# Biologically Active Poly[3-(3,4-Dihydroxyphenyl)Glyceric Acid] from *Borago officinalis* (Boraginaceae)

**T.Kekeishvili, M.Merlani, L.Amiranashvili, L.Gogilashvili, V.Barbakadze**

*Tbilisi State Medical University I. Kutateladze Institute of Pharmacochemistry, 36 P. Sarajishvili str., 0159 Tbilisi, Georgia, E-mail: v.barbakadze@tsmu.edu*

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ABSTRACT. A high-molecular water-soluble preparation from stems of *Borago officinalis* (Boraginaceae family) was isolated. According to data from UV, IR, $^{1}H$, $^{13}C$ NMR, gCOSY and 2D heteronuclear $^{1}H/^{13}C$ gHSQCED experiments, the main chemical constituent of this water-soluble high-molecular preparation from stems of *Borago officinalis* (HMP-BS) was found to be a biologically active caffeic acid-derived polymer, namely poly[oxy-1-carboxy-2-(3,4-dihydroxy-phenyl)ethylene] also referred to as poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA). PDPGA was previously detected in high-molecular preparations of *Symphytum asperum, S. caucasicum, S. officinale, Anchusa italica* and *Cynoglossum officinale*. The detection of this compound in different genera of the Boraginaceae family is interesting as this unusual caffeic acid-derived polymer could be consider 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.

***Key words:*** *Caffeic acid-derived polyether, poly[3-(3,4-dihydroxyphenyl)glyceric acid], poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene], Borago officinalis, Boraginaceae.*

## Introduction

In previous publications we reported that the main chemical constituent of high molecular water-soluble preparations from *Symphytum asperum, S. caucasicum, S. officinale, S. grandiflorum, Anchusa italica* and *Cynoglossum officinale* (Boraginaceae) was biologically active caffeic acid-derived polymer, namely poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene] or poly[3-(3,4-dihydroxyphenyl)glyceric acid] (**PDPGA**) [1-5]. The polyoxyethylene chain is the backbone of this polymer molecule with 3-(3,4-dihydroxyphenyl)glyceric acid residue as the repeating unit (Fig. 1). This compound represents a class of natural polyethers. Within the field of pharmacologically active biopolymers the area of stable polyethers seems rather new and attractive. **PDPGA** as a unique natural polyether contains aliphatic ether groups in its polymer backbone. **PDPGA** is endowed with intriguing *in vitro* and *in vivo* pharmacological properties: anti-inflammatory, antioxidant, anticomplementary and anticancer [6-8].

## Results and Discussion

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 preparation from *Borago officinalis* stems **(HMP-BS)**. The high-molecular (>500 kDa) preparation **HMP-BS** was isolated from the crude polysaccharides by means of ultrafiltration using membrane filters with a cut-off value of 500 kDa, as described in some of our earlier publications [1-5].

The UV spectrum (see materials and methods) of **HMP-BS** showed absorption maxima at 214, 286 nm identical to the UV spectrum of polyether **PDPGA** [1-5]. The IR spectrum of **HMP-BS** showed some absorption bands characteristic of phenols and carboxylic acids. The IR spectrum of **HMP-BS** was also very similar to that of phenolic polyether **PDPGA** [1-5]. Thus, the presence of **PDPGA** in **HMP-BS** was supposed on the basis of its UV and IR spectral data.

Then we tried to identify **PDPGA** in **HMP-BS** by using different techniques of NMR spectroscopy, namely $^{1}H$ and $^{13}C$ NMR, gCOSY and 2D heteronuclear $^{1}H/^{13}C$ gHSQCED. The assignment 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 [1-5] and are listed in TABLE 1.

TABLE 1. The signal assignment in the $^{13}C$ and $^{1}H$ NMR spectra of **PDPGA** from *B.officinalis* stems.

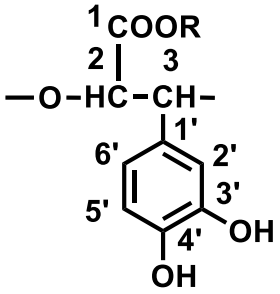
| | C atom no. | $^{13}C$ chemical shift, $\delta_C$, ppm | $^{1}H$ chemical shift, $\delta_H$, ppm |
|---|---|---|---|
|  | | 175.00 (C$OO^-$) | |
| | 1 | 172.00 (C$OOCH_3$) | |
| | | 53.45 ($OCH_3$) | 3.8($OCH_3$) |
| | 2 | 77.48 | 5.16 |
| | 3 | 79.56 | 4.67 |
| | 1' | 130.71 | |
| | 2' | 116.61 | 7.16 |
| | 3' | 143.89 | |
| | 4' | 143.00 | |
| | 5' | 117.82 | 7.06 |
| | 6' | 121.48 | 7.06 |

Fig. 1. The repeating unit of PDPGA; R=H, $CH_3$.

