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Pharmacochemistry

Biologically Active Sugar-Based Biopolyether Poly[3-(3,4-Dihydroxyphenyl)Glyceric Acid] from the Stems and Roots of *Paracynoglossum imeretinum* (Kusn.) M.Pop

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The high-molecular water-soluble mucilage fractions from stems and roots of Paracynoglossum imeretinum (Boraginaceae family) were isolated. According to data of UV, IR, liquid-state 1H, 13C NMR, gCOSY and 2D heteronuclear $^{1}H/^{13}C$ gHSQCED experiments, the main chemical constituent of these water-soluble high-molecular fractions from *Paracynoglossum imeretinum* stems (HMF-PS) and roots (HMF-PR), respectively, was found to be biologically active caffeic acid-derived polymer poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene], which is also referred to as poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA). PDPGA was previously detected in high-molecular mucilage fractions of *Symphytum asperum, S. caucasicum, S. grandiflorum, S. officinale, Anchusa italica, Cynoglossum officinale* and *Borago officinalis.* The detection of this compound in different genera of the Boraginaceae family is interesting, as this unusual caffeic acid-derived polymer could be considered as a chemotaxonomic marker among Boraginaceae plants. Thus, PDPGA is interesting due to the importance of its chemotaxonomic significance, the potential biomedical applications of the Boraginaceae plants and the chemical importance of PDPGA. The presence of poly[3-(3,4-dihydroxyphenyl)glyceric acid] in multiple Boraginaceae species expands the resources of raw materials for this biologically active polymer. *© 2022 Bull. Georg. Natl. Acad. Sci.*

sugar-based biopolyether, caffeic acid-derived polymer, poly[3-(3,4-dihydroxyphenyl)glyceric acid], poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene], Boraginaceae, *Paracynoglossum imeretinum*

Ether bonds are found in a wide variety of natural products – mainly secondary metabolites – including lipids, oxiranes, terpenoids, flavonoids, polyketides, and carbohydrate derivatives or aromatic polymer, such as lignin. Lignin contains ether links between two aromatic rings or between an aromatic

ring and an aliphatic moiety [1,2]. However, reports concerning biopolymers that contain aliphatic ethers as repeating unit were sparse.

In our previous publications we reported that the main chemical constituent of high-molecular water-soluble mucilage preparations from *Symphytum asperum, S.caucasicum, S.officinale, S. grandiflorum, Anchusa italica, Cynoglossum officinale* and *Borago officinalis* (Boraginaceae) was found to be a biologically active caffeic acid-derived polymer poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)-ethylene], which is also referred to as poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA) [3-8]**.** The polyoxyethylene chain is the backbone of this polymeric molecule. Dihydroxyphenyl and carboxyl groups are regular substituents at two carbon atoms in the chain. The hydroxyl groups in positions 3 and 4 of the phenyl ring were unambiguously established. This compound is a representative of a class of natural polyethers with a residue of 3-(3,4-dihydroxyphenyl)glyceric acid as the repeating unit (Fig.).

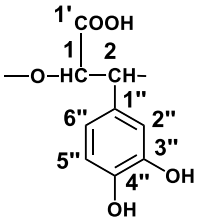

Fig. The repeating unit of PDPGA.

Within the field of pharmacologically active biopolymers the area of stable polyethers seems rather new and attractive. On the other hand **PDPGA** as a 3,4-dihydroxyphenyl derivative of poly(2,3-glyceric acid ether) also belongs to a rare class of carbohydrate-based biopolymers, namely poly(sugar acids). Its basic monomeric moiety, glyceric acid, is a natural three-carbon sugar acid, which is an oxidative form of the simplest of all common aldoses, namely glyceraldehyde. In this case, poly(2,3-glyceric acid ether) chain constitutes the backbone of this polymeric molecule with 3,4-dihydroxyphenyl groups as regular substituents at the 3C positions in the chain. Every repeating structural unit of **PDPGA** contains three reactive functional groups, two *ortho*-related phenolic hydroxyl groups and one carboxyl group. Consequently, the polymeric molecule of **PDPGA** bears many of these functional groups along the polymeric chain. This multifunctionality justifies that **PDPGA** belongs to several important classes of biopolymers. Moreover, multifunctionality should be a reason of its wide spectrum of biological activities, as **PDPGA** is endowed with intriguing *in vitro* and *in vivo* pharmacological properties: anti-inflammatory, antioxidant, anti-complementary and anticancer [9-11]. Medicinal effects of *Symphytum*, *Anchusa*, *Cynoglossum* and *Borago* plants used in folk medicine could be attributed to this polymer, rendering **PDPGA** a unique candidate for many biomedical applications.

Only few papers have been published concerning chemical composition of Georgian endemic *Paracynoglossum imeretinum* [12,13], namely it was documented to synthesis of pyrrolizidine alkaloids heliosupine, echinatine [14] and allantoin [15].

