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ORIGINAL PAPER

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SECONDARY METABOLITES OF *Phaseoli pericarpium*

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ABSTRACT

The pericarp of common bean *Phaseoli Pericarpium* syn. *Fructus Phaseoli sine semine*, is a medicinal raw material that has been long used in traditional folk medicine as an antidiabetic drug and now it is a pharmacopoeial material that belongs to one of the most frequently used plant raw materials that support treatment of diabetes. The aim of this study was to characterize the morphological characteristics of *Phaseoli Pericarpium* of over a dozen bean cultivars and to determine the content of phenolic compounds and the antioxidant properties of pericarp extracts. Bean pericarp used for phytochemical analysis came from field experiments conducted over the period 2010–2011. The experimental material consisted of white-flowered varieties of common bean – 5 cultivars and of runner bean – 6 cultivars. The bean pericarp, *Phaseoli Pericarpium*, was characterized by a high content of secondary metabolites: phenolic acids, flavonoids and tannins, whereas the pericarp extracts also exhibited antioxidant activity in scavenging DPPH. The pericarp of common bean was found to show high variation (*V* – 40.8%) in phenolic acid content, whereas the runner bean pericarp was characterized by high variation *V* (45.1%) in flavonoid content. Among the common bean cultivars studied, the pericarp of cv. 'Laponia' showed the greatest accumulation of biologically active substances – phenolic acids (CAE) – 0.18 mg·g^{-1} DM, flavonoids (QE) – 13.2 mg·100 g^{-1} DM, and tannins 3.43% DM. Likewise, the pericarp of the 'Felicja' runner bean cultivar stood out in terms of the accumulation of these compounds, as it contained the following amounts: phenolic acids – 0.33 mg·g^{-1} DM, flavonoids (QE) – 10.8 mg·100 g^{-1} DM, and tannins – 2.72% DM.

Key words: phenolic acids, flavonoids, tannins, common bean, runner bean, cultivars, variability, AA DPPH

INTRODUCTION

Plants of the family *Fabaceae* are grown in different regions of the world and legume seeds are a major source of protein in human and animal nutrition. The genus *Phaseolus sp*. comprises about 200 plant species widespread in cultivation all over the world, most of them grown under tropical and subtropical conditions.

Within the genus *Phaseolus* sp., the common bean (*Phaseolus vulgaris* L.) is one of the most important and most popular species in cultivation and human nutrition (dry seeds) worldwide and in Europe. At the same time, the pericarp of common bean, empty pods without seeds, i.e. *Phaseoli Pericarpium* syn. *Fructus Phaseoli sine semine*, is a medicinal raw material that has been long used in traditional folk medicine as an antidiabetic drug and now it is a pharmacopoeial material that belongs to one of the most frequently used plant raw materials that support treatment of diabetes [Roman-Ramos et al. 1995, Bazylko and Strzelecka 1997, Polish Pharmacopoeia V 1999, Polish Pharmacopoeia VI 2002, Helmstädter 2007, Modak et al. 2007, Winiarska et al. 2008, Helmstädter

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2010, Lamer-Zarawska 2014, Polish Pharmacopoeia X 2014]. Within this species, there is a great variability in the form and type of plant growth as well as in the usefully important traits of pods and seeds. These differences are primarily due to the genetic characteristics of cultivars, with more than a dozen thousand cultivars being grown. Moreover, environmental factors, which vary under different cropping systems used worldwide, have a significant effect on plant morphology as well as on yield quantity and quality, including the content of major chemical components and also biologically active substances that have therapeutic activity and exhibit a potentially beneficial effect on human health [Łabuda and Papliński 2003, Florez et al. 2009, Romero-Arenas et al. 2013, Aliu et al. 2014, Reichert et al. 2015, Rosales-Serna 2015].

Dry bean seeds have high nutritive and feeding value since they contain more than 20% of protein with a high biological value, 60% of total carbohydrates, with starch accounting for 40% of their total amount and dietary fiber for 16%, and moreover they contain mineral salts – potassium, calcium, phosphorus, magnesium, iron, zinc, copper and manganese, as well as group B vitamins. Non-nutritive substances found in bean seeds, which include, among others, dietary fiber, oligosaccharides, inositol phosphates and phenolic compounds – phenolic acids and flavonoids, have a beneficial health-promoting effect on humans. In nutrition of different population groups, including people with metabolism problems, diabetes and others, it is recommended that they increase the proportion of bean seeds and other legume seeds in their daily diet [Champ 2002, Ribeiro and Salvadori 2003, Venn and Mann 2004, Diaz-Batalla et al. 2006, Duranti 2006, Filipiak-Florkiewicz et al. 2012, Romero-Arenas et al. 2013, Ros et al. 2013, Fan and Beta 2016].

