

RUTIN ANALYSIS BY HIGH-RESOLUTION LIQUID CHROMATOGRAPHY ON REVERSE PHASES IN RASPBERRY FLOWER EXTRACT (*Rubus idaeus* L.)

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ABSTRACT

Rutin belongs to the group of polyphenolic compounds, which consists of hormones, vitamins, and antioxidants in food. It is found mostly in plants and is one of the most biologically active flavonoids. It can act as an antioxidant, antibacterial, and anti-inflammatory and can prevent the formation of a tumor. The concentration of rutin was determined in (*Rubus idaeus* L.) Polka raspberry by applying reverse-phase high-resolution liquid chromatography on reverse phases on Shimadzu Prominence Modular HPLC with UV/Vis detector. The analysis was performed in ethanolic extracts of Polka raspberry flower obtained by the Soxhlet extraction and the ultrasonic methods. Raspberry flowers (*Rubus idaeus* L.) Polka varieties were collected from two different localities in Bosnia and Herzegovina, namely: Starposle near Kakanj and Moševac near Maglaj. Rutin was determined in all analyzed samples of Polka raspberry. The highest rutin concentration was determined in the extract of the Polka raspberry flower from the Kakanj-Starposle site, using the ultrasonic method ($31,46 \pm 13,90 \mu\text{g}/\text{cm}^3$), and the smallest in the Polka raspberry flower from the Kakanj-Starposle site, using Soxhlet extraction ($3,16 \pm 0,08 \mu\text{g}/\text{cm}^3$). The concentration of rutin was higher in the extract of Polka raspberry flower from the Kakanj-Starposle site, which suggests that these flowers of the Polka variety are recommended as better natural sources of rutin and that ultrasonic extraction has proven to be a more efficient method for extraction of rutin in ethanolic extracts, which is the goal of research.

1. INTRODUCTION

Rutin is one of the most active biological flavonoids, also known as vitamin P. Rutin is considered an activating factor for vitamin C. The name rutin comes from the plant *Ruta graveolens*, in which, according to the content of polyphenols, it predominates [1]. Raspberry is a perennial, shrubby, deciduous plant from the family Rosaceae of the genus *Rubus*. It consists of aboveground (leaf, flower, seed and fruit) and underground (root) organs. Polka Raspberry is one of the best varieties of raspberries. It is a permanent raspberry, a newer raspberry variety originating from Poland, created by the crossing of varieties: *Autumn Bliss*, *Lloyd George* and *Rubus crataegifolius*, introduced in 2001 and entered in the official registers in 2003. Raspberry belongs to the group of berries and is a natural source of bioactive compounds that benefit human health. It is rich in compounds such as vitamins, minerals and is one of the richest sources of natural antioxidant compounds such as polyphenols [2]. Rutin is a flavonoid found in citrus fruits, buckwheat, apples, green tea, carrots, grapes, linden and eucalyptus leaves, and various berries. The role of rutin in plants is the transport of cations and the supply of nutrients from the soil. Rutin is a flavonoid that shows numerous positive effects on human health, precisely because of its antioxidant effect [1].

2. EXPERIMENTAL PART

Raspberry flowers (*Rubus idaeus* L.) were collected from two different sites in Bosnia and Herzegovina, namely Starposle near Kakanj and Moševac near Maglaj. The difference between these two sites is the altitude. Moševac near Maglaj is located at an altitude of 169 meters, and Starposle near Kakanj at 470 meters. These two sites are far from industrial plants.

2.1. Obtaining extracts and samples of raspberry flower for HPLC analysis

Dried raspberry leaves were ground in a blender, and as such, used in the Soxhlet apparatus. Ethanol was used as the solvent. The extraction lasted for six hours, after which the obtained extract was evaporated to dryness. The extracts obtained in this way were stored in dark bottles in a refrigerator at a temperature of +4 °C. The obtained extract samples were of resinous consistency, and well soluble in ethanol. Extractions were also performed in the ultrasonic bath under defined conditions: frequency (20-40 kHz), power (250-500 W), temperature (40°C), and extraction time (30 min) [3]. Ethanol was used as the solvent. After treatment, the extracts were filtered and evaporated to dryness. The extracts obtained in this way were stored in dark bottles in a refrigerator at a temperature of +4 °C. The fact that plants contain several thousand secondary metabolites creates a need to develop fast and precise extraction methods. Dry flower extracts (about 0.5 g), obtained by Soxhlet extraction and ultrasonic method, were dissolved in 50% methanol in an ultrasonic bath. Then filtered to remove impurities and transferred to vials.

The content of total phenols was determined spectrophotometrically, on the PerkinElmer, Lambda 650, UV – VIS spectrophotometer device, by the Folin-Ciocalteu method.

