საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 11, №2, 2017
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 11, no. 2, 2017

Pharmacochemistry

Antioxidant Activity of Caffeic Acid-Derived Polymer from *Anchusa italica*

Maia Merlani*, Vakhtang Barbakadze*, Lali Gogilashvili*, Lela Amiranashvili*

** I.Kutateladze Institute of Pharmacochemistry, Tbilisi State Medical University, Tbilisi, Georgia*

(Presented by Academy Member E. Kemertelidze)

ABSTRACT. The main chemical constituent of high-molecular water-soluble preparations from *Symphytum asperum, S. caucasicum, S.officinale* and *Anchusa italica* was found to be a caffeic acid-derived polyether, namely poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA). In contrast to *Symphytum* polymer, most of the carboxylic groups of polymer from *A. italica* are methylated. PDPGA of different species of *Symphytum* revealed pronounced anticomplementary, antioxidant, anti-inflammatory and wound healing activities. Comparative study of antioxidant activity of PDPGA from *A. italica* with its synthetic monomer racemic 3-(3,4-dihydroxyphenyl)glyceric acid and *trans*-caffeic acid against the relatively stable 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$) free radical was investigated. IC_{50} value of PDPGA–AI is 51.5 μg/ml. © *2017 Bull. Georg. Natl. Acad. Sci.*

Key words: poly[3-(3,4-dihydroxyphenyl)glyceric acid], 3-(3,4-dihydroxyphenyl)glyceric acid, caffeic acid, *Anchusa italica*, antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$)

Caffeic acid and its analogues are potential antioxidants with multiple mechanisms involving free radical scavenging, metal ion chelation, and inhibitory actions on specific enzymes that induce free radical and lipid hydroperoxide formation. The structural feature responsible for the antioxidative and free radical scavenging activity of caffeic acid and its analogues is the *ortho* dihydroxyl functionality in the catechol ring. The presence of the electron-donating hydroxyl group at the *ortho*-position also lowers the O-H bond dissociation enthalpy and increases the rate of H-atom transfer to peroxyl radicals [1].

In previous papers we have reported about the isolation and identification of caffeic acid-derived polymer poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA) (Fig. 1) from *Symphytum asperum* – PDPGA–SA, *S. caucasicum* – PDPGA–SC [2-5], *S. officinale* – PDPGA–SO [6] and *Anchusa italica* – PDPGA–AI [7].

This polymer is a representative of a class of natural polyethers with a residue of 3-(3,4-dihydroxyphenyl)glyceric acid as the repeating unit. Besides, the monomer of PDPGA racemic 3-(3,4-dihydroxyphenyl)glyceric acid (RDPGA) was synthesized [8] (Fig. 2).

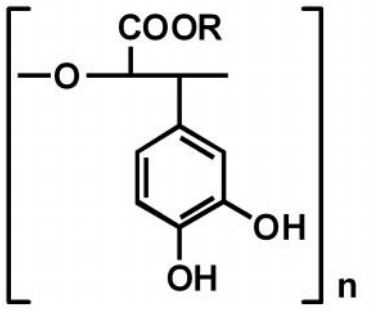

Fig. 1. Poly[3-(3,4-dihydroxyphenyl)glyceric acid] **(PDPGA)** from *S. asperum, S. caucasicum* and *S. officinale* (R = H) and *A. italica* (R = H, CH_3).

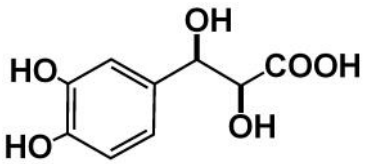

Fig. 2. Synthetic racemic 3-(3,4-dihydroxyphenyl)glyceric acid **(RDPGA).**

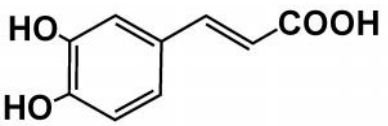

Fig. 3. *Trans*-caffeic acid.

It is necessary to emphasize that in contrast with the *Symphytum* polymers, most of the carboxylic groups (~ 70%) of caffeic acid-derived polymer from *A. italica* are methylated [7].

PDPGA–SA, PDPGA–SC and PDPGA–SO possessed anticomplementary, antioxidant and antiinflammatory activities [6,9,10]. These preparations as well as PDPGA–AI are in fact polycatecholic acids, and due to the presence of *ortho*-dihydroxyl (catechol) groups, could act as donor of hydrogen radicals or electrons what is crucial for enhanced antioxidant efficacy.