Special attention is now paid to the content of polyphenolic compounds in marketable, edible parts of plant products. Polyphenols are secondary plant metabolites with a very diverse chemical structure and they are produced in the plant in response to stress, injury, fungal infection or UV radiation. In the plant, secondary metabolism products perform various important roles, acting as natural protective agents, signal substances and substances that help the plant to adapt to the environment. Secondary metabolites often have the character of physiologically active compounds which can be important in therapeutics. Phenolic compounds commonly occur in the plant world; depending on the number of aromatic rings and how they are bonded together, they are divided into the following classes: flavonoids, phenolic acids, stilbenes, and lignans. A large group of hydroxyl groups found in these compounds give them antioxidant properties and hence these compounds protect organisms against the harmful action of free radicals. Polyphenols also exhibit antiexudative, antiinflammatory, antifungal and antiviral activity as well as they seal blood vessels. Flavonoids are one of the largest groups of phenolic compounds found in different plant organs and thus in food. Flavonoids give color to flowers and fruits, have UV radiation absorbing and protective properties as well as show therapeutic properties. Flavonols and their glycosides, among them primarily quercetin and kaempferol, are flavonoids that are most frequently found in plants. Quercetin is a phytocompound with a wide range of properties: antioxidant and antitumor as well as properties that reduce blood vessel permeability. Quercetin is a component of many diet supplements used in treatment of diabetes and absorption disorders as well as a component of antiallergic agents [Horbowicz 2000, Miean and Mohamed 2001, Kohlmünzer 2007, Kozłowska and Szostak-Węgierek 2014, Lamer-Zarawska et al. 2014, Kobylińska and Janas 2015].

Phenolic acids, in particular hydroxycinnamic and hydroxybenzoic acids, derivatives of cinnamic and benzoic acids, respectively, are secondary metabolites that are found very frequently in the plant world. Phenolic acids often occur as esters or glycosides that are components of lignin and hydrolyzable tannins. Hydroxycinnamic acids belong to the most common phenolic acids in plant tissues. The following hydroxycinnamic acids are most frequently found: caffeic acid, chlorogenic acid, o-, m- and p-coumaric acid, ferulic acid, and sinapic acid. They also occur in free form, as depsides and glycosides. Hydroxybenzoic acids that are most frequently found in plants are the following: gallic acid, p-hydroxybenzoic acid,

protocatechuic acid, vanillic aid, and syringic acid. Phenolic acids show health-promoting properties, predominantly due to their antioxidant properties. Moreover, phenolic acids exhibit wide pharmacological properties: cholagogic, choleretic and antihepatotoxic – acids: caffeic, ferulic, chlorogenic, syringic, vanillic; antibacterial – acids: caffeic, vanillic, p-coumaric, p-hydroxybenzoic; immunostimulating – acids: chlorogenic, caffeic, protocatechuic. Caffeic, chlorogenic, ferulic, ellagic and gallic acids have chemopreventive activity [Gawlik-Dziki 2004, Karamać et al. 2005, Budryn and Nebesny 2006, Chrpova et al. 2010, Parus 2013].

The bean pericarp (*Phaseoli Pericarpium* syn. *Fructus sine semine*) belongs to herbal materials that have been known and used from ancient times in traditional folk medicine in treatment of many ailments, mainly diabetes. Furthermore, the bean pericarp has been and is still used in treatment of diseases such as: gout, urinary tract infections, renal failure, ureterolithiasis, rheumatoid arthritis, and arterial hypertension [Bazylko and Strzelecka 1997, Lutomski and Hasik 2000, Lamer-Zarawska 2014].

The pericarp of bean, *Phaseoli Pericarpium* syn. *Fructus Phaseoli sine semine*, is a pharmacopoeial material that is described in Polish Pharmacopoeia: V [1999], VI [2002], IX [2011], X [2014], and also mentioned in the publication of the European Medicine Agency (EMA/HMPC) – *Phaseolus vulgaris* L., *fructus sine semine* – of 12 March 2013 and 12 November 2013 as well as of 20 September 2016 (224754 and 224755). *Phaseoli Pericarpium* (*Pericarpium Phaseoli*) is also listed in Dz.U. (Journal of Laws) of 2003 No. 125 item 1167, Annex 3, List of unprocessed pharmaceutical raw materials used for medicinal purposes and plant raw materials in shredded form.

The pericarp of common bean (*Phaseolus vulgaris* L.), originating from white-flowered varieties and dried under natural conditions, in a shaded place with air circulation, is a pharmacopoeial material that exhibits diuretic and antidiabetic properties. The raw material should contain not less than 0.01% (0.1 mg g^{-1}) of phenolic acids expressed as caffeic acid equivalents ($C_9H_7O_4$ – MM 180.16). Moreover, the presence of the free amino acid of arginine, identified by TLC (with ninhydrin) [Polish Pharmacopoeia VI 2002], is required in the raw material.

