



Original scientific paper

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

Dejana Kasapović¹, Fehim Korać², Farzet Bikić³

^{1,3} The University of Zenica, Faculty of Metallurgy and Technology, ² The University of Sarajevo, Faculty of Science

ABSTRACT

Gallic acid is a phenolic acid widely spread in various plants, where it is present either in free form or as part of tannins, called gallotannins. It shows several positive effects on human health. It has different biological effects: antibacterial, antiviral, anti-inflammatory. Many scientists are showing great interest in gallic acid precisely because of its antitumor effect. The content of gallic acid 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, mobile phase degasser, pump, autosampler and column oven. 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. Gallic acid was determined in all analyzed samples of Polka raspberry. The highest gallic acid content was determined in the extract of Polka raspberry flower from the Maglaj-Moševac site, using the ultrasonic method (0.1789%), and the smallest in the Polka raspberry flower from the Kakanj-Starposle site, using Soxhlet extraction (0.0995%). The proportion of gallic acid was higher in the extract of Polka raspberry flower from the Maglaj-Moševac site, which suggests that these flowers of Polka variety are recommended as better natural sources of this phenolic acid and that ultrasonic extraction has proven to be a more efficient method for extraction of gallic acid in ethanolic extracts, which is the goal of research.

Keywords: gallic acid, raspberry, HPLC method

Corresponding Author:

Dejana Kasapović,

Faculty of Metallurgy and Technology

Travnička cesta 1, 72 000 Zenica, B&H

Tel.: +387 32 401 831; fax: +387 32 406 903.

E-mail address: dejana.kasapovic@unze.ba

1. INTRODUCTION

Phenolic acids are secondary metabolites of plants, including hydroxybenzoic and hydroxycinnamic acids. Gallic, vanillic, *p*-hydroxybenzoic acid are among the Hydroxybenzoic acids [1].

Gallic acid participates in the formation of hydrolyzing gallotannins, and its condensation produces dimer - ellagic acid,

which is also part of ellagitannin. According to most literature data, one of the most significant compounds from the class of polyphenols in raspberry fruit is ellagic acid and ellagitannins. [2].

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 [3].

Gallic acid (3, 4, 5-Trihydroxybenzoic acid) is one of the best-known representatives of phenolic compounds commonly used to express the mass fraction of total phenols or phenolic subgroups in plant extracts. The most significant sources of gallic acid are apples, olive oil, walnuts, tea, citrus fruits. Gallic acid is a planar molecule consisting of an aromatic ring, three phenolic hydroxyl groups, and a carboxylic acid group. The hydroxyl groups are attached to the aromatic ring in *ortho* position to each other. There are four isomers of gallic acid that differ from each other in the position of the hydroxyl groups. Antioxidant and antiradical activity is influenced by the number and position of hydroxyl groups, other functional groups' presence, and their position in hydroxyl groups. The antioxidant activity of molecule increases with an increasing number of hydroxyl groups attached to the aromatic ring, so many studies have shown that gallic acid shows the most significant antiradical effect among many polyphenols [4]. Gallic acid affects the bioavailability of certain vital minerals, such as iron, zinc, and calcium, creating insoluble complexes. Iron is one of the most abundant transition metals among the various metal ions in the human body. Its binding to polyphenols (gallic acid) leads to reduced iron absorption, leading to a deficiency of this mineral. [4]. Many scientists are showing great interest in gallic acid precisely because of its antitumor effect [5]. Esterification of hydroxyl groups of gallic acid makes it possible to obtain esters with numerous

analogues and extremely significant pharmacological advantages [6].

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, 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) [7]. 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. Galic acid analysis using RP-HPLC-UV/Vis technique

Galic acid 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 galic acid 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 [2]. The galic acid standard was dissolved in 50% methanol.

Chromatograms were recorded at 280 nm for hydroxybenzoic acid derivatives (gallic). Based on the obtained chromatograms and the calibration diagram of the standard galic acid solution, the galic acid contents in the extracts (%) were calculated [2].

The galic acid standard of different concentrations shown in Tables 1 [2] and [7] was used for HPLC analysis. The calibration curve of the analyzed galic acid standard solution is shown in Figure 1.