The aim of current study was to investigate antioxidant activity of PDPGA–AI (Fig. 1) by 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$)-method in comparison with RDPGA (Fig. 2) and *trans*-caffeic acid (Fig. 3).

Experimental

Commercially available reagents *trans*-caffeic acid, 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) were purchased from Aldrich-Sigma Chemical Co. (Sigma-Aldrich, Schnelldorf, Germany). Racemic 3-(3,4-dihydroxyphenyl)glyceric acid (RDPGA) was synthesized as described in [8]. Poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA) from *A. italica* roots was isolated in accordance with [7].

Absorption spectra were run on a Jasco V-560 spectrophotometer (Jasco Co, Tokyo, Japan).

For the determination of radical scavenging activity, $DPPH^{\cdot}$ was used as a stable radical and antioxidant activity of compounds was determined spectrophotometrically ($DPPH^{\cdot}$-method).

The ability of PDPGA–AI, RDPGA and *trans*-caffeic acid to scavenge free radicals or neutral reactive oxygen species was tested against the relatively stable $DPPH^{\cdot}$-radical. Briefly, the absorption of a methanolic solution of 2.0 ml $DPPH^{\cdot}$ (500 μM) and 2.0 ml methanol was measured at 515 nm (blank) and compared with the absorbance of samples containing 2.0 ml $DPPH^{\cdot}$ and 2.0 ml methanolic solutions of sample compounds in concentrations ranging from 0.1 - 250 μg/ml. The absorbance of the samples was measured after the reaction reached a plateau (about 30 min). All measurements were done at room temperature (23°C) and repeated four times. The radical scavenging activity of the samples was expressed in terms of IC_{50} (concentration in μg/ml required for a 50% decrease in absorbance of $DPPH^{\cdot}$-radical) and calculated using the equation $[(A_{blank} - A_{sample}) / A_{blank})]$ x 100), where A_{blank} is the absorbance of the control ($DPPH^{\cdot}$ solution without sample) and A_{sample} the absorbance of the test compound ($DPPH^{\cdot}$ solution plus antioxidant). A plot of absorbance vs. concentration was made to establish the standard curve and the linear regression equations from which the IC_{50} values were calculated [8].

Results and Discussion

Determination of free radical scavenging activity by $DPPH^{\cdot}$ assay is the simplest method to measure the ability of antioxidants to intercept free radicals. The

Table. Comparison of antioxidant activity of PDPGA–AI to RDPGA and caffeic acid

Compound	Antioxidant activity (IC_{50}*, μg/ml) by DPPH•-method
PDPGA–AI	51.5 ± 1.11
Synthetic RDPGA	3.8 ± 0.3
Trans-caffeic acid	12.2 ± 0.7

* IC_{50} ± standard deviation (n=4)

scavenging effects of caffeic acid-derived polymer from *A. italica* (PDPGA–AI) (Fig. 1) and its synthetic monomer racemic 3-(3,4-dihydroxyphenyl)glyceric acid (RDPGA) (Fig. 2) and caffeic acid (Fig. 3), are shown in Table. Their scavenging activity of DPPH$^{\cdot}$-radicals indicates that the DPPH$^{\cdot}$-radical scavenging activity of the test compounds is due to their hydrogen-donating ability. Thus, the nonenzymatic DPPH$^{\cdot}$-radical scavenging activity of test compounds may be attributed to the catechol moieties contained in these preparations.

The unexpected lowest antioxidant activity of polyether PDPGA–AI may be explained with the less stabilizing effects of phenoxide radicals via intra- or inter-molecular hydrogen bonds observed in monomeric products. Such stabilizing effects of phenoxide radicals in phenolic compounds *via* intra- or inter-molecular hydrogen bonds are reported in reference [11].

This hypothesis is strengthened by the similarity of structural features of 3-(3,4-dihydroxyphenyl) glyceric acid and *trans*-caffeic acid. In case of RDPGA, the phenoxide radicals produced during the reaction can be stabilized intra-molecularly *via* hydrogen bonds, while in case of *trans*-caffeic acid, over the extended conjugated system.

Regarding the structure of the PDPGA–AI, it is possible that the observed anti-ulcer property of *Anchusa* extracts [12,13] and their inhibitory activity against pepsin [14] are the results not only of their antioxidant activity, but mainly due to inactivation of enzymes or proteins involved in pepsin [12-14].

Thus, we expect that PDPGA–AI is useful for a therapeutic agent to offer protection against a wide range of free radical-induced and/or enzyme-related inflammatory and vascular diseases and consequently damage of tissue in wound might be reduced.