The chemical composition of the common bean pericarp (*Phaseoli pericarpium*) is already quite well known. The bean pericarp contains guanidine derivatives (amino-β-guanidino-isovaleric acid), indoleacetic acid (IAA), triterpenes (among others, phaseoloside D), organic acids, e.g. pipecolic and traumatic acids, amino acids (including significant amounts of arginine), meso-inositol, phenolic acids, flavonoids, phosphoric and silicic acids, stigmasterine, β- and γ-sitosterol, chromium salts (1ppm), soluble silica, and vitamins (C and E). Bean pericarp extracts reduce hyperglycaemia in mild cases of diabetes and restore carbohydrate balance. Hypoglycemic activity is also shown by compounds such as: amino-β--guanidino-isovaleric acid, arginine, phaseolan, chromium salts and IAA – which inhibits the action of insulin. This plant material shows a wide spectrum of activity in renal, ureteral and urinary bladder diseases as well as in renal and cardiac oedema [Ożarowski and Jaroniewski 1987, Bazylko and Strzelecka 1997, Polish Pharmacopoeia V 1999, Lutomski and Hasik 2000, Kohlmünzer 2007, Papliński 2007, Stepanowska 2013, Zgrajka et al. 2013]. Bean pericarp is a component of herbal mixtures and remedies used in diabetes phytotherapy, among others Diabetex-fix, Diabetosan-fix, Diabetofort, Diabetogran, and Diabetosol, as well as of the more following herbal mixtures: metabolic herbs – species metabolicae and diuretic herbs – species diureticae [Lamer-Zarawska et al. 2014]. Research is also conducted on the preparation of a drug containing bean pericarp extract in the form of tablets [Marczyński et al. 2015]. Bean pericarp is a medicinal material also applied in laryngology where its antiseptic, anti-inflammatory and astringent properties are used; isoprenoids, flavonoids, carbohydrates and related compounds as well as phenols, alkaloids and tannins belong to those active substances that are most frequently mentioned [Jędrzejko et al. 2009]. A study [Zgrajek et al. 2013] shows that the bean pericarp also contains kynurenic acid (KYNA) at an amount of 0.57 ±0.07 mg· g^{-1} DM, and the presence of this acid indicates the antirheumatic properties of this medicinal material.

Numerous reports in the literature dealing with the issue of use of *Phaseoli Pericarpium* as a plant drug, primarily in treatment of diabetes, show that the results of various studies are ambiguous and often contradictory. Bean pericarp has been used in the form of decoction, infusion – tea, and aqueous or ethanol extract injection. These studies analyzed the action of bean pericarp preparations based on different dosage levels and results of blood glucose level analysis [Cerović et al 2001, Petlevski et al. 2001, Pari and Venkateswaran 2003a, b, Winiarska et al. 2008, Berbecaru-Iovan et al. 2016]. However, in the available literature there are few studies aimed to identify and present the content of biological active compounds in *Phaseoli Pericarpium* material originating from various cultivars of *Phaseolus vulgaris* L.

The aim of this study was to characterize the morphological characteristics of *Phaseoli Pericarpium* of over a dozen bean cultivars and to determine the content of phenolic compounds and the antioxidant properties of pericarp extracts.

MATERIAL AND METHODS

Bean pericarp used for phytochemical analysis came from field experiments conducted over the period 2010–2011 in an experimental farm of the University of Life Sciences in Lublin, southeastern Poland (51°23'N, 22°56'E). The bean was grown on a silty soil at a 1.6% organic matter. Fertilization was applied based on soil analysis results. The soil pH (H_2O) was 6.8–7.4), while the available nutrient content in 1 dm^3 was the following: 30 mg $N\text{-}NO_3$, 190–220 mg P, 120–140 mg K, 1100–1270 mg Ca, 100–125 Mg.

The experimental material consisted of white-flowered varieties of common bean – 5 cultivars: 'Aura', 'Igołomska', 'Laponia', 'Longina', and 'Wiejska', and of runner bean – 6 cultivars: 'Blanka', 'Felicja', 'Karo', 'Kontra', 'Westa', and 'Piękny Jaś'. All the bean cultivars studied are domestically bred varieties with white-colored seeds. The seed material came from the following seed breeding and production companies: CNOS Przedsiębiorstwo Nasiennictwa Ogrodniczego sp. z o.o. w Poznaniu – 'Aura'; Krakowska Hodowla i Nasiennictwo Ogrodnicze POLAN sp. z o.o. – 'Igołomska', 'Longina', 'Piękny Jaś'; PlantiCo Hodowla i Nasiennictwo Ogrodnicze Zielonki sp. z o.o. – 'Laponia'; Hodowla Roślin Snowidza sp. z o.o. – 'Wiejska'; PlantiCo Hodowla and Nasiennictwo Ogrodnicze Gołębiew sp. z o.o. – 'Blanka', 'Westa'; „Vera-Agra" sp. z o.o. w Guzowicach – 'Felicja'; PlantiCo Hodowla and Nasiennictwo Ogrodnicze Zielonki sp. z o.o. – 'Kontra'; SPÓJNIA Hodowla i Nasiennictwo Ogrodnicze sp. z o.o. w Nochowie – 'Karo'.