Table 1 Standard solution of galic acid of different concentrations for HPLC analysis

| Standard solution | Concentration 1 (µg/mL) | Concentration 2, (µg/mL) | Concentration 3, (µg/mL) |
|-------------------|----------------------------|-----------------------------|-----------------------------|
| Galic acid | 52.5 | 105 | 420 |

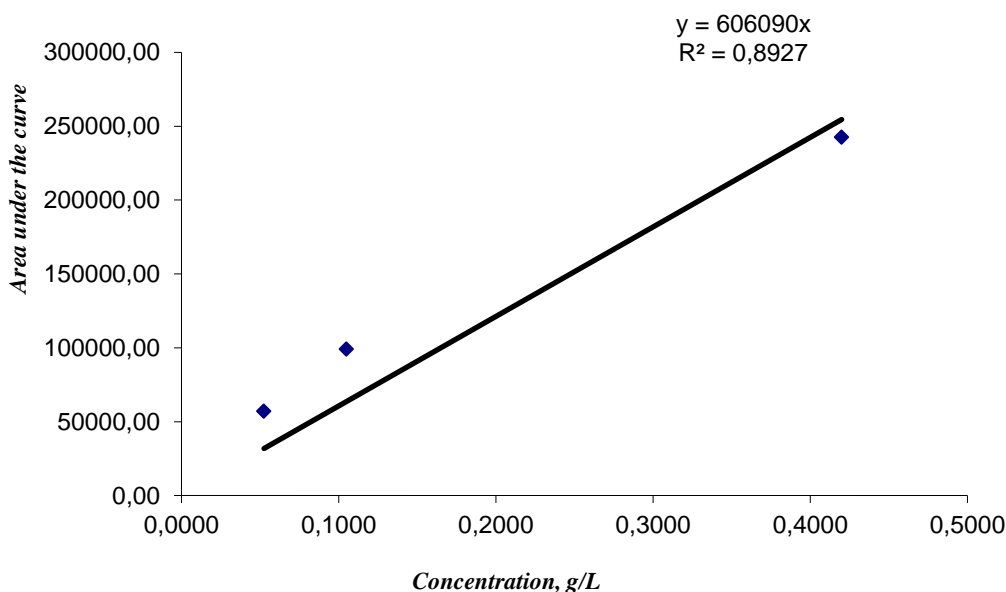


Figure 1. Calibration curve for galic acid

3. RESULTS AND DISCUSSION

Ultrasonic extraction proved to be a better and more cost-effective technique than Soxhlet extraction for Polka raspberry flower extraction. 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.

The HPLC method was used to determine the concentration of galic acid in the tested extracts of Polka raspberry flower. The results of HPLC analyzes of these extracts

are shown in Table 3. Figures 2 - 5 show HPLC chromatograms for gallic acid detected in Polka raspberry flower extracts. Based on the results of spectrometric and HPLC analyzes, it can be concluded that the content of total phenols and gallic acid is much higher in all samples of:

- Maglaj Polka raspberry in relation to the Kakanj Polka raspberry,
- by ultrasound extraction in relation to Soxhlet extraction.

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

| Sample | A ₁ | A ₂ | A ₃ | A ₁ | A ₂ | A ₃ |
|--------|----------------|----------------|----------------|----------------|----------------|----------------|
| | mg/mL | | | mg/g extract | | |
| SCPM | 0.4673 | 0.4211 | 0.3827 | 105.88 | 94.44 | 84.91 |
| SCPK | 0.6817 | 0.6949 | 0.6397 | 63.60 | 64.91 | 59.44 |
| UCPM | 0.6824 | 0.6476 | 0.5939 | 159.17 | 150.55 | 137.24 |
| UCPK | 0.6482 | 0.6242 | 0.6234 | 150.69 | 144.75 | 144.55 |

SCPM - flower Polka Maglaj- Soxhlet; SCPK - flower Polka Kakanj - Soxhlet; UCPM - flower Polka Maglaj - ultrasonic; UCPK- flower Polka Kakanj - ultrasonic; A₁, A₂, A₃ - measured values of concentrations for replica samples

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

| Sample | GALLIC ACID | |
|--------|----------------|------------|
| | Mass sample, g | Content, % |
| SCPM | 0.5033 | 0.1526 |
| SCPK | 0.5040 | 0.0995 |
| UCPM | 0.5064 | 0.1730 |
| UCPK | 0.5056 | 0.1163 |

