SHORT COMMUNICATION

Phenolic acids and antioxidant potential of *Caragana frutex* shoots

Fenolové kyseliny a antioxidační potenciál výhonků *Caragana frutex*

Vitaliy V. Boynik • Svetlana I. Stepanova

Received January 29, 2021 / Accepted May 13, 2021

Summary

The phenolic acid composition of flowering *Caragana frutex* shoots was analyzed by the HPLC method. The quantitative content of seven phenolic acids and their derivatives has been determined: gallic, *p*-hydroxyphenyl acetic, chlorogenic, caffeic, *p*-coumaric, *trans*-ferulic, and sinapic) acids. Sinapic acid (513.0 μg/g) and chlorogenic acid (98.4 μg/g) predominate among phenolic acid derivatives. The antioxidant activity of *Caragana frutex* shoots determined by the ABTS method equaled 9368.51 ± 30.07 μg/g expressed as Trolox equivalent.
Key words: *Caragana fruitex* (Russian pea shrub) • phenolic acids • antioxidant activity • HPLC • ABTS method

Souhrn

Složení fenolové kyseliny kvetoucích výhonků *Caragana frutex* bylo analyzováno metodou HPLC. Byl stanoven kvantitativní obsah sedm fenolických kyselin a jejich derivátů: kyselina galová, *p*-hydroxyfenyloctová, chlorogenová, kávová, *p*-kumarová, *trans*-ferulová, sinapová). Kyselina sinapová (513,0 μg/g) a kyselina chlorogenová (98,4 μg/g) převládají mezi deriváty kyseliny hydroxyskořicové. Antioxidační aktivita výhonků *Caragana frutex* stanovená ABTS metodou činila 9368,51 ± 30,07 μg/g, vyjádřeno jako ekvivalent Troloxu.
Klíčová slova: *Caragana frutex* (ruský hrášek) • fenolové kyseliny • antioxidační aktivita • HPLC • metoda ABTS

Ass. Prof. Svetlana I. Stepanova (✉)
Department of Pharmacology and Pharmacotherapy
National University of Pharmacy of the Ministry of Health of Ukraine
Kulikovska Str. 12, 61002 Kharkiv, Ukraine
e-mail: nutriciologiastepanova@gmail.com

V. V. Boynik
Department of Pharmacognosy
National University of Pharmacy of the Ministry of Health of Ukraine
Kharkiv, Ukraine

Introduction

Oxidative stress has attracted the interest of scientists since its discovery. In the human body, under normal conditions, there is a balance between the natural antioxidant system and reactive oxygen/nitrogen species generated by the body and obtained from exogenous sources. The activation of free radical processes in the body and, as a consequence, oxidative stress may lead to the development of various pathological conditions such as inflammation, carcinogenesis, ischemic and reperfusion tissue damage, atherosclerosis, diabetes, bronchopulmonary and neurodegenerative diseases[1]. Therefore, there is an urgent need for the development of drugs for the overcoming of oxidative stress.

Natural antioxidants are widely used at present, and among them, the main plant antioxidants are represented by phenolic compounds (plants secondary metabolites). They participate in respiration, photosynthesis, formation of cell walls, adaptation and protection of plants from stress factors, and perform a reserve function. Phenolic acids and their derivatives are frequently considered to be among the most valuable antioxidants in plants. They are widespread in the plant world, being present in food products and herbal medicines. Antioxidant activity (AOA) of phenolic compounds depends on the structure, number, and position of hydroxyl groups in the aromatic nucleus and the degree of polymerization. Therefore, phenolic acids possess different antioxidant activity[2]. Numerous studies have shown that such phenolic acids as gallic acid, caffeic acid, and its esters (chlorogenic, chicoric, caftaric, and rosmarinic acids) play the main role in the total AOA of plants and natural products. These compounds have *ortho*-OH groups in the aromatic ring, which are responsible for high AOA[3, 4].

Caragana genus plants of the Fabaceae family caught our attention. They are rich in phenolic compounds such as hydroxycinnamic acids, coumarins, flavonoids, stilbenoids, lignans[5–7]. The antioxidant activity of the individual phenolic compounds and total fractions of biologically active substances of *C. conferta, C. tibetica* and *C. microphylla* was studied by various methods[5, 8, 9].