The experiment was designed as a one-factor experiment in triplicate. Seeds were dressed (the seed dressing T 75 DS/WS + Nomolt 150 SC) and sown in the first 10 days of May at a spacing of 45 cm × 10 cm or 60 cm × 50 cm (runner bean) in 6.75 m^2 plots: length – 5 m and width –1.53 m. After emergence, thinning was done, leaving one plant in each spot. Cv. 'Piękny Jaś' runner bean plants were trained using strings tied to trellises, which were installed in the field at the beginning of June.

During the bean growing season, no crop protection agents were used and weeds were removed by hand. The ripening of common bean occurred in the middle of August, while in the case of runner bean in the second and third 10 days of September. During harvest, the bean plants were cut and initially dried in the field, while subsequently they were dried in a shaded and well-aired shed. After the plants were completely dried, bean pods were stripped from the stem by hand and seeds were also removed from the pods by hand. Pericarp samples for laboratory testing met the requirements for herbal raw material. Bean pericarp of all the cultivars was characterized by uniform color without any spots or damage as well as by a unique bean odor. It was stored in paper bags (500 g each) at a temperature of 15–18°C, protected from light, moisture and foreign odors. The morphological characteristics of the pericarp based on a random sample of 30 pericarps of the studied bean cultivars are shown in Table 1.

Chemical analysis

Chemicals and reagents. All of the chemicals used in this study were purchased from Sigma-Aldrich Chemical Co. (France) and/or Merck Com-

pany (Germany). A Hitachi U-2900 spectrophotometer was used for absorbance measurements.

Total phenolic acids estimation. Total phenolic acids estimation was carried out according to Arnov method [Polish Pharmacopoeia VI 2002]. One milliliter of sample was mixed with 5 ml of distilled water, 1 ml 0,5 M HCl, 1 ml of Arnov reagent and 1 ml 1M NaOH and subsequently completed to 10 ml with distilled water. The absorbance was measured at 490 nm. The total phenolic acid content was expressed as caffeic acid equivalent (CAE).

Total flavonoids estimation. Total flavonoids were estimated according to the spectrophotometric method according to Christ and Müller [Polish Pharmacopoeia IX 2011], expressed as quercetin equivalent (QE). After 45 min the absorbance at 425 nm was measured.

Total condensed tannin contents. The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent, 1 ml of 35 % Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 μg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of % of GAE of extract [Polish Pharmacopoeia IX 2011].

Anti-oxidation activity. Anti-oxidation activity (%) was evaluated on a base of the ability to neutralize the DPPH radicals by means of spectrophotometry according to Chen and Ho (1997): to do this, water extracts were prepared from raw material, extracts were then evaporated till dry and lyophilized. Analyses were performed for 20 $\mu g \cdot ml^{-1}$ concentration. The absorbance measurements were made at λ = 517 nm after 30 minutes wavelength using spectrophotometer UVIKON 932 (Canberra Packard). The percent inhibitio n of DPPH radical was calculated as:

$$DPPH \cdot \text{inhibition } (\%) =$$

$$= [(Abs_0 - Abs_{30})/Abs_0] \times 100\%$$

where: Abs_0 is the absorbance of the control and Abs_{30} is the absorbance of the sample after 30 min.

Statistical analysis

The results obtained from the study conducted during the period 2010–2011 were at a similar level, both in terms of the morphological characteristics of the bean pericarp and laboratory tests, and that is why the two-year means are presented in this paper. The results were statistically analyzed as one-factor results by analysis of variance and T-Tukey's range test, at a significance level of 5%. Statistical calculations were made using Statistica 13.1 software.

RESULTS AND DISCUSSION

The bean pericarp, *Phaseoli Pericarpium*, was characterized by significant variability of its morphological characteristics – weight, length and width as well as color, depending on the varietal and specific characters (tab. 1). In common bean, the pericarp weight (air-dry) ranged from 0.54 g in cv. 'Aura' to 0.79 g in cv. 'Laponia', whereas the average weight for this species was 0.69 g. The pericarp in the 'Laponia' cultivar had the lowest length – 11.0 cm, but it was characterized by the largest width – 10.0 mm. The pericarp of cv. 'Igołomska' was longest – 13.3 cm, but its pods were the most narrow – 5.9 mm. The pericarp of the runner bean cultivars showed significantly higher parameters in terms of weight and width compared to the pericarp of common bean. The weight of the runner bean pericarp was from 0.73 g in cv. 'Felicja' to 1.33 g in cv. 'Piękny Jaś', whereas the pericarp width was from 13.3 mm in cv. 'Felicja' to 19.6 mm in cv. 'Piękny Jaś'. On average for the runner bean cultivars, the pericarp weight was 0.96 g, the width 15.9 mm, while the pericarp length 11.4 cm, and on average this latter parameter was significantly smaller than the average for the common bean cultivars, in the case of which it was 12.2 cm. Bean pericarp color at physiological maturity stage varied depending on the varietal traits and was characteristic for the genetic characters of the cultivars. The bean pericarp, *Phaseoli Pericarpium*, of common bean was characterized by lighter color, that is, from bright crème to sand/crème, compared to