Within our ongoing search for biologically active caffeic acid-derived polymers in plant species belonging to different genera of the Boraginaceae family, we have carried out the isolation and structure elucidation of a main chemical constituent of water-soluble high-molecular mucilage fractions (Mr>500 kDa) from *P.imeretinum* stems (**HMF-PS)** and roots (**HMF-PR)**.

Results and Discussion

In our previous studies we found that the mucilage crude polysaccharides obtained from *Symphytum*, *Anchusa*, *Cynoglossum* and *Borago* plants essentially consist of mixtures of biologically active **PDPGA** and biologically inactive polysaccharides [3-8]. **HMF-PS** and **HMF-PR** were isolated from water mucilage extracts of *P.imeretinum* by means of ultrafiltration using

membrane filter with a cut-off value of 500 kDa, as described in some earlier publications [3-8]. During the ultrafiltration process to monitor the removal of biologically inactive ballast polysaccharides, we detected sugars in ultrafiltrates by phenol-sulfuric acid reaction [16].

The UV spectra (see materials and methods) of **HMF-PS** and **HMF-PR** showed absorption maxima at 212, 282 (shoulder), 286 nm identical to the UV spectrum of polyether **PDPGA** [3-8]. The IR spectra of **HMF-PS** and **HMF-PR** showed absorption bands characteristic of phenols and carboxylic acids. The IR spectra of **HMF-PS** and **HMF-PR** were also very similar to that of phenolic polyether **PDPGA** [3-8]. Thus, the presence of **PDPGA** in **HMF-PS** and **HMF-PR** was supposed on the basis of its UV and IR spectral data.

Then we tried to identify **PDPGA** in **HMF-PS** and **HMF-PR** using different techniques of NMR spectroscopy, namely liquid-state ^{1}H and ^{13}C NMR, gCOSY and 2D heteronuclear $^{1}H/^{13}C$ gHSQCED. The assignments of the complete set of resonances signals for **PDPGA** in the ^{13}C NMR and ^{1}H NMR spectra, based on correlations between protons and carbon atoms by means of the 2D $^{1}H/^{13}C$ gHSQCED spectra, was carried out as described in the previous papers [3-8] and are listed in Table.

Table. The signal assignment in the ^{13}C and ^{1}H NMR spectra of PDPGA from *P.imeretinum*

C atom no.	^{13}C chemical shift, δ_C, ppm	^{1}H chemical shift, δ_H, ppm
1'	175.0	
1	77.5	5.7
2	79.6	5.1
1"	130.7	
2"	116.6	7.6
3"	143.9	
4"	143.0	
5"	117.8	7.5
6"	121.5	7.4

The gCOSY spectrum showed a cross peak between the signals at 5.7 and 5.1 ppm, which was consistent with the coupling between H-1 and H-2 of **PDPGA** (Table 1, Fig. 1).

Thus, according to different techniques of NMR spectroscopy we found that the main chemical constituent of **HMF-PS** and **HMF-PR** is **PDPGA.**

Conclusion

Thus, according to data of UV, IR and different techniques of NMR spectroscopy, the main chemical component of water-soluble high-molecular mucilage preparations from *P.imeretinum* stems (**HMF-PS)** and roots (**HMF-PR)** similarly to *Symphytum*, *Anchusa*, *Cynoglossum* and *Borago* plants, was found to be a representative of regular carbohydrate-based biopolyethers, namely poly[3-(3,4-dihydroxyphenyl)glyceric acid] (**PDPGA**) (Fig. 1) [3-8]. The detection of **PDPGA** in the genus *Paracynoglossum* shows that its biosynthesis is a unique feature not only for the following genera *Symphytum*, *Ancusa*, *Cynoglossum* and *Borago*, but the *Paracynoglossum* (Boraginaceae) genus as well. The presence of **PDPGA** in different genera of Boraginaceae family would be interesting from the chemotaxonomic point of view. This unusual caffeic acid-derived polymer could be a chemotaxonomic marker among Boraginaceae plants. The biosynthetic pathway responsible for this compound might also be unique for different genera of Boraginaceae plants. Further detection of **PDPGA** amongst other members of the Boraginaceae family is interesting due to the importance of the chemotaxonomic significance, the potential biomedical applications of the Boraginaceae plants and the chemical importance of **PDPGA**. Thus, the results of this study support the previous research that **PDPGA** can be used as a chemotaxonomic marker. The presence of poly[3-(3,4-dihydroxyphenyl)glyceric acid] in multiple Boraginaceae species expands the resources of raw materials for this biologically active polymer.

