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ORIGINAL PAPER

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ELLAGIC ACID CONTENT IN SELECTED WILD SPECIES OF FRUIT ROSES

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ABSTRACT

Ellagic acid (EA) is a natural antioxidant, belonging to the group of polyphenolic compounds. It displays a broad spectrum of pro-health effects, ranging from the prevention of cancer to antiviral properties. It is present in many fruit from the rose family (*Rosaceae*): strawberries, raspberries, blackberries and walnuts as well as cranberries and grapes. The available literature states that the fruit of roses, in addition to their aesthetic and functional applications, also show therapeutic properties, which, among other things, are associated with a high content of polyphenols, including ellagic acid derivatives. The aim of this research was to determine the differences in the content of free ellagic acid in the fruit of selected rose species. The test material consisted of freeze-dried sublimation and ground fruit from the following species of roses: *R. canina*, *R. moyesii*, *R. pendulina.* The fruit was separated into two fractions: flesh and seeds. Quantitative analysis of free ellagic acid was carried out by spectrofluorimetry. The free EA was present both in the flesh and in the seeds of the studied species of roses. Its content in the mentioned fractions varied depending on the species. The flesh of *R. pendulina* was characterised by the highest level of EA in free form (247.72 $\mu g \cdot g^{-1}$ of dry weight). The seeds of *R. moyesii* proved to be the most abundant in free EA (105.69 $\mu g \cdot g^{-1}$ of dry weight).

Key words: *Rosa* L., wild rose, rose fruits, medicinal plant, ellagic acid, spectrofluorimetric analysis

INTRODUCTION

The growing expectations of consumers and the competitive market force fruit producers to offer new products that are attractive in terms of their sensory and pro-health benefits. Food processors often reach for little known exotic fruit. Meanwhile, in Poland we have many undervalued and forgotten plant species. They usually grow wild or are grown in home gardens mainly for their decorative qualities. Some of them were very popular in home food processing a few decades ago. Currently we have refocused our interest on these plants, especially because of their strong antioxidant properties. They produce fruit which, in their composition, include flavonoids and polyphenols which are considered as anti-cancer agents. Such fruit include Japanese quince (*Chaenomeles japonica* (Thunb.) Lindl. ex Spach.), dogwood (*Cornus mas* L.), ginkgo biloba (*Ginkgo biloba* L.), one and two-seeded hawthorn (*Crataegus monogyna* Jacq., *Crataegus laevigata* (Poir.) DC.), elderberries (*Sambucus nigra* L.) and wild roses [Nowak 2006, Laskowska and Pogorzelski 2007, Buchwald et al. 2007, Leja et al. 2007, Stoilova et al. 2007, Gasik et al. 2008, Fecka 2009, Zawirska-Olszańska et al. 2010, Kostić et al. 2012, Teleszko et al. 2012, Bratu et al. 2012, Milala et al. 2013,

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Tahirović and Bašić 2014, Doroszko et al. 2018]. At the present time, due to the great interest in ellagic acid, wild roses deserve attention as the fruit of these shrubs are characterised by a high abundance of EA.

The genus *Rosa* L. includes about 200 species of shrubs and vines located in the temperate zone of the Northern Hemisphere. Most wild rose varieties occur in China. However, these shrubs do not only grow in Asia, but also in Europe, especially in the Mediterranean Basin, and in North America [Ercisli et al. 2007, Meyer 2008]. Approximately 30 native species of roses were recorded in Europe, of which over half of them were present in Poland [Zieliński 1987, Nowak 2006, Wissemann et al. 2006, Popek 2002, Popek 2007, Buchwald et al. 2007, Kobus and Pogorzelski 2008, Nowak 2011, Cendrowski et al. 2012, Monder 2017].

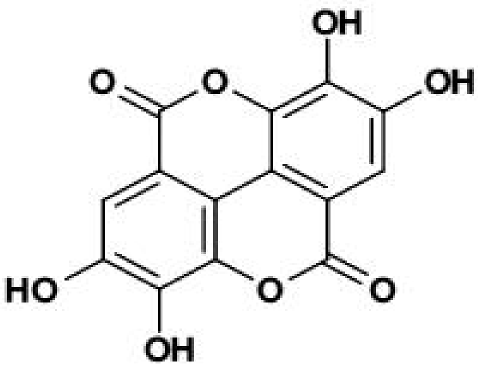

Fig. 1. Ellagic acid – a natural phytochemical compound from the polyphenol group found in the fruits of wild roses

The great interest of rose hip is observed in Poland. The main recipients of this raw material are the food and processing as well as phytopharmaceutical and cosmetics industries. In domestic herbal cultivars, rose plants bearing fruit do not represent a large share – the area of crops is currently estimated at approximately 250 ha. However, an upward trend is observed in this industry, as the data from 1999 shows the area of cultivation in the range of 150–200 ha. These fruit are obtained mostly from natural habitats, while the demand for them is constantly growing [Marosz 2016].