Table 1. Morphological characteristics of the studied bean pericarp cultivars

Species/Cultivar	*Phaseoli Pericarpium*			
	Weight (g) mean ± SD	Length (cm) mean ± SD	Width (mm) mean ± SD	Color
Common bean				
Aura	0.54 ±0.09	12.0 ±0.6	9.3 ±1.1	bright creme
Igołomska	0.72 ±0.16	13.3 ±1.0	5.9 ±0.9	bright creme
Laponia	0.79 ±0.10	11.0 ±0.8	10.0 ±1.4	yellow/creme
Longina	0.66 ±0.16	12.1 ±1.2	9.5 ±1.3	bright creme
Wiejska	0.73 ±0.11	12.5 ±0.9	9.2 ±1.0	sand/creme
Runner bean				
Blanka	0.75 ±0.15	10.5 ±0.8	14.0 ±2.2	bright brown
Felicja	0.73 ±0.14	10.2 ±1.0	13.3 ±2.1	sand
Karo	0.84 ±0.15	10.2 ±1.0	13.3 ±2.1	sand yellow
Kontra	1.16 ±0.33	12.6 ±1.4	18.6 ±2.7	sand/creme
Westa	0.92 ±0.16	11.0 ±0.9	15.9 ±2.4	yellow/creme
Piękny Jaś	1.33 ±0.19	13.3 ±1.3	19.6 ±2.3	bright brown
$LSD_{0.05}$	0.18	1.19	1.9	
Mean common bean	0.69 ±0.15a	12.2 ±1.3a	8.9 ±1.9a	
Mean runner bean	0.96 ±0.29b	11.4 ±1.3b	15.9 ±3.1b	

Means in the same column with different letters are significantly different $p \leq 0,05$ (Tukey test)
*SD – standard deviation

Table 2. Dry matter content of *Phaseoli pericarpium* cultivars

Species/Cultivar	Dry mater (%)			
	mean ± SD	min	max	V (%)
Common bean				
Aura	91.03 ±0.16	90.79	91.25	0.17
Igołomska	91.01 ±0.33	90.52	91.54	0.37
Laponia	90.08 ±0.26	89.61	90.40	0.29
Longina	91.90 ±0.11	91.78	92.10	0.13
Wiejska	91.69 ±0.54	90.92	92.31	0.59
Runner bean				
Blanka	91.20 ±0.32	90.63	91.56	0.35
Felicja	92.60 ±0.32	92.27	93.03	0.35
Karo	92.70 ±0.20	92.33	92.95	0.22
Kontra	92.65 ±0.23	92.35	92.94	0.25
Westa	92.73 ±0.19	92.55	93.09	0.21
Piękny Jaś	92.81 ±0.18	92.66	93.06	0.19
$LSD_{0.05}$	0.54			

* SD – standard deviation
V – variability coefficient

Table 3. Total phenolic acid content expressed as caffeic acids equivalents (mg·g^{-1}) in the dry matter of *Phaseoli pericarpium* cultivars

Species/Cultivar	Total phenolic acid (mg·g^{-1})			
	mean ± SD	min	max	V (%)
Common bean				
Aura	0.11 ±0.08	0.04	0.19	71.7
Igołomska	0.14 ±0.05	0.09	0.20	39.3
Laponia	0.18 ±0.02	0.15	0.20	10.1
Longina	0.17 ±0.03	0.14	0.21	17.5
Wiejska	0.11 ±0.06	0.06	0.18	57.4
Runner bean				
Blanka	0.25 ±0.03	0.22	0.29	11.6
Felicja	0.33 ±0.09	0.23	0.42	30.1
Karo	0.20 ±0.01	0.18	0.22	7.0
Kontra	0.21 ±0.01	0.20	0.23	5.7
Westa	0.23 ±0.01	0.22	0.25	3.6
Piękny Jaś	0.27 ±0.07	0.18	0.34	27.6
$LSD_{0.05}$	0.016			

* SD – standard deviation
V – variability coefficient

Table 4. Flavonoid content expressed as quercetin equivalents (mg·100 g^{-1}) in the dry matter of *Phaseoli pericarpium* cultivars

