Journal of Pharmaceutical and Biomedical Analysis 155 (2018) 56–69

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Chemical characterization with *in vitro* biological activities of *Gypsophila* species

Dimitrina Zheleva-Dimitrova[a,*], Gokhan Zengin[b], Vessela Balabanova[a], Yulian Voynikov[c], Valentin Lozanov[d], Irina Lazarova[c], Reneta Gevrenova[a]

[a] Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Sofia, Bulgaria
[b] Department of Biology, Faculty of Science, Selcuk University, Turkey
[c] Department of Chemistry, Faculty of Pharmacy, Medical University of Sofia, Bulgaria
[d] Department of Medical Chemistry and Biochemistry, Faculty of Medicine, Medical University of Sofia, Bulgaria

ARTICLE INFO

Article history:
Received 14 November 2017
Received in revised form 16 March 2018
Accepted 17 March 2018
Available online 21 March 2018

Keywords:
Gypsophila
Natural products
Flavonoids
Antioxidant
Enzyme inhibitor

ABSTRACT

Methanol-aqueous extracts from the aerial parts of *Gypsophila glomerata* (GGE), *G. trichotoma* (GTE) and *G. perfoliata* (GPE) were investigated for antioxidant potential using different *in vitro* models, as well as for phenolic and flavonoid contents. The possible anti-cholinesterase, anti-tyrosinase, anti-amylase and anti-glucosidase activities were also tested. The flavonoid variability was analyzed using ultra high-performance liquid chromatography (UHPLC) coupled with hybrid quadrupole-Orbitrap high resolution mass spectrometry (HRMS). Eleven *C*-glycosyl flavones and 4 *O*-glycosyl flavonoids, including 2"-*O*-pentosyl-6-*C*-hexosyl-apigenin/methylluteolin, as well as their mono(di)-acetyl derivatives were found in GGE. Both GGE and GTE shared 2"-pentosyl-6-*C*-hexosyl-luteolin together with the common saponarin, homoorientin, orientin, isovitexin and vitexin, while di *C*-glycosyl flavones were evidenced only in GPE.

The highest radical scavenging in both ABTS and DPPH assays was noted in GPE, as well as ferric and cupric reducing abilities. However, GTE had the strongest metal chelating activity (17.44 ± 0.51 mg EDTAE/g extract). GPE and GGE were more potent as acetylcholinesterases inhibitors witnessed by 2.09 ± 0.02 mg GALAE/g extract and 1.59 ± 0.09 mgGALAE/g extract, respectively. All flavonoids were found in *G. glomerata* for the first time. Therefore, further isolation and structural elucidation of newly described acetylated flavonoids are needed in order to determine their relevance in the beneficial properties of the plant.

1. Introduction

Gypsophila L. (Caryophyllaceae) species are highly valued and important medicinal herbs widely spread in Asia and Europe [1,2]. Mainland China is one of the most important centers of biodiversity of the genus. Native species in China have been known for their significant ethno-medicinal properties such as *G. oldhamiana*, *G. paniculata* and other localized species which have application in traditional Chinese medicine to treat fever, consumptive disease, and infantile malnutrition syndrome [3]. *G. oldhamiana* roots are recommended in the folk medicine for the treatment of diabetes [3], while *G. elegans* is used as a traditional medicine for immune disorders and liver diseases [4]. In Turkey both *G. bicolor* and *G. arrostii*, var. *nebulosa* roots are widely used as emulgators in the production of "tahini halvah" [5].

Indeed, triterpenoid saponins from *Gypsophila* roots are exploited for a variety of purposes including as medicines, detergents and adjuvants [1]. In the last few years our studies on *Gypsophila trichotoma* native to Bulgaria led to the isolation of a variety of triterpenoid saponin bidesmosides [6,7]. Their synergistic cytotoxicity in combination with type I ribosome-inactivating protein (RIP-I) was evaluated *in vitro* and used to derive a quantitative structure – activity relationship (QSAR) [8].