The geographical distribution and share of particular species in Polish flora are very varied. *Rosa pendulina* – alpine rose is predominantly a mountain species, growing spontaneously in the Alps. In Poland, it occurs in the Bieszczady, Sudetes and Tatra mountains. The remaining species grow mainly in the lowlands. The most common taxon covering the entire country is *R. canina* – dog rose. It grows widely on the boundaries, roadsides and wasteland, both in thickets and on the edge of forests. However, *R. moyesii* – Moyes rose is a wild species originating from China. A rarely occurring species limited to the northern and western part of the country and foothill regions. It grows in thickets on the banks of lakes and rivers, and on the sunny outskirts of forests [Henker and Rosa 2000, Zieliński and Popek 2001, Popek 2002, Król et al. 2012].

Nowadays, we have a wide range of various preparations used to treat an ever-increasing number of diseases. The cancer problem has affected civilisation as a whole, although many of its variants are already well recognised and methods of its effective treatment have been developed. In this era of looking for new drugs, there is an increased interest in herbal preparations that often contain a mixture of various active compounds which function harmoniously. Recently, much attention has been devoted to flavonoids acting as anti-cancer agents [Ball 2000, Birt et al. 2001, Taylor 2005, Conclin et al. 2007, Chen and Ping Dou 2008]. Another active substance with a multidirectional and beneficial effect on the human body is ellagic acid (EA), belonging to the group of polyphenols (Fig. 1) [Sroka and Cisowski 2003, Beliveau et al. 2005, Komańska and Żesławska 2012, Komańska and Żesławska 2013, Krauze-Baranowska et al. 2014, Baranowska et al. 2015]. This compound occurs in plants in free form and (more often) as ester-linked with glucose, forming hydrolysing tannins (ellagotannins) [Kwiatkowska 2010, Milala et al. 2013, Nurzyńska-Wierdak 2016]. The sources of ellagic and ellagotannins include oak, walnut and chestnut wood, some seeds, nuts, pomegranates, ginkgo, dogwood and, above all, berries: gooseberries, blueberries, cranberries, aronia and rose family fruit, e.g. strawberries, blackberries and raspberries [Chapus et al. 2005, Soong and Barlow 2006, Nowak 2006a, Mohan Rao et al., 2007, Aguilera-Carbo et al. 2008, Kwiatkowska 2010, Montazeri et al. 2011, Gherib 2011, Kucharska 2012, Teleszko et al.

2012, Deng et al. 2013, Zalega and Szostak-Węgierek 2013, Baraniak and Kania 2015].

Numerous studies have shown that ellagic acid has antiatherosclerotic and anti-inflammatory effects, as well as displays antiproliferative and antioxidant properties, which makes it an interesting research subject on its potential health benefits on humans. As a natural plant ingredient it has unique multidirectional phytopharmacological effects and promising antioxidant properties [Özkan et al. 2004, Adams et al. 2010, Gao et al. 2011, Wang et al. 2012, Parus 2013, Mármol et al. 2017]. It was also found to be a potent dietary inhibitor of carcinogenesis, lowering the risk of cancer, hence its content in plant products was and still is of interest to scientists [Mohan Rao et al. 2007, Gaber et al. 2009, Bagavan et al. 2012]. Some authors also report on the coordinated action of ellagic acid in combination with some of the medicines used to treat malaria [Soh et al. 2009].

In conclusion, the results so far have proved that EA undoubtedly has a beneficial effect on human health. It also presents many positive properties in the treatment of various diseases. The antioxidant, antimalarial and antineoplastic effects of this molecule are the most promising.

