

## DETERMINATION OF ELLAGIC ACID IN PSEUDOFRUITS OF SOME SPECIES OF ROSES

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**Abstract:** Ellagic acid (EA) is known as a naturally occurring dietary antimutagen and anticarcinogen with strong antioxidant and anti-inflammatory activities. An SPE RP HPLC method was optimized and applied for identification and determination of ellagic acid in hips of fourteen species of roses, wildy growing in Poland. A large amount of total EA, ranged from 487.2 to 1065.2 µg/g of dry material was found. The results suggest that studied materials are good sources of dietary ellagic acid and could be used as a natural antioxidant and functional food.

**Keywords:** Ellagic acid, *Rosa* L. hips, HPLC analysis

Recently, there has been considerable interest in nutraceutical industry and in preventive medicine in the quest for natural antioxidants from plant material. Various phytochemical components are known to be responsible for the antioxidant capacity of plants.

Among them ellagic acid (EA) is a phenolic compound that exhibits both antimutagenic and anticarcinogenic activity in a wide range of assays *in vitro* and *in vivo* (1, 2). It can act as an antioxidant, and has been found to cause apoptosis (cell death) in cancer cells (3). Ellagic acid has also been said to reduce heart disease, birth defects, liver problems, and to promote wound healing (4). There are also reports that it may help the liver to break down or remove some cancer-causing substances from the blood (5). Several studies have found that ellagic acid can inhibit the growth of skin, esophagus, lung, and other tumors caused by carcinogens (6,7). Recently Italian researchers found that ellagic acid seemed to reduce the side effects of chemotherapy in men with advanced prostate cancer, although it did not help to slow disease progression or improve survival (8). However, further studies would be needed to confirm these results and to determine if other results apply to humans.

The highest levels of ellagic acid are found in some fruits, especially in raspberries, strawberries, and pomegranates (9). Eating berries or other natural sources of ellagic acid should be a part of balanced diet including several sources of fruits and vegetables each day. Now, ellagic acid is available in supplement as a food additive form functioning as an antioxidant, too (10).

There is some literature data about antioxidative capacity of extracts from rosehips and high

content of phenolic compounds in these plants (11, 12).

In view of the increasing interest of *Rosa* L. sp. in recent years and of the growing evidence of their free-radical-induced deleterious effects (13), the present investigation was undertaken with the aim of analyzing the amounts of ellagic acid in some Polish roses, in order to find new potential source of this compound. This study continues early investigations concerning determination of phenolic acids in pseudofruits of roses (14).

### EXPERIMENTAL

#### Plant material

The study material – whole pseudofruits – Fructus Rosae cum semine Polish Pharmacopoeia IV and places of their collection have been described earlier (14).

#### Chemicals

Standard of ellagic acids was purchased from Roth (Karlsruhe, Germany). Compound was dissolved in methanol to obtain a stock solution (0.5 mg×mL<sup>-1</sup>).

All solvents used were of analytical or HPLC grade (Merck, Darmstadt, Germany).

#### HPLC conditions

A used HPLC system (Knauer, Berlin, Germany) consists of a HPLC Pump K-1001, Solvent Organizer K-1500, UV-VIS Detector Fast Scanning Spectrophotometer K-2600, Degasser K-5004, Column Thermostat and 20 µL sample injector (Rheodyne, Cotati, CA, USA). Chromatographic

data were collected and recorded using a computer program Eurochrom 2000.

The analytical column was Hypersil (200 × 4.6 mm I.D., 5 µm; Agilent Technologies, Germany) with guard column (5 µm, 125 mm × 4.6 mm I.D.). A 10 µL volume of sample was injected into chromatographic system.

After preparation, the mobile phases were filtered through 0.45 µm filter (J.T. Baker, Phillipsburg, NY, USA). The sample solutions were filtered through a 0.45 µm filter before HPLC.