The gCOSY spectrum showed a cross peak between the signals at 5.16 and 4.67 ppm, which was consistent with the coupling between H-2 and H-3 of **PDPGA** (TABLE 1, Fig. 1).

Thus, we found that the main chemical constituent of **HMP-BS** was **PDPGA.**

## Conclusion

Thus, according to UV, IR and NMR data, the main chemical component of water-soluble high-molecular preparation from *B.officinalis* stems **(HMP-BS)**, similarly to *Symphytum, Anchusa* and *Cynoglossum* plants, was found to be a representative of regular carbohydrate-based biopolyethers, namely poly[3-(3,4-dihydroxyphenyl)glyceric acid] (**PDPGA**) (Fig. 1) [1-5]. Similarly to *A. italica* and *S. grandiflorum* biopolymers, most of the carboxylic groups of this caffeic acid-derived polymer of *B. officinalis* are methylated. The detection of **PDPGA** in the genus *Borago* shows that its biosynthesis is a unique feature not only for the following genera *Symphytum, Ancusa and Cynoglossum,* but the *Borago* (Boraginaceae) genus as well. 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 among Boraginaceae plants. The presence of poly[3-(3,4-dihydroxy-phenyl)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 spectrum in KBr disc was obtained on a FT-IR spectrophotometer (Jasco, FT/IR-4600, Tokyo, Japan). All NMR spectra of 1% solutions in $D_2O$ at 80°C and with acetone-$d_6$ were recorded in a Varian NMR System 500 MHz (Palo Alto, CA, USA). 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**: *B. officinalis* was cultivated near Tbilisi, Georgia. Fresh stems of *B. officinalis* were collected in June of 2019. A voucher specimen (TBPH №19568) was deposited at the Tbilisi State Medical University I. Kutateladze Institute of Pharmacochemistry.

**Extraction and isolation:** 33.5 g of air-dried and ground stems of *B. officinalis* were preliminary pretreated in a Soxhlet apparatus with chloroform and methanol, sequentially. Hot water extraction of preliminary pretreated 24.4 g of stems of *B. officinalis* followed by dialysis [1-5] afforded 6 g of crude polysaccharides of *B. officinalis* stems **(CP-BS)**. Yield of **CP-BS** was 17.9 %, based on air-dried biomass (6/33.5g). Further fractionation procedure of 0.794 g of **CP-BS** by ultrafiltration on a membrane filter with a cut off value of 500 kDa [1-5] afforded 0.087 g of high-molecular (>500 kDa) preparation of *B. officinalis* stems **HMP-BS**. Yield of **HMP-BS** based on

crude polysaccharides (0.087/0.794g) was 11 %. Ultrafiltration of 6 g **CP-BS** afforded 0.66 g **HMP-BS.** Yield of **HMP-BS** based on air-dried biomass (0.66/33.5 g) was 2 %.

UV spectrum of **HMP-BS** ($H_2O$, $\lambda_{max}$, nm): 214, 236 (shoulder), 282 (shoulder), 286.

IR spectrum of **HMP-BS** (KBr, υ, $cm^{-1}$): 3553.7, 3477.0, 3414.8 (st OH); 2923 (CH); 1740 (–$COOCH_3$); 1636.8 (–COOH); 1618.5, 1508.5, 1440 (aromatic C=C); 1422.7, 1013 (phenols); 1266.5, 1147, 1099 (R-O-R'); 894 (C-H in the aromatic ring with one isolated hydrogen atom); 821.5 (C-H in the aromatic ring with two neighboring hydrogen atoms).