2.2. Rutin analysis using RP-HPLC-UV/Vis technique

Rutin analysis of Polka raspberry flower extracts from two different sites was performed by high-performance liquid chromatography on reversed phases on Shimadzu Prominence Modular HPLC with UV/Vis detector, mobile phase degasser, pump, autosampler, and column oven. Analysis of rutin was performed on a Nucleosil C18 column (250 mm × 4.6 mm, particle size 5 µm; Macherey-Nagel). As the mobile phase, a solvent system was used: A (1% formic acid) and B (acetonitrile) at a flow rate of 1 ml/min and using the following linear gradient: 0–10 min from 10 to 25% A; 10–20 min linear rise to 60% A, 20–30 min linear rise to 70% A. The column was balanced to initial conditions, 10% A, 10 min with an additional 5 min for stabilization [4,5]. The rutin standard was dissolved in 50% methanol. Chromatograms were recorded at 360 nm for flavonoids (rutin). Based on the obtained chromatograms and the calibration diagram of the standard rutin solution, the rutin concentration in the extracts was calculated ($\mu\text{g}/\text{cm}^3$) [4,5].

The rutin standard of different concentrations is shown in Table 1 and was used for HPLC analysis[3,4].

Table 1 Standard solution of rutin of different concentrations for HPLC analysis

Standard solution	Concentration ($\mu\text{g}/\text{cm}^3$)			
Rutin	4	5	6	100

The calibration curve of the analyzed rutin standard solution is shown in Figure 1.

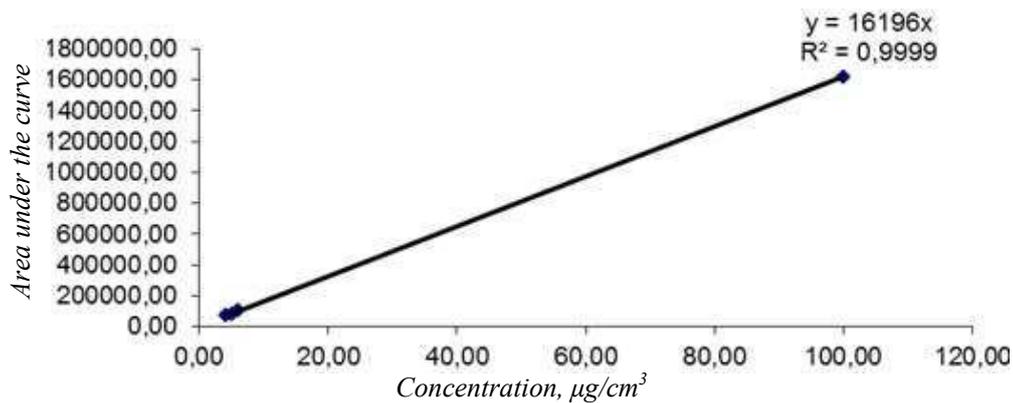


Figure 1. Calibration curve for rutin

3. RESULTS AND DISCUSSION

Extracts of Polka raspberry flower obtained by ultrasonic extraction showed a significantly higher total phenol content than extracts obtained using Soxhlet extraction, as shown in Table 2. Based on these results, it is safe to say that ultrasonic extraction leads to a higher yield of phenolic compounds in a shorter time, reducing energy consumption and phenol degradation. Ultrasonic extraction has been shown a better and more cost-effective technique than Soxhlet extraction for the extraction of Polka raspberry flower.

Table 2. Content of total phenols in Polka raspberry flowers extracts obtained by methods of Soxhlet and ultrasonic extractions

Plant extracts	Content of total phenols (mg GA/g extract)
SCPM	95,08±8,57
SCPK	62,65±2,33
UCPM	148,99±9,02
UCPK	146,66±2,85

SCPM - flower Polka Maglaj – Soxhlet; SCPK - flower, Polka Kakanj - Soxhlet UCPM - flower Polka Maglaj – ultrasonic; UCPK- flower Polka Kakanj – ultrasonic

All experiments were repeated three times. Results are expressed as mean ± SD (n = 3). The HPLC method was used to determine the concentration of rutin in the tested extracts of the Polka raspberry flower. The results of HPLC analyzes of these extracts are shown in Table 3. Figures 2 - 5 show HPLC chromatograms for rutin detected in Polka raspberry flower extracts.