Russian pea shrub (*Caragana frutex* (L.) K. Koch) is a deciduous branched shrub up to 2.0 m tall. Stipules are spiny and short. Leaves are palmate due to the reduced rachis, with two pairs of sessile, glabrous leaflets. Leaflet blades are obovate or truncate with an entire margin and a spiny pointed tip. Pea-like flowers are axillary, solitary, or in fascicles of two to three. The calyx is tubular or bell-shaped, with five teeth. Corolla is yellow. A pod is cylindrical, glabrous, many-seeded[5, 10].

Caragana frutex has a natural Eurasia area and forms thickets in forest, forest-steppe and steppe zones within European Russia, Ukraine, Moldova, Romania, Bulgaria, Russian Caucasus, western Siberia, Kazakhstan, Kyrgyzstan, western China[11].

Russian peas hrub shoots contain vitamins; nitrogen-containing compounds (allantoin, allantoic acid), amino acids, alkaloids, hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic, chlorogenic, neochlorogenic), coumarins (umbeliferon, esculetin, scopoletin, bergapten, xanthotoxin), flavonoids (acacetin, 3-methylquercetin, isorhamnetin, kaempferol, quercetin, myricetin, isorhamnetin-3-*O*-glucoside, acacetin-7-*O*-glucoside, linarin, narcissin, isoquercitrin, rutin); condensed tannins; steroidal saponins. *Caragana frutex* is used in traditional Chinese and Tibetan medicine to treat a traumatic injury, sore and superficial infections, lumps in breasts, and menstrual disorders. Experimental pharmacological studies have shown that Russian peashrub possesses anti-inflammatory, membrane-, vaso-, cerebro- and hepatoprotective effects[5, 6, 12–17].

The quantitative content of phenolic acids in the *Caragana frutex* shoots has not been determined previously. It should be noted that these phenolic compounds can contribute to a higher antioxidant activity of plant materials and phytopreparations.

The purpose of our study is to determine the qualitative composition and quantitative content of phenolic acids in the *Caragana frutex* shoots and to evaluate the plant material antioxidant activity by ABTS method.

Experimental part

Plant material

The object of the study was the flowering shoots of *Caragana frutex* (L.) K. Koch collected in May 2020 in the Kharkiv region of Ukraine. The samples were identified, and the voucher specimen has been deposited at the Department of Pharmacognosy, National University of Pharmacy, Ukraine. Collected plant material was air-dried without access to light. Before the preparation of extracts *Caragana frutex* shoots were ground in a cross beater mill.

Determination of phenolic acids

Phenolic acids were qualitatively and quantitatively determined by the HPLC method using a liquid chromatograph Agilent Technologies 1200. The separation of substances was carried out on a chromatographic column Zorbax SB-Aq (150 mm × 4.6 mm, 3.5 µm), a diode-array detector was used for detection[18].

Samples of raw materials were prepared and analyzed as follows:

The quantity of approximately 0.5 g of the dry crushed plant material was accurately weighed and extracted with 10 mL of 60% methanol in an ultrasonic water bath for 4 hours. The extract was centrifuged at 3000 RPM and filtered through a disposable membrane filter with pores of 0.22 µm and then chromatographed.

Methanol (A) and 0,1% formic acid solution in water (B) were used the mobile phase. The elution was carried out in this gradient mode: 0–25 min – A : B (1 : 3), 25 min – A : B (3 : 1), 27 min – A (100%), 35 min – A (100%). The flow rate through the column was 0.5 mL/min, the thermostat temperature was 30 °C, and the injection volume was 4 µL. The detection was carried out using a diode-array detector with signal recording at 250 and 275 nm and recording absorption spectra in the range of 210–700 nm. Identification and quantitative analysis were performed using standard solutions of phenolic acids (gallic, *p*-hydroxyphenyl acetic, chlorogenic, caffeic, syringic, *p*-coumaric, *trans*-ferulic, and sinapic).

The content of phenolic acids was expressed as the average mean of three replicates. The research results are shown in the table 1.