Species/Cultivar	Flavonoid (mg·100 g^{-1})			
	mean ± SD	min	max	V (%)
Common bean				
Aura	9.2 ±1.8	7.5	10.9	20.1
Igołomska	9.3 ±2.2	6.5	11.3	24.0
Laponia	13.2 ±2.7	10.7	15.9	20.6
Longina	8.1 ±1.7	6.6	9.7	20.5
Wiejska	8.7 ±2.1	6.7	10.8	24.5
Runner bean				
Blanka	9.8 ±1.8	8.0	11.5	18.6
Felicja	10.8 ±0.4	10.5	11.6	3.8
Karo	7.1 ±4.4	3.1	11.3	61.9
Kontra	5.8 ±4.9	1.1	10.4	85.6
Westa	8.8 ±1.6	7.3	10.7	18.6
Piękny Jaś	6.4 ±4.5	1.9	10.7	69.7
$LSD_{0.05}$	0.6			

* SD – standard deviation
V – variability coefficient

the pericarp of the runner bean cultivars with yellow/crème to bright brown color. The pericarp dimensions and weight in the bean cultivars varied depending on the investigated cultivars of common bean and runner bean, which is justified by and strictly associated with the usefully important traits of dry bean seeds, i.e. seed size, expressed by thousand seed weight, and seed shape. A study [Łabuda and Witek 2004] shows that the thousand seed weight of common bean cultivars ranges 305–650 g; in cv. 'Igołomska' it was 392 g, while for cv. 'Longina' it was highest – 650 g. Likewise, for the runner bean species the differences in seed size are large, depending on the cultivar, and the thousand seed weight is from 995 g (cv. 'Felicja') to 1805 g (cv. 'Piękny Jaś'). The study also revealed that the coefficient of variation *V* of thousand seed weight was 28.3% for the common bean species and 22.7% for the runner bean species.

The conducted analysis of dry matter content in the pericarp of the bean cultivars shows low variation in this trait (tab. 2). The dry matter content ranged from 91.01% to 92.81%; it was lowest for the pericarp of cv. 'Igołomska' (common bean) and highest for cv. 'Piękny Jaś' (runner bean). The variation in pericarp dry matter content of the bean cultivars studied was low; the coefficient of variation (*V*) was from 0.13% for cv. 'Longina' to 0.59% for cv. 'Wiejska'.

The results of the conducted laboratory tests of the bean pericarp, *Phaseoli Pericarpium*, revealed that phenolic acid content (expressed as caffeic acid equivalents) significantly varied, depending on the studied cultivars belonging to the different species (tab. 3). The phenolic acid content in the bean pericarp of all the studied cultivars complied with the requirements of Polish Pharmacopoeia and it was at least 0.1 mg·g^{-1}, i.e. 0.01%. On average over the study period, the phenolic acid content in the common bean pericarp ranged from 0.11 mg·g^{-1} DM in the cultivars 'Aura' and 'Wiejska' to 0.18 mg·g^{-1} DM in cv. 'Laponia'. A higher phenolic acid content was found for the runner bean pericarp, which was from 0.20 mg·g^{-1} DM in cv. 'Karo' to 0.33 mg·g^{-1} DM in cv. 'Felicja'. The calculated coefficients of variation *V* of phenolic acid content in the pericarp of over a dozen bean cultivars varied (tab. 3). The highest values of the variation coefficient *V* were found for the common bean cultivars 'Aura' and 'Wiejska', which was 71.7 and 57.4%, respectively. The variation in phenolic acid content in the pericarp, *Phaseoli Pericarpium*, of runner bean showed lower values, as the coefficient of variation *V* ranged 3.6–30.1%. Among the runner bean cultivars evaluated, the highest values of the coefficient of variation *V* of pericarp phenolic acid content were found for the cultivars 'Felicja' – 30.1% and 'Piękny Jaś' – 27.6%. The study results demonstrated that the level of phenolic acid content in the bean pericarp was lower compared to the results of the previous study which had evaluated the pericarp of some of the common bean and runner bean cultivars investigated in the present study [Łabuda and Papliński 2004]. These authors showed that the pericarp phenolic acid content in different bean cultivars was on average 0.34–0.45 mg·g^{-1}, depending on year; a higher content was found under less favorable conditions during the pod maturation period as well as in the case of occurrence of pathogen-induced plant infections and delayed harvest. Synthesis and accumulation of secondary metabolites in the plant is largely dependent on environmental factors – temperature, precipitation and UV radiation during the growing season, which may have a modifying effect on the content of biologically active substances [Strzałka 2002]. In the bean pericarp, *Phaseoli Pericarpium*, free hydroxybenzoic acids – p-hydroxybe- nzoic acid, vanillic acid, and β-resorcylic acid, have been identified by HPLC [Łabuda and Papliński 2004].

