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Der Pharmacia Lettre, 2016, 8 (6):73-76
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Phytochemical investigation of bird cherry fruits

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ABSTRACT

Bird cherry, *Prunus padus* is widely distributed in Ukrainian forests. Fresh and dried fruits are highly reputed in Russian, Belarus and Ukrainian ethnoscience medicine for astringent, nutritive and digestive properties and recommended for common cold, strengthening stomach in gastritis, colic, diarrhea, as anesthetic and disinfectant, to improve complexion and eyesight. Content of phenolic compounds, hydroxycinnamic acids, flavonoids were determined in bird cherry fruits by spectrophotometry. Investigation of fruits by HPLC method was carried out. Presence and content one caffeic acid derivative, two anthocyanins and two flavonols were determined. Research is continued for further standardization of bird cherry fruits.

Keywords: *Prunus padus*, fruits, flavonoids, anthocyanins, hydroxycinnamic acids.

INTRODUCTION

In Ukraine bird cherry, *Prunus padus* L, is known mainly under synonym *Padus avium* Mill [1, 2]. It belongs to family *Rosaceae*. The geographical distribution of European bird cherry is wide. The northern border of European distribution follows 71° north parallel and the shore of Arctic Ocean. Asian distribution follows 65°-70° north parallel. The main boundaries in the north are tundra zone in the northeast of Asia Minor and western Siberia and the timberline of coniferous forest in central and eastern Siberia and Far East (Amur and Kamtschatka). The southern most European populations have been found in Scotland, northern England, Wales, mountainous Spain and Portugal, the island of Madeira, Morocco, the northwest of Italy, Croatia, Bulgaria and northern Balkan Peninsula in Europe [1-3]. The southernmost populations thrive in Turkish Armenia, Afghanistan, Caucasus, southern Ural, Tien-Shan, the Himalayas, northern Japan, Korea and northern China in Asia [3]. In Ukraine this tree is widely distributed in forests in Polesie. In Carpathian Mountains the plant rises to the upper boundary of forest, it is less common in the forest-steppe areas mainly in river valleys and ravines, in the steppe - very rare. The fruit may be harvested in Zakarpatskiy, Lviv, Ivano-Frankivsk, Chernivtsi, Ternopil, Khmelnytsky, Volyn, Rivne, Zhytomyr, the Kiev, Chernihiv, Sumy and Kharkiv regions [4-7].

Fresh and dried fruits are highly reputed in Russian, Belarus and Ukrainian ethnoscience medicine for astringent, nutritive and digestive properties and are recommended for common cold, strengthening stomach in gastritis, colic, diarrhea, anesthetic and disinfectant, improve complexion and eyesight [4, 5, 8].

The aim of our research was to determine content and qualitative composition of phenolic compounds in dried *Padus avium* fruits harvested in Ukraine.

MATERIALS AND METHODS

The object of the study was bird cherry fruits *Padi fructus* harvested in July 2015 in the Kharkiv region and dried under room condition.

For preliminary identification of biologically active substances of the plant material, such generally accepted methods as qualitative reactions, paper chromatography (PC) and thin layer chromatography (TLC) were used. Flavonoids, and among them anthocyanins, were studied by PC and TLC with valid samples of flavonoids in the solvent systems: 1% hydrochloric acid, 15 % acetic acid, butanol-glacial acetic acid-water (4:1:2), glacial acetic acid-water-ethyl acetate (20:20:60), chloroform-ethyl acetate-glacial acetic acid (3:5:1). Obtained chromatograms were treated by ammonia solution; 1-5% aqueous solution of sodium hydroxide; solution of amino ethyl ester of diphenyl boric acid in methanol, and then by macrogol solution with heating for 10 min and viewed in daylight. [9]. For identification of hydroxycinnamic acids, the method of two-dimensional PC was used in solvent systems: I-st direction – n-butanol-acetic acid - water (4: 1: 2), and II-nd direction - 15% acetic acid. Obtained chromatograms were treated by ammonia solution and azo coupling reagent [9, 10].