Table 1. Content of phenolic acids and their derivatives in the Caragana frutex shoots, µg/g of dry plant material

№	Phenolic acid	Retention time, min	Substance content
1	Gallic acid	4.76	13.6 ± 0.68
2	*p*-hydroxyphenylacetic acid	8.22	8.2 ± 0.41
3	Chlorogenic acid	9.96	98.4 ± 4.3
4	Caffeic acid	10.40	20.6 ± 1.2
5	Syringic acid	12.46	Not detected
6	*p*-coumaric acid	13.89	53.6 ± 2.4
7	*Trans*-ferulic acid	15.17	6.8 ± 0.39
8	Sinapic acid	15.61	513.0 ± 15.3

ABTS radical scavenging assay

The free radical scavenging activity of samples was estimated by ABTS radical cation assay. The method is based on the reaction of the interaction of antioxidants with the radical cation of 2,2′-azinobis(3--ethylbenzothiazolin-6-sulphonic acid) diammonium salt (ABTS)[19, 20].

The quantity of approximately 0.5 g of the dry crushed plant material was accurately weighed and extracted with 10 ml of 50% ethanol solution on a boiling water bath for 40 min. Prepared extracts were filtered through a 0.45 μm membrane filter.

The stock solution included 7 mM ABTS solution and 2.4 mM potassium persulfate solution mixed in equal quantities, stored for 16 h in the dark at room temperature before use. The working solution was then prepared by diluting the stock solution with water to obtain an absorbance of 0.900 at 734 nm against the blank (water).

For the analysis, 100 μL of test solution was added to 9.9 mL of ABTS working solution. In 30 minutes, the absorbance was measured at 734 nm using a spectrophotometer 'Evolution-60S'. An appropriate solvent blank was run in each assay. All determinations were repeated at least three times.

To determine the antiradical activity of the *Caragana frutex* shoots a standard calibration curve was constructed using a standard sample of Trolox. ABTS radical cation scavenging activity of the samples was expressed as antioxidant Trolox equivalents (*TE*) per gram of dry plant material. It was calculated using the formula:

$$TE_{ABTS} = \frac{(C \times V)}{m}, \mu g/g$$

where *C* – Trolox concentration from the calibration curve (μg/L), *V* – the volume (L), *m* – the exact weight of the dry material (g).

Radical scavenging activity equaled 9368.51 ± 30.07 μg/g.

The antioxidant activity is expressed as the average mean of three replicates.

Results and discussion

The specific HPLC procedure has been used for the qualitative and quantitative analyses of phenolic acids in the *Caragana frutex* shoots. To obtain good separation and a stable peak retention value, the extraction and chromatographic conditions have been optimized.

As the result of HPLC analysis, the content of seven phenolic acids and their derivatives has been determined in the *Caragana frutex* shoots.

Sinapic acid (513.0 μg/g), and chlorogenic acid (98.4 μg/g) predominate among phenolic acid derivatives.

Gallic, *p*-hydroxyphenyl acetic, and sinapic acids have been identified in the *Caragana frutex* for the first time.

According to the literature, many classes of phenolic compounds have antioxidant properties[2–5, 19–21]. Polar solvents are commonly used to extract phenolic compounds with antioxidant activity from plant raw material[20, 21]. As shown previously, 50% ethanol extracted most of the phenolic compounds (hydroxycinnamic acids and flavonoids) from *Caragana frutex* shoots[16]. Therefore, it was expedient to determine the antioxidant activity of the investigated raw materials using 50% ethanol as the extractant. It could be assumed that phenolic acids are the potential antioxidant components in *Caragana frutex* shoots.

The antioxidant activity of Russian pea shrub shoots has been determined by the ABTS method equaling 9368.51 ± 30.07 μg/g expressed as Trolox equivalent.

The obtained results indicate that *Caragana frutex* shoots are rich in phenolic acids and possess a high antioxidant scavenging capacity.

Knowledge of the antioxidants composition in the plant raw materials will help to select objects for further research, develop technology for obtaining phytopreparations, and suggest possible pharmacological effects for other screening studies.

Conflict of interest: none.