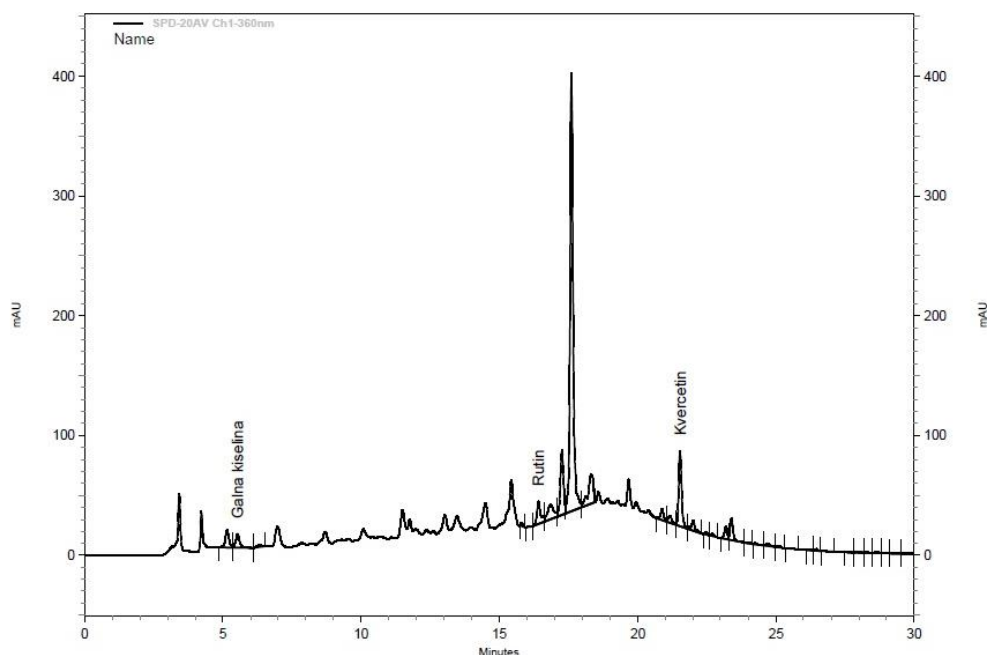


Figure 2. HPLC chromatograms of Maglaj Polka flower - Soxhlet extraction

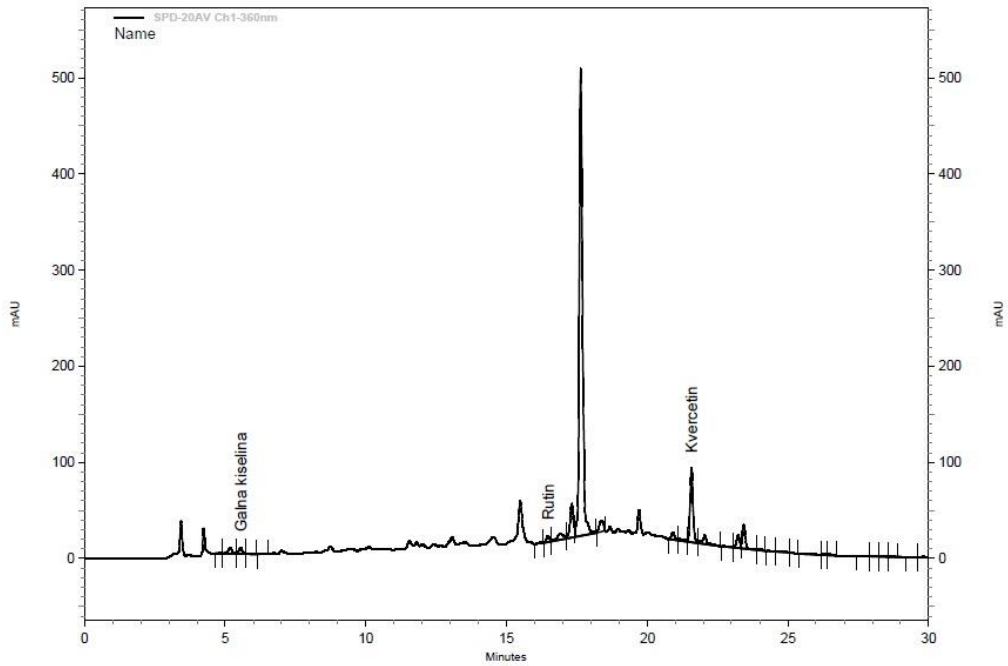


Figure 3. HPLC chromatograms of Kakanj Polka flower - Soxhlet extraction

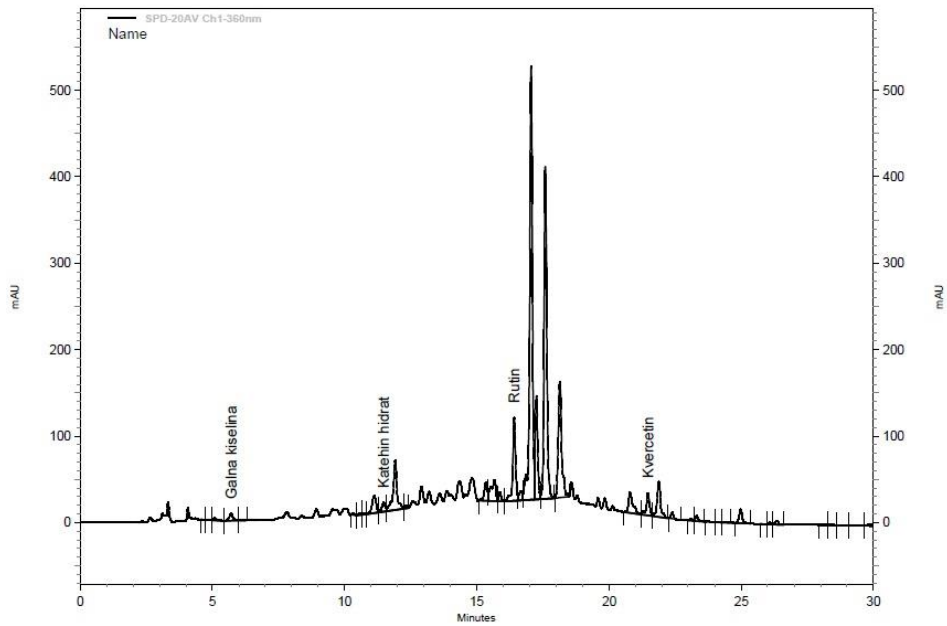


Figure 4. HPLC chromatograms of Maglaj Polka flower - ultrasonic extraction

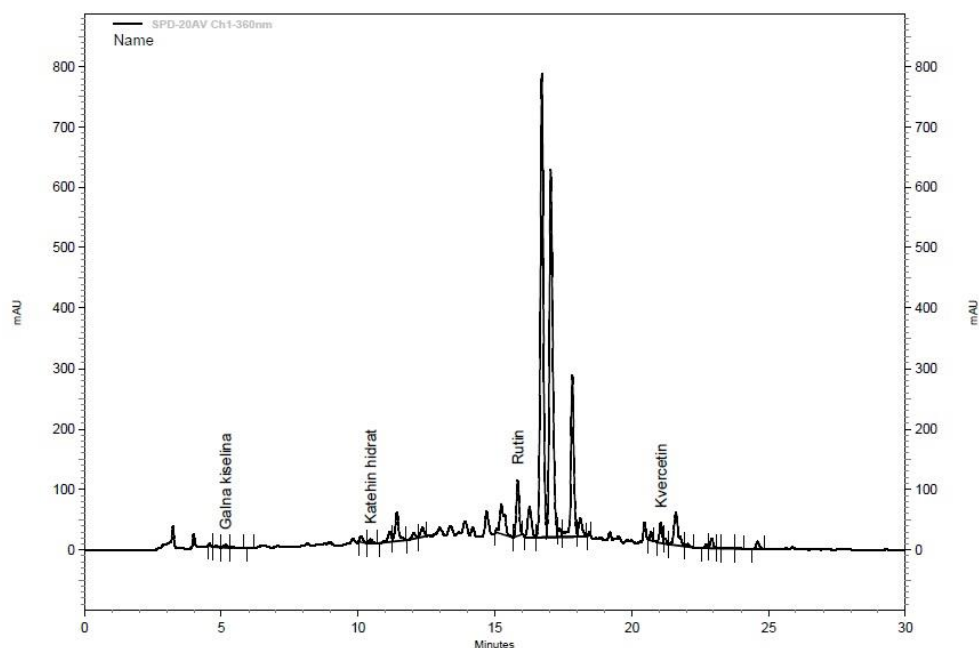


Figure 5. HPLC chromatograms of Kakanj Polka flower - ultrasonic extraction

4. CONCLUSIONS

Gallic acid was determined in all analyzed samples of Polka raspberry. The highest gallic acid content was determined in the extract of Polka raspberry flower from the Maglaj-Moševac site, using the ultrasonic method (0.1789%), and the smallest in the Polka raspberry flower from the Kakanj-Starposle site, using Soxhlet extraction (0.0995%). The proportion of gallic acid was higher in the extract of Polka raspberry flower from the Maglaj-Moševac site, using the ultrasonic method, which suggests that these flowers of Polka variety are recommended as better natural sources of this phenolic acid.

Conflicts of Interest

The authors declare no conflict of interest.

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