Positive research results on the effectiveness of pro-health effects of ellagic acid prompt a search for new, natural sources of this valuable compound. Taking into account the fruit with the highest content of EA, the attention should be mainly given to fruit-bearing roses. The ellagotannins contained in the fruit of these plants is present in considerable concentrations [Nowak 2006, Buchwald et al. 2007, Fecka 2009, Teleszko et al. 2012, Milala et al. 2013].

Most often, the raw material is obtained from dog roses (*R. canina*), as well as wrinkled rose (*R. rugosa*), *R. hybrida* 'Konstancin', *R. pomifera* 'Karpatia', apple rose (*R. villosa*), glaucous dog rose (*R. dumalis*) and others. This raw material is treated as equivalent [Kozłowski et al. 2009, Milala et al. 2013, Marosz 2016], however, the detailed comparative researches indicates large interspecies phytochemical diversity within the genus [Nowak 2006a, Adamczak et al. 2011, Adamczak et al. 2012, Król et al. 2012, Rutkowska et al. 2012, Jiménez et al. 2017].

Thus far, the pseudo-fruit of rose was treated mainly as a valuable raw material in terms of phytochemical. However, rose seeds (true fruit, achenes, nuts), according to scientific reports, were often considered as waste material [Őzcan 2002, Nowak 2005, Ercisli et al. 2007, Barros et al. 2010]. Insightful analyses performed by scientists have proven that this raw material is a valuable source of, among other things, fatty acids and ellagic acid [Nowak 2005, Nowak 2006, 2006a, Milala et al. 2013]. Rose petals are also a source of EA [Nowak and Gawlik-Dziki 2007, Ochir et al. 2010, Nowak et al. 2014, Zawiślak and Michalczyk 2015]. Nowak and Gawlik-Dziki [2007] the presence of EA in the leaves of roses has also been proven.

The great importance of the rose for both the food and pharmaceutical industry justifies the need for conducting the interdisciplinary researches that will allow the designation of a form or variety containing a particularly high content of biologically active compounds.

The aim of the research was to determine the differences in the content of free ellagic acid in the true (hard achenes) and pseudo-fruits (fleshy hypanthia) of the selected species of wild roses.

MATERIAL AND METHODS

Research material. Fruit from selected species of wild roses were used in this research*: R. canina, R. moyesii, R. pendulina*. The choice of wild rose species was prompted by reports from the scientific literature which revealed that research material prepared from wild plants or plants grown in organic farming show a higher content of polyphenols [Nowak 2006a, Wojdyło 2010, Cendrowski et al. 2012].

The research material came from the Experimental Farm of Felin Department of Ornamental Plants and Landscape Architecture in Lublin (Poland, 51°23'N, 22°56'E) – *R. canina* and from the UMCS Botanical Garden in Lublin (Poland, 51°16'N, 22°30'E) – *R. moyesii, R. pendulina*. The fruit were collected between the third decade of September and first decade of October in 2016 in their maturity phase, i.e. at the time of complete ripeness, growth and colouring of fruit with peduncles easily detaching from the fruit-bearing stalk. The research material was separated into two fractions: fleshy hypanthia and hard achenes. The raw material prepared in this manner was dried by lyophilisation using the Alpha 1–4 type equipment from Martin Christ (Germany). The choice of drying method was dictated by reports from the literature which indicated that lyophilisation is a more effective method for obtaining levels of organic acids and polyphenols [Aaby et al. 2005, Adamczak et al. 2010, Rutkowska et al. 2012]. The content of free acid in the pseudo-fruit of roses is expressed in μg of EA in a gram of lyophilisate.

Before sublimation the fruit were frozen in nitrogen until the temperature reached –20°C, followed by a process using sublimation at a temperature of 33°C during a course of 22.5 hours. Three samples were made in the drying process. The dried material was ground using a laboratory grinder.

In the obtained research material, the differences in the content of free ellagic acid in the fruit – achenes and of pseudo-fruit – hypomas were determined by the species of roses submitted for analysed.

Spectrofluorimetric analysis

Analysis was conducted using the spectrofluorimetric method based on fluorescence of ellagic acid-borax complex in methanolic solution [Sádecká and Tóthová 2012]. The extraction of free ellagic acid from rose matrix was adjusted to spectrofluorimetric analysis, because the ellagic acid do not have native fluorescence.

Reagents. Ellagic acid standard in grade ≥98% was purchased from LKT, Laboratories, Inc (USA), borax (sodium tetraborate decahydrate) was used in an analytical reagent grade (Chempur, Poland) and methanol was used in gradient grade (Merck, Germany).

