.
The following step gradient profile was used: 5 % B for 1.0 min, increased linearly to 25 % B in 14 min, held isocratic at 25 % B for 2.0 min, increased linearly to 50 % B in 1.0 min, held isocratic at 50 % B for 2.0 min, increased linearly to 95 % B in 2.0 min, held isocratic for

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2.0 min, finally brought back down to 5 % B over 0.5 min. The flow was 0.3 mL min–1. Data were processed with Xcalibur software ver. 3.0 (Thermo Scientific).

The total phenolic and flavonoid content was determined by classical spectrophotometric methods expressed as gallic acid (mgGAE/g extract) and rutin (mgRE/g extract), respectively.

Results and discussion

Based on the retention times, MS and MS/MS accurate measurements, fragmentation patterns and comparison with reference standards and literature data, 14 *C, O* – glycosyl flavones were tentatively identified/dereplicated. A variety of 2″-*O*-glycosyl-*C*-hexosyl-flavones was evidenced in *G. tekirae* including 2″-*O*-pentosyl/deoxyhexosyl/hexosyl-*C*-hexosyl-flavones. It should be noted that both *Gypsophila* species shared saponarin (6-*C*-7-*O*-diglucosyl-apigenin) and 2″-*O*-pentosyl-6-*C*-hexosyl-luteolin. The chromatographic profile of *G. trichotoma* was dominated by saponarin, while the main flavonoids in *G. tekirae* were 2″-*O*-deoxyhexosyl-6-*C*-hexosyl-apigenin and 2″-*O*-acetyldeoxyhexosyl-6-*C*-hexosyl-apigenin. Moreover, 2″-*O*-deoxyhexosyl-6-*C*-hexosyl-luteolin was found as well. The aforementioned compounds could be associated with 2"-*O*-rhamnosyl-isovitexin/homoorientin, previously isolated from *G. pacifica* (Jakimiuk et al., 2021). In addition, 2″-*O*-hexosyl-6-*C*-hexosyl-luteolin/apigenin were evidenced in *G. tekirae*; the former flavonoid was previously found in *G. perfoliata* (Zheleva-Dimitrova et al., 2018). *O*-hexosyl-(2″-*O*-pentosyl-6-*C*-hexosyl)-apigenin from *G. tekirae* was also annotated in *G. glomerata* and *G. paniculata* (Ferreres et al., 2007; Zheleva-Dimitrova et al., 2018). For the first time, three acetylated derivatives of 2″-*O*-deoxyhexosyl-6-*C*-hexosyl-flavones along with 2″-*O*-diacetylhexosyl-6-*C*-hexosyl-apigenin were tentatively identified in the genus.

The occurrence of 2″-*O*-glycosyl-*C*-hexosyl-flavones in *Gypsophila* species has chemophenetic significance, especially 2″-*O*-pentosyl/deoxyhexosyl/hexosyl-6-*C*-hexosyl-flavones (Ferreres et al., 2007; Zheleva-Dimitrova et al., 2018).

Commonly, the presence of acetylated *C, O*-glycosyl flavones is considered as a valuable chemophenetic marker. The literature survey of Jakimiuk et al. (2021) showed a general homogeneity of *C*- and *C, O* - flavonoid glycosides composition of aerial parts of Caryophyllaceae taxa.

The data revealed that the total phenolic and flavonoid content of *G. tekirae* was 27.29 mgGAE/g and 44.73 mgRE/g, respectively.

Conclusion

The identified *C, O* –glycosyl flavones favor the release of *G. tekirae* as a distinct species.

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References

Barkoudah, Y.I., Chater, A.O., Akeroyd, J.R., 1993. *Gypsophila* L., in: Tutin, T.G., Burges, N.A., Chater, A.O., Edmondson, J.R., Heywood, V.H., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), Flora Europaea 1, second ed. Cambridge University Press, Cambridge, pp. 219–222.

Ferreres, F., Gil-Izquierdo, A., Andrade, P.B., Valentão, P., Tomás-Barberán, F., 2007. Characterization of *C*-glycosyl flavones *O*-glycosylated by liquid chromatography–tandem mass spectrometry. J. Chromatogr., A 1161, 214–223. https://doi.org/10.1016/j.chroma.2007.05.103.

Gevrenova, R., Joubert, O., Mandova, Tsv, Zaiou, M., Chapleur, Y., Henry, M., 2014. Cytotoxic effects of four Caryophyllaceae species extracts on macrophages cell lines. Pharm. Biol. 52, 919–925. https://doi.org/10.3109/13880209.2013.868492.

Gevrenova, R., Bardarov, V., Bardarov, K., Voutquenne-Nazabadioko, L., Henry, M., 2018. Selective profiling of saponins from *Gypsophila trichotoma* Wend. by HILIC separation and HRMS detection. Phytochem. Anal. 29, 250-274. doi: 10.1002/pca.2739

Gevrenova R., Zengin G., Balabanova, V., Voynikov, Y., Zheleva-Dimitrova, D., 2021. *C, O* – flavonoid glycosides and oleanane-type bidesmosides from *Gypsophila perfoliata* L. "*tekirae*" (Caryophyllaceae): Chemophenetic implications. Biochem. System. Ecol. 99: 104353. https://doi.org/10.1016/j.bse.2021.104353.

Jakimiuk, K., Wink, M., Tomczyk, M., 2021. Flavonoids of the Caryophyllaceae. Phytochemistry Rev. https://doi.org/10.1007/s11101-021-09755-3.

Valev, St., 1966. Gypsophila L., in: Jordanov, D. (Ed.), Flora Republicae Popularis Bulgaricae. Vol III. Aedibus Academiae Scientiarum Bulgaricae, Sofia, p. 388.

Zheleva-Dimitrova, D., Zengin, G., Balabanova, V., Voynikov, Y., Lozanov, V., Lazarova, I., Gevrenova, R., 2018. Chemical characterization with *in vitro* biological activities of Gypsophila species. J. Pharmaceut. Biomed. Anal. 155, 56–69. https://doi.org/10.1016/j.jpba.2018.03.040.