Quantitative determination of hydroxycinnamic acids, flavonoids, and polyphenolic compounds was performed by spectroscopy [9, 11-13]. Absorbance was measured in the cuvette with a layer thickness of 10 mm on a spectrophotometer Hewlett Packard 8453. The measurements were carried out 5 times. Statistical analysis of the results was performed according to the requirements of SPU [14]. The determination of phenolic compounds calculated as gallic acid equivalent was performed by modified method [9, 12], flavonoids - by method of State Pharmacopoeia of the USSR XI ed. and calculated as rutin equivalent in % of dried plant raw material [13]. Quantitative determination of hydroxycinnamic acids, was calculated as chlorogenic acid equivalent in % of dried plant raw material. Maximum absorption of chlorogenic acid reference solution occurred at 327 nm, so the measurements were carried out at this wavelength by modified method [11].

Qualitative composition and content of phenolic compounds in the bird cherry fruits were studied by HPLC, with the Agilent Technologies chromatograph (model 1100) completed with continuous-flow vacuum degasifier G1379A, 4-channel pump of low pressure gradient G13111A, automatic injector G1313A, column oven G13116A, diode array detector G1316A [10]. The chromatographic column ZORBAX-SB C-18, 2.1 × 150 mm, filled with octadecyl silyl sorbent graded 3.5 microns was used for analysis.

Analysis was performed under the following conditions: temperature of the thermostat was 35 °C; mobile phase flow rate - 0.25 ml / min. Gradient regime of chromatography was used with a mobile phase solution A (0,1% H₃PO₄, in water) and solution B (MeOH) in a ratio of 90:10 (the first 10 min), 70:30 (10 to 25 min), 20:80 (25 min). From 26 to 30 min, only the solution B was used, and after again, solutions A and B in a ratio of 90:10 (30 to 35 min) were used. The operating pressure of the eluent was 240-300 kPa. These conditions refer to the known method and was performed with some modifications to the gradient [10].

Parameters of the analysis were the following: scale of measurement – 1.0; scan time - 0.5 seconds; options metering spectrum – each peak of 190-600 nm. Identification of phenolic compounds was performed by the retention time of standards of hydroxycinnamic acids, anthocyanins and flavonoids and their spectral characteristics. Each peak detected in this investigation was identified by comparing retention time and UV spectra given by the diode array detector with the standards. Standards of phenolic compounds were dissolved in 50% methanol. The flavonoids, anthocyanins, hydroxycinnamic acids were quantified by calibration with the standards. Sample of bird cherry fruits was extracted in triplicate and analyzed by HPLC.

The statistical processing of results was carried out using the package Statistica 6.0. The error did not exceed 5%.

RESULTS AND DISCUSSION

After our preliminary study of biologically active substances in the bird cherry fruits by TLC and PC with authentic samples, the presence of hydroxycinnamic acids, anthocyanins and flavonoids, including rutin and cyanidin-3-glycoside, was established. Content of phenolic compounds determined by spectrophotometry was (%) 2.09± 0.03; hydroxycinnamic acids – 0.20± 0.01, flavonoids – 0.64± 0.01.

Graphical result of determination of phenolic compounds by HPLC can be seen on figure 1. The amount of compounds was given in mg per 100g dry plant raw material (Table 1).