Sample preparation. The extracts of lyophilised and powdered plant material (0,5g) were prepared in triplicate in 50 ml plastic Falcon type tubes with 25 ml of 0.04 $mol \cdot l^{-1}$ borax solution in methanol. The following procedure was applied: 1 min hand-shaken, 30 sec vortex-shaken, 15 min see-saw rocker at 70 oscillations per minute (OPM) (Stuart SSL4), and then 5 min centrifuging at 8.000 rpm (Eppendorf 5804). Collected supernatants were transferred to spectrofluorimetric analysis.

Spectrofluorimetric analysis. Due to matrix effect, to analysis of obtained methanolic extracts of ellagic acid-borax complex the method of standard additions was applied. Four levels of ellegic acid concentrations, i.e. 0.1, 0.5, 1.0 and $1.5 \cdot 10^{-6}$ $mol \cdot l^{-1}$, were spiked to adequate diluted plant material extracts. Analysis was performed with using of spectrofluorimeter F-2000 Hitachi (Japan) in 10 × 10 × 45 quartz cuvette (Hellma). The fluorescence intensity was measured in triplicate after excitation at wavelenght λ_{ex} = 383 nm, and emission λ_{em} = 456 nm [Huerga-González et al. 2015, Sádecká and Tóthová 2012]. The concentration of ellagic acid-borax complex was calculated from linear curve, related to dilution of extracts and weight of plant lyophylisate, and expressed as microgram of free ellagic acid to gram of lyophilisate [$\mu g \cdot g^{-1}$]. The illustrative graph of linear relationship between the intensity of fluorescence radiation of spiked borax-methanolic sample extract and ellagic acid-borax concentration is presented on Figure 2.

RESULTS AND DISCUSSION

The potential of rose hips is based on its antioxidant effects caused or associated with its phytochemical composition, which includes, inter alia, ellagic acid. This biologically active compound is in turn an important source of medicinal products that prevent, alleviate or treat many diseases [Nowak 2006, Fecka 2009, Milala et al. 2013, Jiménez et al. 2017]. The flesh of roses from the research, contained on average 36.71–247.72μg·of free ellagic acid g^{-1} of dry weight. In contrast, true fruit (achenes) 26.39–105.69 $\mu g \cdot g^{-1}$ of dry weight.

The results obtained coincide with the data published by Nowak [2006]. The author researched 14 varieties of hypanthia roses from the Lublin region. The free ellagic acid in the analysed raw material was at a much higher level corresponding to 101.1–631.3 $\mu g \cdot g^{-1}$ of dry weight. However, the author conducted researched on other rose taxon, and the literature states that depending on the species and varieties of roses, the proportions of the content of individual components, and thus the chemical composition may be different [Nowak 2006a, Adamczak et al. 2011, Adamczak et al. 2012, Król et al. 2012, Rutkowska et al. 2012, Jiménez et al. 2017]. In addition, research conducted by Kołodziej and Drożdżal [2011] regarding *Sambucus nigra* L. fruit, prove that the content of compounds also depends on the location from which the raw materials were obtained.

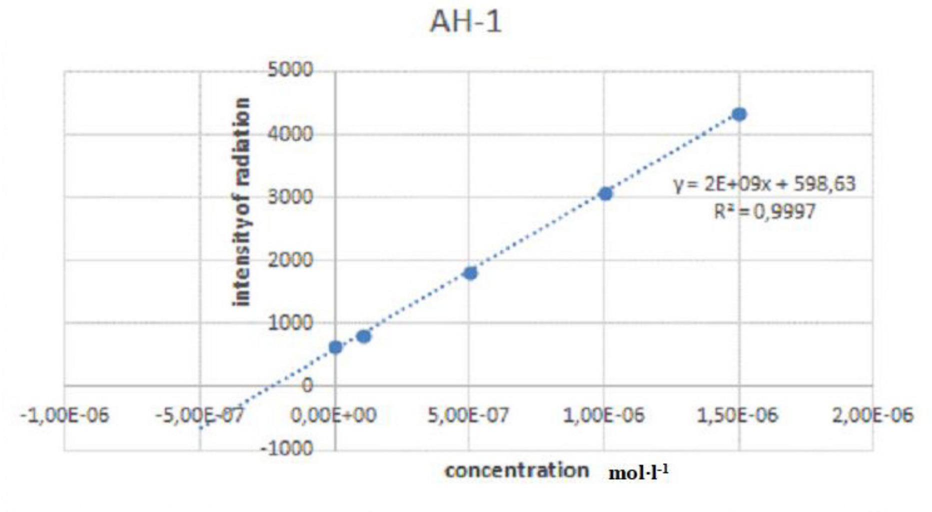

Fig. 2. Standard addition method curve to calculate the concentration of ellagic acid-borax complex in methanolic extract of AH-1 sample (*Rosa canina*, hypanthia) in adequate dilution, one of the three replicates