Flavonoid content expressed as quercetin equivalents in the bean pericarp, *Phaseoli Pericarpium*, significantly varied depending on the varietal and specific traits of bean (tab. 4). The study also demonstrated a high variation, as expressed by the coefficient of variation *V*, in the content of this compound in the pericarp, with the highest values found for the runner bean cultivars 'Kontra', 'Piękny Jaś', and 'Karo'. The values of the coefficients of variation *V* were 85.6, 69.7 and 61.9%, respectively. The flavonoid content in the bean pericarp ranged from 5.8 mg·100 g^{-1} DM (cv. 'Kontra', runner bean) to 13.2 mg·100 g^{-1} DM (cv. 'Laponia', common bean). In the other common bean cultivars, the content of this

Table 5. Total tannin content (%) in the dry matter of *Phaseoli pericarpium* cultivars

Species/Cultivar	Total tannin (%)			
	mean ± SD	min	max	V (%)
Common bean				
Aura	2.71 ±0.37	2.10	3.22	13.9
Igołomska	2.74 ±0.46	1.93	3.19	16.9
Laponia	3.43 ±0.70	2.61	4.19	20.4
Longina	2.93 ±0.09	2.82	3.04	3.4
Wiejska	2.49 ±0.32	2.11	2.90	12.7
Runner bean				
Blanka	2.39 ±0.31	2.06	2.76	12.9
Felicja	2.72 ±0.48	2.06	3.18	17.8
Karo	2.48 ±0.35	2.00	2.90	14.2
Kontra	2.91 ±0.19	2.68	3.15	6.7
Westa	2.77 ±0.42	2.25	3.21	15.4
Piękny Jaś	2.73 ±0.35	2.28	3.10	12.7
$LSD_{0.05}$	0.55			

* SD – standard deviation
V – variability coefficient

Table 6. Antioxidant activity (AA) of *Phaseoli pericarpium* extract determined by the DPPH on depending of cultivars

Species/Cultivar	AA by DPPH inhibition (%)			
	mean ± SD	min	max	V (%)
Common bean				
Aura	11.01 ±0.19	10.85	11.22	1.73
Igołomska	9.99 ±0.08	9.79	10.14	1.80
Laponia	12.78 ±0.12	12.65	12.91	0.99
Longina	9.94 ±0.18	9.79	10.15	1.83
Wiejska	9.44 ±0.09	9.34	9.53	1.01
Runner bean				
Blanka	16.18 ±0.18	15.97	16.29	1.13
Felicja	11.78 ±0.11	11.66	11.90	1.00
Karo	15.83 ±0.15	15.67	15.99	1.00
Kontra	11.52 ±0.06	11.45	11.57	0.52
Westa	14.86 ±0.23	14.59	15.07	1.60
Piękny Jaś	18.97 ±0.26	18.38	18.90	1.42
$LSD_{0.05}$	0.208			

* SD – standard deviation
V – variability coefficient

Table 7. Comparison of secondary metabolites content of *Phaseoli pericarpium* and antioxidant activity their extract of common bean (*Phaseolus vulgaris* L.) and runner bean (*Phaseolus coccineus* L.)

Compounds	Species	Means ± SD	V (%)
Dry mater (%)	Common bean	91.15 ±0.72	0.79
	Runner bean	92.45 ±0.61	0.67
Total phenolic acid (CAE) ($mg \cdot g^{-1}$ DM)	Common bean	0.14 ±0.06	40.86
	Runner bean	0.25 ±0.06	25.68
Total flavonoids (QE) ($mg \cdot 100g^{-1}$ DM)	Common bean	9.69 ±2.69	27.84
	Runner bean	8.11 ±3.66	45.10
Total tannin (GAE) (% DM)	Common bean	2.86 ±0.52	18.13
	Runner bean	2.67 ±0.38	14.34
AA by DPPH Inhibition (%)	Common bean	10.63 ±1.24	11.66
	Runner bean	14.81 ±2.59	17.49

* SD – standard deviation,
V – variability coefficient

compound in the pericarp was 8.1–9.3 $mg \cdot 100\ g^{-1}$ DM, whereas the value of the coefficient of variation *V* ranged 20.1–24.5%. Flavonoids belong to secondary metabolites that commonly occur in the plant world, but the content of these biologically active compounds is low, ranging 15–30 mg·kg FM, except for *Alium cepa* L., for which it is up to 1.5 $g \cdot kg^{-1}$ [Miean and Mohamed 2001]. Oomah et al. [2015] revealed a significant variation in the content of flavonoids (quercetin, kaempferol and myricetin) in bean seeds depending on the genetic characteristics of the cultivars. Moreover, it was confirmed that high temperature and high sunshine duration during the reproductive stage of common bean had a significant effect on increased accumulation of bioactive substances in common bean seeds.

Phenolic content in bean seeds is variable, depending on the seed part (seed coat, cotyledons) and seed color. Colored-seeded bean cultivars are characterized by a greater concentration of phenolic compounds, which are predominantly accumulated in the seed coat. It has also been shown that the antioxidant activity of extracts from the seed coat of colored-seeded cultivars is generally higher [Drużyńska 2002, Drużyńska and Klepacka 2004, Łabuda and Papliński 2007, Stasiak and Ulanowska 2008, Oomah et al. 2010].