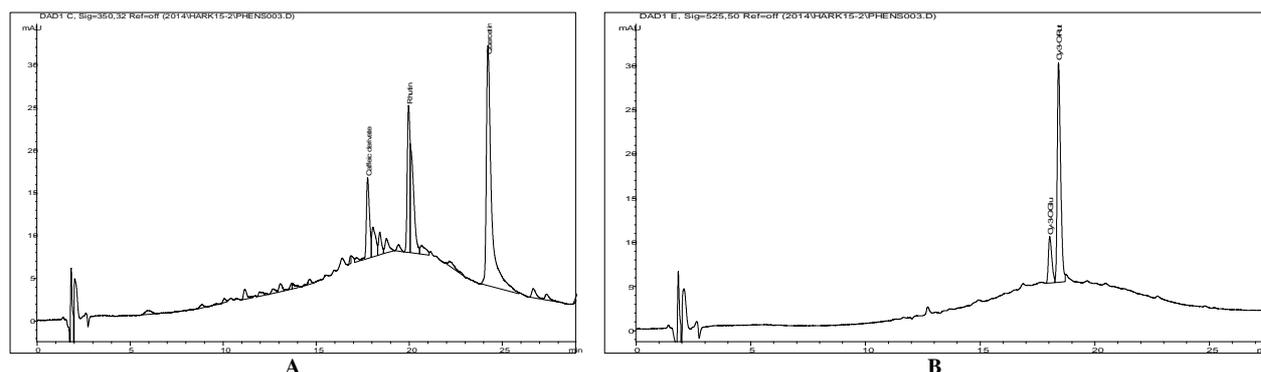


Figure 1. Graphical result of HPLC investigation of phenolic compounds in bird cherry fruit A and B parts

Table 1. The compounds of bird cherry fruits determined by HPLC

No	Compounds	Retention time, min	Content (mg/100g)
1	Caffeic acid derivative	17.76	6.4
2	Cyanidin-3-O-glycoside	18.03	2.1
3	Cyanidin-3-O-rutinoside	18.42	9.7
4	Rutin	19.96	9.8
5	Quercetin	24.23	56.9

As a result of the study of the bird cherry fruits 5 phenolic compounds were determined as derivatives of hydroxycinnamic acids, anthocyanins and flavonols. The total amount of phenolic compounds determined by this method was 84.9 mg/100g. As can be seen from table hydroxycinnamic acids, anthocyanins and flavonols present in amount (mg/100g): 6.4, 11.8, 66.7 that mins (%) – 7.54, 13.90 and 78.56 of total determined compounds respectively; the predominant component was quercetin (56.9 mg/100g). Content of quercetin consist was 67% of total phenolics determined by HPLC.

The research established presence of hydroxycinnamic acids, anthocyanins and flavonols and content of phenolic compounds in bird cherry fruits. According to our result bird cherry fruits contain only two cyanidin derivatives: cyanidin-3-O-glycoside (2.1 mg/100g) and cyanidin-3-O-rutinoside (9.7 mg/100g). The ratio was 18:82. This result confirmed investigation of other scientist obtained earlier. According to their data the pigment composition was very simple, the anthocyanins – cyanidin-3-rutinoside (60%) and cyanidin-3-glucoside (40%) were determined using chromatographic and spectroscopic methods [15]. Among the biological active compounds rutin and quercetin were present as dominant. The biological and pharmacological role of rutin and quercetin revealed by recent studies [18]. They demonstrate the potential in mitigating radiation induced mortality, and cytogenetic damage, rutine can induce bone formation, improve endothelia function by augmenting nitric oxide production, it exhibited significant antidiabetic activity, presumably by inhibiting inflammatory cytokines, and improved the antioxidant and plasma lipid profiles in high fat diet + streptozotocin-induced type 2 diabetic model [18, 19]. Rutin protects against the neurodegenerative effects of prion accumulation by increasing the production of neurotropic factors and inhibiting apoptotic path-way activation in neuronal cells. These results suggest that rutin may have clinical benefits for prion diseases and other neurodegenerative disorders [20]. Common flavonoids, quercetin and its glycone rutin have protective role, against high cholesterol diet and inflammation [21]. Rutin and quercetin are potent antioxidant and have antihypertensive property [22]. Anthocyanins are also very prospective compounds and together with flavonols rutin and quercetin may play role as antioxidant, anti-inflammatory agents and inhibit tumor cells growth [16, 17].

CONCLUSION

Content of phenolic compounds, hydroxycinnamic acids, flavonoids were determined in bird cherry fruits. Investigation of bird cherry fruits by HPLC method was carried out.

Presence of hydroxycinnamic acids, anthocyanins and flavonols was determined. Research is continued for further standardization of bird cherry fruits and development new phyto medicine.

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