Table 1. Free ellagic acid content in different roses are presented in table

Sample	Content of free ellagic acid (μg·g^{-1} lyophylisate)			
	Hypanthia		Achenes	
A (*R. canina*)	AH-1	36.28	AN-1	27.53
	AH-2	36.59	AN-2	24.51
	AH-3	37.27	AN-3	27.11
	AH mean	36.71	AN mean	26.39
	SD	0.50	SD	1.64
	RSD	1.37 %	RSD	6.21 %
B (*R. moyesii*)	BH-1	67.55	BN-1	109.07
	BH-2	68.85	BN-2	103.27
	BH-3	68.48	BN-3	104.73
	BH mean	68.29	BN mean	105.69
	SD	0.67	SD	3.02
	RSD	0.98 %	RSD	2.85%
C (*R. pendulina*)	CH-1	245.77	CN-1	55.02
	CH-2	251.46	CN-2	55.84
	CH-3	245.93	CN-3	54.78
	CH mean	247.72	CN mean	55.21
	SD	3.24	SD	0.55
	RSD	1.31 %	RSD	1.00 %

AH – *R. canina* hypanthia, BH – *R. moyesii* hypanthia, CH – *R. pendulina* hypanthia, AN – *R. canina* achenes, BN – *R. moyesii* achenes, CN – *R. pendulina* achenes, SD – standard deviation, RSD – relative standard deviation

The material for my own research was obtained from organic crops not subjected to fertiliser and spraying.

In my own research, free EA was most abundant in the hypanthia of the alpine rose – *R. pendulina.* The flesh of this species contained an average of 247.72 $\mu g \cdot g^{-1}$ of dry weight (Tab. 1). The content of phenolic acid in Moyes rose – *R. moyesii* was over three times lower and was only at an average of 68.29 $\mu g \cdot g^{-1}$ of dry weight. The lowest amounts of the level of compound in its free form was found in the pseudo-fruit of *R. canina*, i.e. dog rose (36.71 $\mu g \cdot g^{-1}$ of dry weight).

Seeds of the researched roses (Tab. 1.) were a rich source of ellagic acid. The ones most rich in free ellagic acid were achenes *R. moy*esii (105.69 $\mu g \cdot g^{-1}$ of dry weight). The content of this phenolic acid in its free form exceeded its presence in the taxon of the flesh undergoing research by 2 times. A significant amount of compound was indicated in the true fruit of *R. pendulina.* The EA level in this material was lower by half when compared to *R. moyesii* and amounted to 55.21 $\mu g \cdot g^{-1}$ of dry weight. of the lyophilisate. The lowest level of free ellagic acid was found in the material of *R. canina* (26.39 $\mu g \cdot g^{-1}$ of dry weight). The content of this biologically active compound in its free form found in the seed lyophilisate was 4 times lower compared to *R. moyesii* and 2 times lower when compared to *R. pendulina.* The obtained results can show that the EA in the seeds of *R. canina* occurs mainly in a bound form.

In the lyophilisate of *R. pedulina* hypanthia a high content of free ellagic acid was found corresponding to the level of 245.5 μg^{-1} g of dry weight, while the seeds contained more than 4 times less of this compound, at only 55.21 μg^{-1} g of dry weight. It can therefore be assumed that ellagic acid in the achenes of *R. pedulina* occurs mainly in bound form.

The results are confirmed in the literature. Milala et al. [2013] proved that the flesh of *Rosa pomifera* 'Karpatia' contained an average of 25 ±2 $mg \cdot 100\ g^{-1}$ of dry weight of free ellagic acid. The seeds contained an average of 7 ±1 $mg \cdot 100\ g^{-1}$ of dry weight of this compound. The share of free ellagic acid in hypanthia was approximately 3-, 4 times higher than in achenes.