To facilitate effective resource utilization, aerial parts of several *Gypsophila* species including *G. elegans, G. trichotoma, G. pacifica,* have been investigated for flavonoids [9–11]. Recently, the hepatoprotective and antioxidant properties of *C*-flavonoids isoorientin-2"-*O*-α-L-arabinopyranosyl and isoorientin, isolated from *G. elegans*, were evidenced in alcohol- and carbon tetrachloride-induced injuries in rats [4,12]. It has been shown that saponarin isolated from *G. trichotoma* aerial parts is effective

* Corresponding author.
E-mail address: dzheleva@pharmfac.mu-sofia.bg (D. Zheleva-Dimitrova).

https://doi.org/10.1016/j.jpba.2018.03.040
0731-7085/

in attenuating hepatic damage in the paracetamol-, cocaine- and tetrachloride-induced *in vitro*/*in vivo* models [10,13,14]. Moreover, the potential of isoorientin-2"-*O*-α-L-arabinopyranosyl to induces apoptosis in liver cancer HepG2 cells via mitochondrial-mediated pathway has been reported [15]. In addition, phenylethanoid glycoside verbascosides was identified in *G. pilulifera* as the main free-radical scavenger present in this species [16]. However, there are only a few studies with respect to the antioxidant potential of *Gypsophila* species related to *C*-flavonoid glycosides [4,10,12]. Moreover, little data is available on the enzyme inhibitory activities of the genus *Gypsophila* [17].

Although a large number of *Gypsophila C*-flavonoids have been characterized, there are no reports concerning their profiling by LC-ESI/MS. Six *C*- and *O*-glycosylflavones from *G. paniculata* were analyzed by HPLC-DAD-ESI-IT MS and tentative assignment of their structures was proposed [18]. Therefore, it was of interest to extend our assay for the evaluation of antioxidant properties and enzyme inhibitory potential of *G. trichotoma* and *G. glomerata* native to Bulgaria, and *G. perfoliata* originating from Turkey.

Based on all above mentioned studies, we aimed at investigating the flavonoid variability in *G. trichotoma*, *G. glomerata* and *G. perfoliata* aerial parts using UHPLC-ESI/HRMS. In addition, our objective was to evaluate their antioxidant potential using different *in vitro* models and to test the possible enzyme inhibitory activities linked to Alzheimer's disease and diabetes mellitus.

2. Materials and methods

2.1. Plant material

G. trichotoma Wend. aerial parts were collected in August 2004 at the Black Sea coast (Kavarna region) (43°25′ N–28°19′ E) in Bulgaria, while *G. glomerata* aerial parts were collected in September, 2010 at Ognyanovo village (Pazardjik region) (42°15′ N–24°42′ E). The plants were identified by Dr. R. Gevrenova (Faculty of Pharmacy, Medical University-Sofia, Bulgaria). Voucher specimen of *G. trichotoma* was deposited in the Herbarium of the Faculty of Pharmacy of Nancy, Universite de Lorraine, France (HP101), while voucher specimen of *G. glomerata* was deposited at Institute of Biodiversity and Ecosystems Research, Bulgarian Academy of Sciences, Sofia, Bulgaria (SOM 171499). *G. perfoliata* var. *perfoliata* was collected from Kayseri-Turkey (Ali Mount) during of flowering season (June 2015). Taxonomic identification of the plant material was confirmed by the senior taxonomist Dr. Murad Aydin Sanda, from the Department of Biology, Selcuk University [19,20]. The voucher specimen was deposited at the Herbarium of the Department of Biology, Selcuk University, Konya-Turkey (GZ 1513).

Air-dried powdered aerial parts were extracted with 80% methanol (1:25 w/o) (×3) by sonication for 15 min at room temperature to yield the crude extracts (*G. glomerata*, GGE; *G. trichotoma*, GTE; *G. perfoliata*, GPE).