The following mobile phases were used: in isocratic elution – mobile phase A, methanol – water – phosphoric acid (49,5:49,5:1, v/v/v), in gradient elution phases: B, methanol – water – phosphoric acid (199,5:799,5:1, v/v/v), C, methanol – water – phosphoric acid (599,5:399,5:1, v/v/v) and the elution profile: 0–5 min: 100% B to 50% B / 50% C; 5–10 min: 50% B / 50% C to 20% B / 80% C; 10–15 min 20% B / 80% C to 100% C; 15–25 min 100% C (isocratic), 25–30 min: 100% C to 100% B; post-time 5 min before the next injection. Flow rate was 1.0 mL/min, column temperature 25°C, UV detection at  $\lambda=254$  and 360 nm.

The identification of compound was accomplished by comparison of their retention time and UV spectra with those of appropriate standard of EA. The UV spectra were recorded between 200 and 400 nm.

The quantitative determination of ellagic acid was performed using system elution A, external standard method and calculating the peak areas. The calibration curve was obtained with six samples in

the concentration range 0.1–100 µg/mL using linear regression analysis. Each sample was measured three times.

#### Determination of free ellagic acid content

10 g of crushed plant material was twice extracted using 60 mL and next 40 mL of 70% aqueous methanol in hot water bath for 1 h. The combined extracts were filtered and placed in 100 mL volumetric flasks and filled up to the mark. Next, the SPE modified method of Amakura et al. (15) was used. 10 mL of each extract was placed in volumetric flask and filled up with 0.1% HCl in water to 25 mL. 10 mL portion of diluted extract was passed through octadecyl SPE microcolumns (500 mg, J.T. Baker Inc., Phillipsburg, USA) previously conditioned with methanol (10 mL), then with water (10 mL) and 30/70 methanol/water in 0.1% HCl. After the application of samples the individual microcolumn was washed with 2 mL of water. The ellagic acid adsorbed on the packing was eluted with 10 mL of methanol. The solution was evaporated in vacuum to 1 mL volume and analyzed by HPLC. Each procedure was repeated two times.

#### Estimation of total ellagic acid content

The extraction method applied for the total ellagic acid determination was based on the method of Häkkinen et al. with some modifications (16).

1 g of each sample was hydrolyzed with 2M HCl in 50% methanol (25 ml) with TBHQ (0.01 g). The mixture was refluxed for 5 h at 90°C. The cool extract was filter and diluted to 50 mL with water.

Table 1. The content of ellagic acid in the investigated rose pseudofruits (in µg/g of dry weight).

	Species	Contents of ellagic acid (µg/g of dry material)	
		Free	Total
1.	<i>R. rugosa</i>	631.3 ± 4.05	1461.2 ± 1.28
2.	<i>R. rubiginosa</i> var. <i>rubiginosa</i>	192.1 ± 4.83	605.1 ± 14.84
3.	<i>R. rubiginosa</i> var. <i>umbellata</i>	211.4 ± 6.11	594.7 ± 8.38
4.	<i>R. villosa</i>	390.7 ± 8.23	996.6 ± 1.30
5.	<i>R. tomentosa</i>	159.4 ± 8.13	487.2 ± 16.23
6.	<i>R. inodora</i>	151.5 ± 3.67	577.6 ± 15.87
7.	<i>R. canina</i> var. <i>canina</i>	224.4 ± 4.26	964.1 ± 10.49
8.	<i>R. canina</i> var. <i>corymbifera</i>	146.2 ± 3.35	509.3 ± 4.36
9.	<i>R. canina</i> var. <i>dumalis</i>	159.8 ± 0.96	498.5 ± 0.93
10.	<i>R. vosagiaca</i>	127.6 ± 1.82	620.6 ± 0.18
11.	<i>R. caryophyllacea</i>	147.7 ± 1.27	1065.2 ± 10.50
12.	<i>R. subcanina</i>	161.9 ± 3.61	707.2 ± 6.09
13.	<i>R. coriifolia</i>	109.2 ± 6.56	549.1 ± 13.17
14.	<i>R. subcollina</i>	101.1 ± 4.65	708.6 ± 33.49

Linear equations of calibration curves EA (for eluent A):  $c = 0.000309A - 0.000138$ ; determination limit  $LD = 0.5 \text{ mg} \times \text{L}^{-1}$ . Values are mean ± SD of six replicates (two extractions and three injections of each one).