## *Borago officinalis*-is (Boraginaceae) ბიოლოგიურად აქტიური პოლი[3-(3,4-დიჰიდროქსიფენილ)გლიცერინის მჟავა]

**თ. კეკეიშვილი, მ. მერლანი, ლ. ამირანაშვილი ლ. გოგილაშვილი ვ. ბარბაქაძე**

*თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ი.ქუთათელაძის ფარმაკოქიმიის ინსტიტუტი, 36 პ.სარაჯიშვილის ქ., 0159 თბილისი, საქართველო*

UV, IR, $^1H$, და $^{13}C$ NMR, gCOSY და 2D ჰეტერობირთვული $^1H/^{13}C$ gHSQCED ექსპერიმენტების მონაცემების საფუძველზე დადგენილი იქნა, რომ *Symphytum asperum, S. caucasicum, S. officinale*, *S. grandiflorum, Anchusa italica* და *Cynoglossum officinale*-ის (Boraginaceae) მაღალმოლეკულური ფრაქციების მსგავსად *Borago officinalis*-ის ღეროების წყალში ხსნადი მაღალმოლეკულური პრეპარატის (MMP-BO) 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). პდფგმ-ის 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-s 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.

## REFERENCES

1. Barbakadze V., Kemertelidze E., Targamadze I., Mulkijanyan K., Shashkov A.S., Usov A. I. (2005) Poly[3-(3,4-dihydroxyphenyl)glyceric acid], a new biologically active polymer from *Symphytum asperum* Lepech. and *S.caucasicum* Bieb. (Boraginaceae). *Molecules,* **10**, 9: 1135-1144. https://doi.org/10.3390/10091135

2. Barbakadze V., van den Berg A.J.J., Beukelman C.J., Kemmink J., Quarles van Ufford H.C. (2009) Poly[3-(3,4-dihydroxyphenyl)glyceric acid] from *Symphytum officinale* roots and its biological activity. *Chem. Nat. Compds.,* **45**, 1: 6-10. https://doi.org/10.1007/s10600-009-9221-5

3. Barbakadze V., Gogilashvili L., Amiranashvili L., Merlani M., Mulkijanyan K., Churadze M., Salgado A., Chankvetadze B. (2010) Poly[3-(3,4-dihydroxyphenyl)glyceric acid] from *Anchusa italica* roots. *Nat. Prod. Commun.,* **5**, 7: 1091-1095. https://doi.org/10.1177/1934578X1000500722
4. Gokadze S., Gogilashvili L., Amiranashvili L., Barbakadze V., Merlani M., Bakuridze A., Salgado A., Chankvetadze B. (2017) Investigation of water-soluble high molecular preparation of *Symphytum grandiflorum* DC (Boraginaceae). *Bull. Georg. Natl. Acad. Sci.,* **11**, 1:115-121. http://science.org.ge/bnas/vol-11-2.html
5. Gogilashvili L., Amiranashvili L., Merlani M., Salgado A., Chankvetadze B., Barbakadze V. (2020) Poly[3-(3,4-Dihydroxyphenyl)Glyceric Acid] from *Cynoglossum officinale* L. (Boraginaceae). *Bull. Georg. Natl. Acad. Sci.,* **14**, 1: 108-113. http://science.org.ge/bnas/vol-14-1.html
6. Barthomeuf C.M., Debiton E., Barbakadze V., Kemertelidze E.P. (2001) Evaluation of the dietetic and therapeutic potential of a high molecular weight hydroxicinnamate-derived polymer from *Symphytum asperum* Lepech. Regarding its antioxidant, antilipoperoxidant, antiinflammatory, and cytotoxic properties. *J. Agric. Food Chem.*, **49**, 8: 3942-3946. https://doi.org/10.1021/jf010189d
7. Barbakadze V., Kemertelidze E.P., Mulkijanyan K.G., van den Berg A.J.J., Beukelman C.J., van den Worm E., Quarles van Ufford H.C., Usov A.I. (2007) Poly[3-(3,4-dihydroxyphenyl)glyceric acid] from *Symphytum officinale* roots and its biological activity. *Pharm. Chem. J.*, **41,** 1: 14-16. http://dx.doi.org/10.1007/s11094-007-0040-3
8. Shrotriya S., Deep G., Ramasamy K., Raina K., Barbakadze V., Merlani M., Gogilashvili L., Amiranashvili L., Mulkijanyan K., Papadopoulos K., Agarwal C., Agarwal R. (2012) Poly[3-(3, 4-dihydroxyphenyl) glyceric] acid from comfrey exerts anti-cancer efficacy against human prostate cancer via targeting androgen receptor, cell cycle arrest and apoptosis. *Carcinogenesis,* **33**, 8: 1572–1580. https://doi.org/10.1093/carcin/bgs202