Materials and Methods

Apparatus: The UV spectra were recorded on a UV/VIS spectrophotometer (Mecasys Optizen Pop, Mecasys Co., Ltd., Daejeon, Korea). The IR spectra in KBr disc was obtained on a FT-IR spectrophotometer (Jasco, FT/IR-4600, Tokyo, Japan). NMR spectra were taken on a Bruker Avance III 400 spectrometer for 1% solutions in D_2O at 80°C using acetone-d_6 (δ_H 2.69 ppm, δ_C 30.64 ppm vs. Me_4Si) as the internal standard. All NMR spectra were processed with the MestReNOVA software (version 14.2.1, Mestrelab Research, S. L., Santiago de Compostela, Spain). The ultrafiltration fractionation procedure was carried out in a stirred ultrafiltration cell (model 8200, Millipore Corporation, Billerica, MA, USA), fitted with a Biomax-500 ultrafiltration disc (500 000 NMWL).

Plant material: *P.imeretinum* was collected on 14 August of 2016. A voucher specimen (TBPH №1551) was deposited at Tbilisi State Medical University, I. Kutateladze Institute of Pharmacochemistry.

Extraction and isolation: 33.25 g and 40.06 g of air-dried and ground *P. imeretinum* stems and roots, respectively, were preliminary pretreated in a Soxhlet apparatus with chloroform, methanol and acetone, sequentially, and afforded 29.68 g (89.3%) and 37.25 g (92.98%) stems and roots, respectively. Quadruple hot water extraction for 15.45 g and 15.04 g of preliminary pretreated of stems and roots, respectively, afforded 800 ml each of mucilage water extracts which directly subjected to ultrafiltration and subsequently freeze-dry.Yields of **HMF-PS and HMF-PR** were 0.41 g (2.37%) and 0.8 g (4.9%) based on air-dried biomass.

UV spectrum of **HMF-PS** and **HMF-PR** (H_2O, λ_{max}, nm): 212, 282 (shoulder), 286.

IR spectrum of **HMF-PS** and **HMF-PR** (KBr, υ, cm^{-1}): 3416 (OH); 2929,82 (CH); 1617,98 (ionized carboxyl); 1508 and 1448 (aromatic C=C); 1417,42 and 1218,79 (phenols); 1267,97, 1125,74 and 1075 (R–O–R'); 871,908 (C–H in the aromatic ring with one isolated hydrogen atom); and 819,357 (C–Hin the aromatic ring with two neighboring hydrogen atoms).

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ფარმაკოქიმია

Paracynoglossum imeretinum (Kusn.) M.Pop-ის ღეროებისა და ფესვების ბიოლოგიურად აქტიური შაქარზე დაფუძნებული ბიო-პოლიეთერი პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავა]

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**სან-დიეგოს სახელმწიფო უნივერსიტეტი, თბილისი, საქართველო
§ბმრ სპექტროსკოპიის ცენტრი – ალკალას უნივერსიტეტის ფარმაცევტული ფაკულტეტი, მადრიდი-ბარსელონა ალკალა დე ჰენარესი, მადრიდი, ესპანეთი
#აკადემიის წევრი, ივანე ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, ფიზიკური და ანალიზური ქიმიის ინსტიტუტი, ზუსტი და საბუნებისმეტყველო მეცნიერებათა ფაკულტეტი, თბილისი, საქართველო

შრომაში დადგენილია, რომ *Paracynoglossum imeretinum*-ის (Boraginaceae) ღეროების და ფესვების წყალში ხსნადი მაღალმოლეკულური პრეპარატების ძირითადი კომპონენტია ბიოლოგიურად აქტიური კოფეინის მჟავას წარმოებულის პოლიმერი, კერძოდ, პოლი[ოქსი-1-კარბოქსი-2-[3,4-დიჰიდროქსიფენილ)ეთილენი], ანუ პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავა] (პდფგმ). აღნიშნული ბიოპოლიმერის სტრუქტურა დადგენილია უი, იწ, ^{1}H და ^{13}C ბმრ, gCOSY და 2D ჰეტერობირთვული ^{1}H/^{13}C gHSQCED ექსპერიმენტების მონაცემების საფუძველზე. ადრე ჩატარებული კვლევების მიხედვით *Symphytum asperum, S. caucasicum, S. officinale, S. grandiflorum, Anchusa italica, Cynoglossum officinale* და *Borago officinalis*-ის (Boraginaceae) მაღალმოლეკულური ფრაქციების ძირითადი კომპონენტი აგრეთვე არის პდფგმ. მისი დეტექტირება Boraginaceae-ს ოჯახის სხვადასხვა გვარში საინტერესოა ქემოტაქსონომიური თვალსაზრისით. კოფეინის მჟავას წარმოებულის უჩვეულო პოლიმერი შეიძლება იყოს Boraginaceae-ს მცენარეების ქემოტაქსონომიური მარკერი. ამგვარად, პდფგმ საინტერესოა ქიმიური და ქემოტაქსონომიური თვალსაზრისით, ხოლო Boraginaceae-ს მცენარეები მათი პოტენციური ბიოსამედიცინო გამოყენების მნიშვნელობის გამო. პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავას] არსებობა Boraginaceae-ს მრავალ სახეობაში აფართოებს ამ ბიოლოგიურად აქტიური პოლიმერის ნედლეულის რესურსების სიას.

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