2.2. Chemicals

The standards of orientin, homoorientin, vitexin, isovitexin, kaempferol-3-glucoside (astragalin), apigenin, luteolin, diosmetin and kaempferol (≥99% HPLC purity), saponarin and kaempferol-3-rutinoside (≥98% HPLC purity), isorhamnetin 3-glucoside (≥95% HPLC purity) were provided from Extrasynthese (Genay, France). 6, 8-diC-glucosyl-apigenin (Vicenin-2) (95% HPLC) was isolated in our laboratory [21]. The stock standard solutions of appropriate concentration were prepared in methanol and were stored at 4 °C in the dark. Acetonitrile (hypergrade for LC–MS), formic acid (HPLC-grade) and methanol (analytical grade) were purchased from Merck (Darmstade, Germany).

2.3. UHPLC-ESI/HRMS

The LC–MS analyses were performed on a Q Exactive Plus heated electrospray ionization (HESI-II) – high resolution mass spectrometer (HRMS) (ThermoFisher Scientific, Inc., Bremen, Germany) equipped with an ultra high-performance liquid chromatography (UHPLC) system Dionex Ultimate 3000RSLC (ThermoFisher Scientific, Inc.).

Operating conditions for the HESI source used in a negative ionization mode were: −2.5 kV spray voltage, 320 °C capillary temperature, 300 °C probe heater temperature, sheath gas flow rate 38 units, auxiliary gas flow 12 units (units refer to arbitrary values set by the Exactive Tune software) and S-Lens RF level 50.00. Nitrogen was used for sample nebulization and collision gas in HCD cell. The LC–MS method was operating in Full scan-dd MS^2/Top 5 with the following settings: 70000 FWHM resolution (at *m*/*z* 200), AGC target 1e6, max. IT 50 ms and mass range 100–1000 *m*/*z*, while ddMS^2 conditions were set to resolution 17500 FWHM (at *m*/*z* 200), AGC target 1e3, max. IT 50 ms, isolation window 2.0 *m*/*z* and normalased collision energy (NCE) 30. The UHPLC separations were performed on a Kromasil EternityXT C18, 1.8 μm, 2.1 × 100 mm (AkzoNobel, Sweeden) with a binary mobile phase consisting of solution A: 0.1% HCOOH and solution B: MeCN (0.1% HCOOH). The following step gradient profile was used: 5% B for 1.0 min, increasing up to 25% B in 14 min, held isocratic at 25% B for 2.0 min, increasing up to 50% B in 1.0 min, held isocratic at 50% B for 2.0 min, increasing up to 95% B in 2.0 min, held isocratic for 2.0 min finally brought back down to 5% B over 0.5 min [22]. Equilibration time was 4.5 min, the flow rate was 0.3 mL/min. The column compartment temperature was set at 40 °C. Data were processed with Xcalibur software ver. 3.0. The calculation of the exact masses and mass measurement errors, prediction of molecular formulas and simulation of monoisotopic profiles were carried out with Xcalibur 3.0 software (ThermoScientific).

2.4. Total phenolics and flavonoids content

The total phenolics content was determined by the Folin-Ciocalteu method [23] with slight modification and expressed as gallic acid equivalents (GAE/g extract), while total flavonoids content was determined using $AlCl_3$ method [24] with slight modification and expressed as rutin equivalents (RE/g extract).

2.5. Biological activities evaluation

Antioxidant (DPPH, ABTS radical scavenging, reducing power (CUPRAC and FRAP), phosphomolybdenum and metal chelating (ferrozine method) and enzyme inhibitory assays (cholinesterase (Elmann's method), tyrosinase (dopachrome method), α-amylase (iodine/potassium iodide method) and α-glucosidase (chromogenic PNPG method) were reported in our previous papers [24,25]. Antioxidant capacities were expressed as equivalents of Trolox and EDTA (for metal chelating). The enzyme inhibitory activities were evaluated as equivalents of standard inhibitors per gram dry extract (galantamine for AChE and BChE, kojic acid for tyrosinase and acarbose for α-amylase and α-glucosidase inhibition assays).

3. Results and discussion

3.1. UHPLC-ESI/HRMS analysis of G. glomerata, G. trichotoma and G. perfoliata extracts

The methanol extracts from G. *glomerata* (GGE), *G. trichotoma* (GTE) and *G. perfoliata* (GPE) were analyzed by UHPLC gradient elution with Orbitrap-HRMS detection for the first time. According