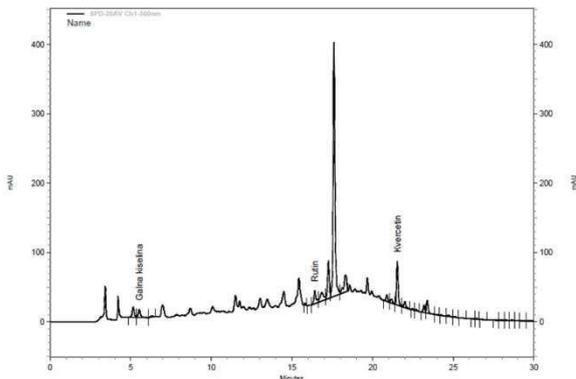


Figure 2. HPLC chromatograms of Maglaj Polka flower - Soxhlet extraction (SCPM)

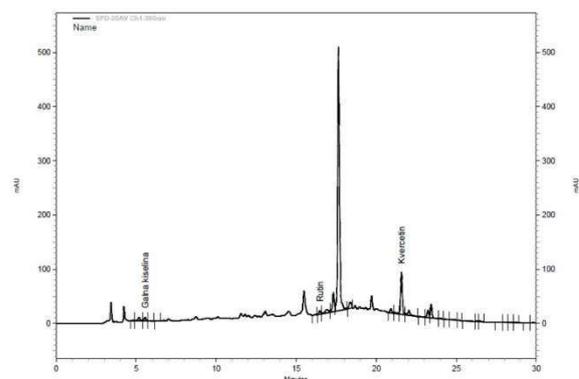


Figure 3. HPLC chromatograms of Kakanj Polka flower - Soxhlet extraction (SCPK)

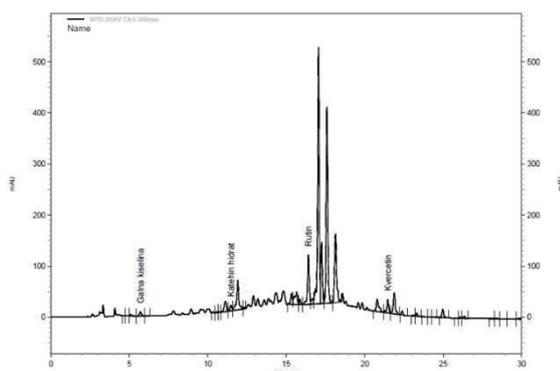


Figure 4. HPLC chromatograms of Maglaj Polka flower - ultrasonic extraction (UCPM)

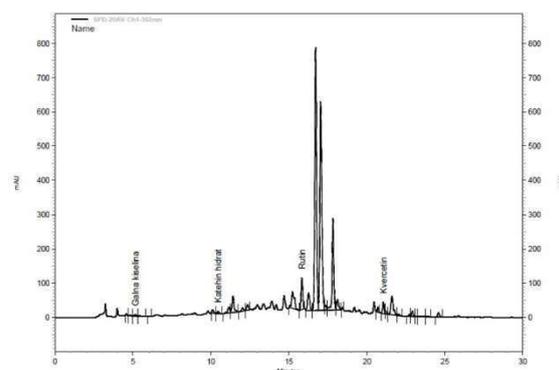


Figure 5. HPLC chromatograms of Kakanj Polka flower - ultrasonic extraction (UCPK)

HPLC chromatograms showed the presence of rutin in extracts of the Polka raspberry flower.

Table 3. Results of HPLC analysis of rutin in Polka raspberry flower extracts obtained by Soxhlet and ultrasonic extractions

Plant extract	RUTIN
	Concentration, $\mu\text{g}/\text{cm}^3$
SCPM	10,38±0,79
SCPK	3,16±0,08
UCPM	29,88±21,22
UCPK	31,46±13,90

Based on the results of spectrometric and HPLC analyzes, it can be concluded that the content of total phenols and rutin is much higher in all samples of:

- Maglaj Polka raspberry in relation to the Kakanj Polka raspberry obtained by Soxhlet Extraction;
- By ultrasound extraction in relation to Soxhlet extraction.

4. CONCLUSIONS

Rutin is a flavonoid that shows numerous positive effects on human health, precisely because of its antioxidant effect. Rutin was determined in all analyzed samples of Polka raspberry. The highest rutin concentration was determined in the extract of the Polka raspberry flower from the Kakanj-Starposle site, using the ultrasonic method ($31,46 \pm 13,90 \mu\text{g}/\text{cm}^3$), and the smallest in the Polka raspberry flower from the Kakanj-Starposle site, using Soxhlet extraction ($3,16 \pm 0,08 \mu\text{g}/\text{cm}^3$). The concentration of rutin was higher in the extract of Polka raspberry flower from the Kakanj-Starposle site, which suggests that these flowers of the Polka variety are recommended as better natural sources of rutin and that ultrasonic extraction has proven to be a more efficient method for extraction of rutin in ethanolic extracts.

5. REFERENCES

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