References

1. **Sies H.** Oxidative stress: concept and some practical aspects antioxidants (Basel) 2020; 9(9), 1–6, Article 852. https://www.mdpi.com/2076-3921/9/9/852 (28.04.2021).
2. **Kumar N., Goel N.** Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnol. Rep. (Amst.). 2019; 24, 1–10, e00370. https://www.sciencedirect.com/science/article/pii/S2215017X19302383 (28.04.2021).
3. **Razzaghi-Asl N., Garrido J., Khazraei H., Borges F., Firuzi O.** Antioxidant properties of hydroxycinnamic acids: a review of structure-activity relationships. Curr. Med. Chem. 2013; 20(36), 4436–4450.
4. **Kahkeshani N., Farzaei F., Fotouhi M., Alavi S. S., Bahramsoltani R., Naseri R., Momtaz S., Abbasabadi Z., Rahimi R., Farzaei M. H., Bishayee A.** Pharmacological effects of gallic acid in health and diseases: A mechanistic review. Iran. J. Basic Med. Sci. 2019; 22(3), 225–237.
5. **Meng Q., Niu Y., Niu X., Roubin R. H., Hanrahan J. R.** Ethnobotany, phytochemistry and pharmacology of the genus Caragana used in traditional Chinese medicine. J. Ethnopharmacol. 2009; 124(3), 350–368.
6. **Zhanga T., Songa Y., Zhou D., Li X., Li N.** Studies on the chemical components and biological activities of the genus of Caragana. A. J. Traditional Medicines 2019; 14(2), 101–124.
7. **Wang J-R., Ding L., Zhang Y-Y., Chang Z-Y., Li Z-W.** Testing of active constituents in the stems and leaves of

ten Caragana plants. Acta Bot. Boreali-Occidentalia Sin. 2005; 25, 2549–2552.

8. **Xiang T., Uno T., Ogino F. Ai C., Duo J., Sankawa U.** Antioxidant Constituents of Caragana tibetica. Chem. Pharm. Bull. (Tokyo) 2005; 53(9), 1204–1206.
9. **Wei Q., Wei Y., Wu H., Yang X., Chen H., Zhang H.** Chemical Composition, Antioxidant, and Antimicrobial Activities of Four Saline Tolerant Plant Seed Oils Extracted by SFC. J. Am. Oil. Chem. Soc. 2016; 7, 1–10.
10. WFO (2021): Caragana frutex (L.) K. Koch. http://www.worldfloraonline.org/search?query=Caragana+frutex (28.04.2021).
11. Caragana frutex (L.) K. Koch. https://pfaf.org/user/Plant.aspx?LatinName=Caragana+frutex (28.04.2021).
12. **Boinik V. V., Kovalev V. N., Komissarenko N. F., Dikhtyarev V. I.** Coumarins of the epigeal part of Caragana frutex. Chem. Nat. Compd. 1983; 19, 742–742.
13. **Barnaulov O. D., Belodybrovskaya G. A.** Comparative estimation vasssel- and hepatoprotective properties galenic plant drugs of 16 Caragana Lam. species. Reviews on Clinical Pharmacology and Drug Therapy 2018; 16(3), 60–67.
14. **Barnaulov O. D.** The comparative estimation cerebroprotective activity decoctions of plants genus Caragana Lam. Reviews on Clinical Pharmacology and Drug Therapy 2020; 18(1), 71–76.
15. **Stepanova S. I., Boynik V. V., Gontova T. M., Kozyra S. A.** Determination of ureid content in shoots of russian pea shrub and siberian pea tree. Farmatsevtychnyi zhurnal 2020; 2, 59–66.
16. **Boinik V. V., Kovalev V. N.** Flavonoids of Caragana frutex. Chem. Nat. Compd. 1987; 23, 504.
17. **Partilkhaev V. V., Tankhaeva L. M., Olennikov D. N.** Phenolic compounds content in shoots of siberian Caragana species. Chem. Plant Raw Mater. 2013; 1, 143–150.
18. **Sumere B. R., de Souza M. C., Dos Santos M. P., Bezerra R. M. N., da Cunha D. T., Martinez J., Rostagno M. A.** Combining pressurized liquids with ultrasound to improve the extraction of phenolic compounds from pomegranate peel (*Punica granatum* L.). Ultrason. Sonochem. 2018; 48, 151–162.
19. **Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C.** Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 1999; 26(9–10), 1231–1237.
20. **Bezruk I. V., Grudko V. O., Georgiyants V. A., Ivanauskas L.** Screening of the antioxidant activity of extracts from Hedera helix leaves using the HPLC/ABTS method. Clin. Pharm. 2020; 24(3), 47–52.
21. **Dirar A. I., Alsaadi D. H. M, Wada M., Mohamed M.A., Watanabe T., Devkota H. P.** Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. S. Afr. J. Bot. 2019; 120, 261–267.