It is noted that different parts of fruit may contain varied amounts of ellagic acid. For example, raspberry seeds contained about 87.5% ellagic acid, and the flesh about 12.2% [Aaby et al. 2005]. Other sources indicate that white grape varieties contained from 879–1620 $mg \cdot kg^{-1}$ ellagic acid, while dark varieties 592–1900 $mg \cdot kg^{-1}$ in the skin of the fruit. Much smaller amounts were noted in the flesh of the fruit. The content of EA in the juice of these grapes was much higher in the white variety compared to the dark varieties. In light varieties, the acid content amounted to 105–162 $mg \cdot kg^{-1}$and in dark ones 187–322 $mg \cdot kg^{-1}$ [Lee 2004]. Strawberry seeds (nuts) constitute about 1% of fruit weight, but contain up to 11% of the total polyphenolic compounds present in strawberry fruit [Aaby et al. 2005]. Based on the above results, it can be concluded that ellagic acid concentrates mainly in seeds and the skin of fruit.

The literature reported by Fecka [2009] indicates the absence of the presence of EA in the seeds of of the wild rose. It was proven that the test compound in *R. canina* is bound in the form of tellimagrandin I and II and rugosin A, B, D and E.

In my own research, EA in free form in *R. canina* achenes was at the level of $\mu g \cdot g^{-1}$ of dry weight. However, in *R. moyesii* this phytochemical compound corresponded to the level of 105.69 $\mu g \cdot g^{-1}$ of dry weight, and its content was more than 1,5 times greater than the EA value indicated in the flesh of fruit (hypanthia) (68.29 $\mu g \cdot g^{-1}$ of dry weight).

A significant effect on the chemical composition of the fruit, corresponding to their harvesting time, was found. Pale yellow dogwood fruit contained the most phenols and tannins and had the highest antioxidant activity [Gunduz et al. 2013]. Dogwood fruit, which were harvested later, contained a much higher amount polyphenolic compounds [Kucharska 2012]. Reports by Williner et al. [2003], show that the content of the EA decreases with the degree of maturity of the fruit.

The fruit of roses for my own research were collected in their phase of full maturity, i.e. well-coloured, firm and easily detached from the stalk.

The technological processes used in the production process of fruit products are also important. It appears that they significantly affect losses in the content of ellagic acid in final products. Häkkinen et al. [2000] reported that the storage of raspberries and strawberries at a refrigeration temperature of –20°C resulted in the decrease in the amount of ellagic acid by 30–40%. Jiménez et al. [2017] examining the hypanthia of *R. pouzinii*, *R. corymbifera*, *R. glauca and R. canina* and observed a relationship between the freshness of rose hip and the concentration of polyphenols.

Comparing the obtained results with the data presented by other authors, it was found that, despite the differences in EA content in the research material, the true and accessory fruit of roses: *R. canina, R. moyesii and R. pendulina* can be considered a promising source of this biologically active compound.

The majority of fruit consumed on a daily basis contains a significantly lower level of ellagic acid in comparison with the alpine rose (247.72 μg of the free EA according to its lyophilisate). Bananas contain about 20 μg EA according to of dry weight, mandarins and pears 40 μg, on average apples and plums 70 μg and cranberry 120 μg [Williner et al. 2003, Kwiatkowska 2010].

Authors of many reports suggest that differences in flavonoid content may result from phylogenetic relations between different taxon, and research conducted on the content and qualitative composition of flavonoids should be continued.

CONCLUSIONS

1. Fruit from roses are a valuable source of free ellagic acid.

2. Free EA is present both in the hypanthia and in the achenes of the researched species of roses. Its content varies depending on the species.

3. The highest content of free form ellagic acid was indicated in the mountain rose species, with native sites in the Bieszczady, Sudetes and Tatra mountains (247.72 $\mu g \cdot g^{-1}$ of dry weight). The content of free form phenolic acid in this species was three times higher than the concentration of this compound in comparison with the taxa commonly occurring in Poland, i.e. dog rose – *R. canina* (36.71 $\mu g \cdot g^{-1}$).

4. Depending on the seeds, the achenes of the roses which were researched are a valuable source of free ellagic acid. Achenes of *R. moyesii* contained 3 times more EA when compared to *R. canina* and twice as much when compared to *R. pendulina.*

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