ფარმაკოქიმია

Anchusa italica-ს კოფეინის მჟავას წარმოებულის პოლიმერის ანტიოქსიდანტური აქტივობა

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

** თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ი. ქუთათელაძის ფარმაკოქიმიის ინსტიტუტი, თბილისი, საქართველო*

(წარმოდგენილია აკადემიის წევრის ე. ქემერტელიძის მიერ)

შესწავლილია *Anchusa italica*-ს კოფეინის მჟავას წარმოებულის პოლიმერის პოლი [3-(3,4-დიჰიდროქსიფენილ) გლიცერინის მჟავას] (პდფგმ), მისი სინთეზური მონომერის რაცემული 3-(3,4-დიჰიდროქსიფენილ) გლიცერინის მჟავას და *ტრანს*-კოფეინის მჟავას შედარებითი ანტიოქსიდანტური აქტივობა სტაბილური 2,2-დიფენილ-1-პიკრილჰიდრაზილის (დფპჰ˙) თავისუფალი რადიკალის მიმართ. *A.italica*-ს პდფგმ-ის IC_{50} მნიშვნელობა ტოლია 51,5 მგ/მლ.

REFERENCES

1. Son S., Lewis B. A. (2002) Free radical scavenging and antioxidative activity of caffeic acid amide and ester analogues: structure"activity relationship. *J. Agric. Food Chem.* **50,** 3: 468-472.
2. Barbakadze V., Kemertelidze E., Usov A.I., Kroes B.H., Quarles van Ufford H.C. et al. (1999) Evaluation of immunomodulatory activity of some plant polysaccharides. *Proc. Georgian Acad. Sci. Biol. Ser.* **25**, 4-6: 207-216.
3. Barbakadze V.V., Kemertelidze E.P., Shashkov A.S., Usov A.I. (2000) Structure of a new anticomplementary dihydroxycinnamate – derived polymer from *Symphytum asperum (Boraginaceae)*. *Mendeleev Commun.* **10**, 4: 148-149.
4. Barbakadze V.V., Kemertelidze E.P., Targamadze I.L., Shashkov A.S., Usov A.I. (2002) A new biologically active polymer from two comfrey species Symphytum asperum and S. caucasicum (Boraginaceae). *Russ. J. Bioorg. Chem.* **28**, 4: 326-330.
5. 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.
6. 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.
7. Barbakadze V., Gogilashvili L., Amiranashvili L., Merlani M., Mulkijanyan K., et al. (2010) Poly[3-(3,4-dihydroxyphenyl)glyceric acid] from *Anchusa italica* roots. *Nat. Prod. Commun.* **5**, 7: 1091-1095.
8. Merlani M., Barbakadze V., Amiranashvili L., Gogilashvili L., Yannakopoulou E., Papadopoulos K., Chankvetadze B. (2010) Enantioselective synthesis and antioxidant activity of 3-(3,4-dihydroxyphenyl)-glyceric acid - basic monomeric moiety of a biologically active polyether from Symphytum asperum and S. caucasicum. *Chirality.* **22**, 8: 717-725.

9. Barthomeuf C.M., Debiton E., Barbakadze, V.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.
10. Barbakadze V.V., Kemertelidze E.P., Mulkijanyan K.G. van den Berg A.J.J., Beukelman C.J., et al. (2007) Antioxidant and anticomplementary activity of poly[3-(3,4-dihydroxyphenyl) glyceric acid] from *Symphytum asperum* and S. caucasicum. *Pharm. Chem. J.* **41**, 1: 14-16.
11. Siquet C., Paiva-Martins F., Lima J.L., Reis S., Borges F. (2006) Antioxidant profile of dihydroxy-and trihydroxyphenolic acids-A structure–activity relationship study. *Free Rad. Res.* **40,** 4: 433-442.
12. Disi A.M., Tamimi S.O., Abuereish G.M. (1998) Effects of Anchusa strigosa root aqueous extract on gastric ethanol-induced ulcer in laboratory animals. *J. Ethnopharmacol.* **60**, 3: 189–198.
13. Abbas M., Disi A., Al-Khalil S. (2009) Isolation and identification of anti-ulcer components from Anchusa strigosa root. *Jordan J. Pharmaceut. Sci.* **2,** 2: 131-139.
14. Abuereish G.M. (1998) Pepsin inhibitor from roots of Anchusa strigosa. *Phytochem.* **48,** 2: 217-221.

Received October, 2017