10 mL of the extraction solution was filtered through a C<sub>18</sub> SPE cartridge (Octadecyl, 500 mg, J.T. Baker, Phillipsburg, NJ, USA) early preconditioned with water, methanol and 25% methanol in 0, 1% HCl, respectively. Microcolumn was washed with 2 mL of water and then ellagic acid was eluted with 10 mL of methanol. After evaporation to 1 mL volume EA was analyzed using HPLC system. Each hydrolysis and SPE procedure was repeated two times.

### Recovery

Recovery tests for the SPE method were performed. A stock solution of standard EA (1 mL) was diluted to obtain 10 mL,  $c = 0.05 \text{ mg} \times \text{mL}^{-1}$  solution in 30% methanol in 0.1% HCl. 2 ml of this standard solution were added to selected extracts and analyzed by means of the above described method. The results enabled the calculation of recoveries.

## RESULTS AND DISCUSSION

Ellagic acid is known as a naturally occurring dietary antioxidant, antimutagen and anticarcinogen with strong anti-inflammatory activities.

Different chromatographic techniques have been developed for the analysis of ellagic acid, mainly in plant foods in the past (15–18). The aim of this work was to find the best HPLC method for the analysis of ellagic acid content in roses.

The simple and rapid SPE HPLC method, based on the isocratic elution with UV detection

was elaborated. Sample preparation was carried out by solid phase extraction with a recovery  $97.5 \pm 3.1\%$  ( $\pm$  S.D.:  $n = 3$ ) for free EA and  $95.8\% \pm 3.8\%$  ( $\pm$  S.D.:  $n = 3$ ) for total EA, calculated from the comparison of peak areas of normal and spiked samples as well as the standard solution of ellagic acid and the same samples after the SPE procedure. For total EA contents, the long time of hydrolysis (5 h) in 2 M HCl was used. After SPE separation, the analyzed compound was quantitatively investigated by the RP HPLC method. Good separation of EA, characterized by a high, symmetrical peak, was obtained in a short time of analysis (12 min in isocratic elution and 25 min in gradient elution) (see Figures 1 and 2).

As a result, ellagic acid was detected and quantified in extracts of fruits from fourteen rose species. Hips free ellagic acid content ranged from 101.1 to 631.3  $\mu\text{g/g}$  (mean 208.2) for examined rose species (Table 1). Total ellagic acid contents in material estimated after hydrolysis ranged from 487.2 to 1461.2  $\mu\text{g/g}$  of dry weight (mean 738.9).

Large differences in ellagic acid level were found among species (Figure 3). Especially rich in EA were pseudofruits from *R. rugosa*, *R. caryophyllaceae* and *R. villosa*. The lowest content of EA was estimated in hips of *R. tomentosa* and mostly varieties of *R. canina*. It seemed that fruits of roses are very rich source of dietary ellagic acid.

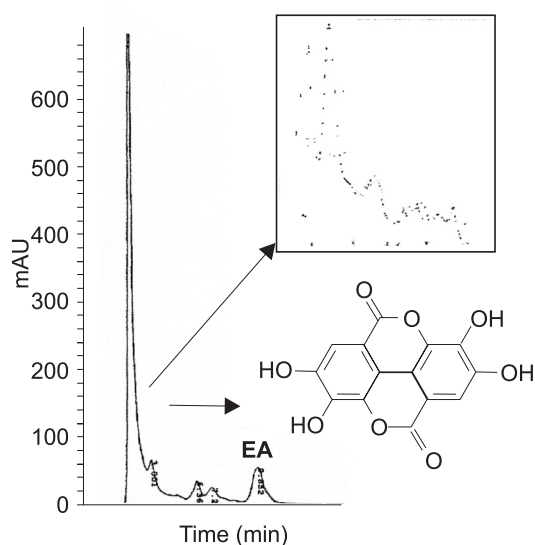


Figure 1. HPLC chromatogram of *R. villosa* sample – free ellagic acid (isocratic elution).