Tannin content in the bean pericarp, *Phaseoli Pericarpium*, significantly varied depending on the cultivar and species (tab. 5). The tannin content in the pericarp was from 2.39% DM in cv. 'Blanka' (runner bean) to 3.43% DM in cv. 'Laponia' (common bean). The tannin content in the common bean pericarp was higher and ranged 2.49–3.43% DM, whereas in the runner bean pericarp it was 2.39–2.91% DM. The calculated coefficients of variation *V* exhibit low variation in pericarp tannin content in the cultivars 'Longina' (common bean) and 'Kontra' (runner bean), and the values of these coefficients of variation *V* were 3.4 and 6.7%, respectively. It was also demonstrated that cv. 'Laponia', with the highest tannin content in the pericarp, was at the same time characterized by the greatest variation in the accumulation of this compound, with the coefficient of variation standing at 20.4%.

The results of investigation of the antioxidant activity of extracts from the bean pericarp, *Phaseoli Pericarpium*, of over a dozen cultivars in scavenging DPPH free radicals are shown in Table 6. The antioxidant activity of these bean pericarp extracts was found to range 9.53–18.90%, and it was lowest in the case of the pericarp extract of cv. 'Wiejska' (common bean) and highest for the pericarp extract of cv.

'Piękny Jaś' (runner bean). The variation in antioxidant activity of the pericarp extracts of the different cultivars was low, with the coefficient of variation *V* standing at 0.52–1.83%.

Table 7 shows a comparison of the study results concerning the average content of biologically active substances in the pericarp of the different cultivars for the two species in question – *Phaseolus vulgaris* L. and *Phaseolus coccineus* L. On average over the two-year study period for 5 common bean cultivars and 6 runner bean cultivars, the study results, based on an analysis of the plant material, *Phaseoli Pericarpium*, showed that the content of phenolic acids, flavonoids and tannins exhibited high and variable variation depending on the bean species. The common bean pericarp showed greater variation in the content of phenolic acids, with the coefficient of variation *V* standing at 40.86%, whereas the average content of these compounds was lowest – 0.14 $mg \cdot g^{-1}$ DM. The pericarp of runner bean was characterized by a significantly higher average content of phenolic acids – 0.25 $mg \cdot g^{-1}$ DM, but lower variation in the content of these compounds was found, with the coefficient of variation *V* standing at 25.68%. The average flavonoid content in the runner bean pericarp was slightly lower (8.13 $mg \cdot 100g^{-1}$ DM) than in common bean and the coefficient of variation *V* (45.10%) in flavonoid content in the pericarp of this species was also shown to be high. The average tannin content in the pericarp of the species compared was at a similar level (2.67–2.86% DM), but common bean was found to have higher capacity to accumulate these compounds and higher variation in their accumulation was revealed. The antioxidant ability of the bean pericarp extracts to scavenge DPPH free radicals was slightly higher in the case of runner bean (14.81%) but, at the same time, the pericarp extracts of this species were shown to have higher variation in antioxidant activity.

CONCLUSIONS

The bean pericarp, *Phaseoli Pericarpium*, was characterized by a high content of secondary metabolites being biologically active substances – phenolic acids, flavonoids and tannins, whereas the pericarp extracts also exhibited antioxidant activity in scavenging DPPH free radicals. According to Polish Pharmacopoeia, the content of phenolic acids expressed as caffeic acid equivalents has a determining effect on the usefulness of *Phaseoli Pericarpium* as a pharmacopoeial material, but the bean pericarp of different cultivars exhibits high variability in terms of their content. The pericarp of common bean was found to show high variation (*V* – 40.8%) in phenolic acid content, whereas the runner bean pericarp was characterized by high variation *V* (45.1%) in flavonoid content. The content of tannins in the pericarp of the bean species compared was at a similar level, while the variation *V* in the content of these compounds ranged 11.6–17.4%. Among the common bean cultivars studied, the pericarp of cv. 'Laponia' showed the greatest accumulation of biologically active substances – phenolic acids (CAE) – 0.18 $mg \cdot g^{-1}$ DM, flavonoids (QE) – 13.2 $mg \cdot 100\ g^{-1}$ DM, and tannins (GAE) 3.43% DM. Likewise, the pericarp of the 'Felicja' runner bean cultivar stood out in terms of the accumulation of these compounds, as it contained the following amounts: phenolic acids – 0.33 $mg \cdot g^{-1}$ DM, flavonoids (QE) – 10.8 $mg \cdot 100\ g^{-1}$ DM, and tannins (GAE) – 2.72% DM. The results of the present study show that further detailed experiments need to be conducted in this area.

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