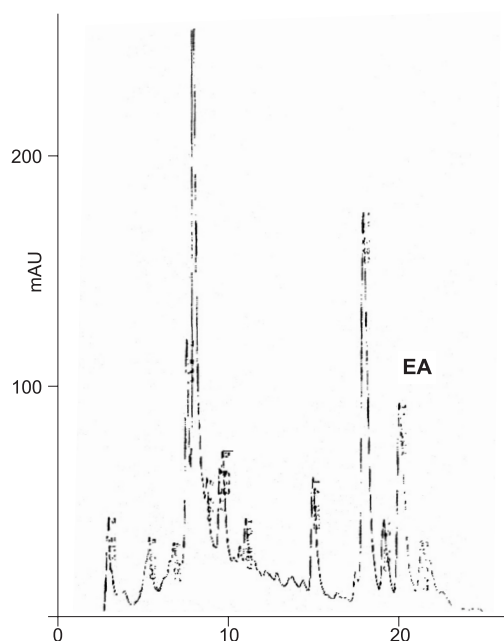


Figure 2. HPLC chromatogram of *R. rugosa* sample – total ellagic acid (gradient elution –  $t_R = 19.88$  min).

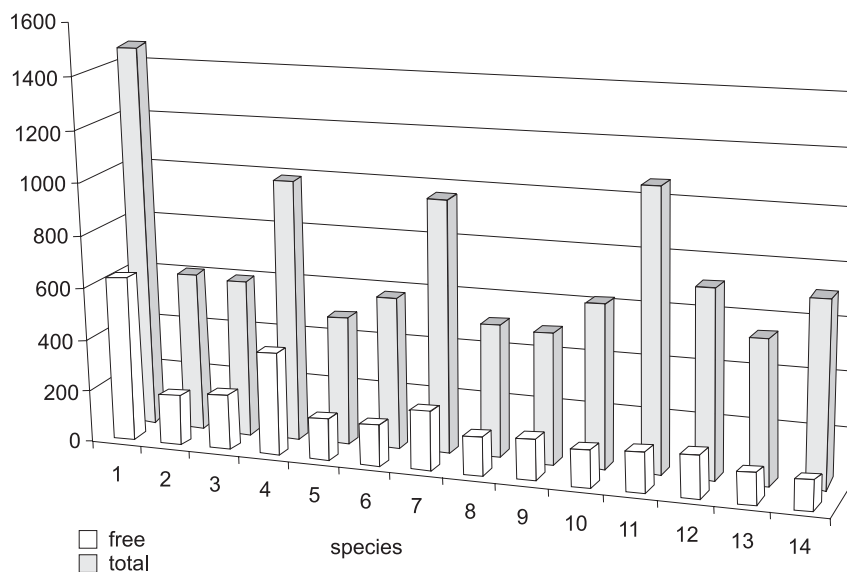


Figure 3. Comparison of content of free and liberated after hydrolysis ellagic acid in the investigated rose pseudofruits (in  $\mu\text{g/g}$  of dry weight).

The estimated results are comparable to that obtained for fruits by other authors. For example, extracts from red raspberry are said to contain the highest levels of ellagic acid (2). EA was the major phenol determined in red raspberries, greater than 160 mg/100 g. Strawberries also contained high ellagic acid levels (greater than 40 mg/100 g) and considered to be one of the most potent sources of EA (9).

However, the estimated levels of EA in roses fruits are lower than in red raspberries, but comparable to their level in strawberries, which are available as dietary supplements in capsule, powder, or liquid forms.

Considering the obtained results, rose hips are very interesting and promising plant material which could be used as natural source of ellagic acid and as antioxidant and functional food.

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