

Study of *Rubus Idaeus* Juice Ellagitannins and Its Antimicrobial Activity

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Abstract: Medicines on the basis of herbs are increasingly used for treating of many diseases, providing the following actions antimicrobial, antiviral, anticancer, anti-enzymatic and immunomodulatory. Ellagitannins are a group of polyphenolic compounds of plant origin, which exhibiting a strong antibacterial effect in virtue of their ability to form a bond with the microbial membrane. Raspberry contains a large amount of ellagitannins, so it is advisable to study their composition and content in raspberry juice, obtained in Ukraine and determine its antimicrobial activity. As raw material Ukrainian variety 'Phenomenon' was taken for the research. It was bred in 1991 in Kharkiv region by crossing the varieties 'Stolichnaya' and 'Odarka'. Analysis of raspberry juice ellagitannins was performed by HPLC method. 4 ellagic acid derivatives were identified and their contents were established. Unbound ellagic acid (55% of all) and sanguin H-6 (21% of all) had the highest content among them. Antimicrobial activity of raspberry juice was studied by agar diffusion method. The juice showed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans*. The antimicrobial activity of the juice was higher than the comparison drug "Chlorophyllipt" for more than twice in relation to all strains. The greatest effect was manifested relative to *Staphylococcus aureus*, which can be the cause of purulent-inflammatory processes in almost all human organs with severe consequences. This is especially true in connection with the increasing resistance of strains not only to traditional antibiotics, but also to new groups.

Keywords: Raspberry, Juice, Ellagitannins, Antimicrobial Activity

1. Introduction

Plant tannins are one of the most common groups of antioxidant polyphenols found in food and beverages. For a long time, they attracted special attention of scientists because of their multifunctional role in human health. Great progress in confirmation of the structures of many tannins of food, beverages and medicinal plant raw materials has occurred since the 1980s. The biological activity of ellagitannins was studied *in vitro* and *in vivo* [1-3].

The high affinity of ellagitannins to the various biopolymers, such as enzymes, is the cause of their diverse biological effects. The analysis of literature data about the

biological effects of ellagitannins showed following types of activity: antimicrobial, antiviral, anticancer, antienzyme, and immunomodulating [4-9]. The literature data also provides information about the presence of substances of ellagitannins nature in the fruits of raspberry, but no information about their qualitative and quantitative composition in raspberry, collected in Ukraine [10-13].

Along with synthetic medicines, antimicrobials of plant origin are becoming increasingly important. These biologically active substances are similar in structure to the natural components of the human body and therefore their use leads to less side effects.

The aim of the present work was to study raspberry juice ellagitannins by HPLC method, to compare the results with

those of other researchers as well as determination of raspberry juice antimicrobial activity.

2. Materials and Methods

2.1. Object

The object of our research was the juice obtained from the *Rubus idaeus* fruits variety 'Phenomenon'. This was a mid-ripening raspberry variety bred in 1991 in the Institute of Horticulture of the NAASU (National Academy of Agrarian Science of Ukraine), at the Krasnokutsk experimental station by crossing the varieties 'Stolichnaya' and 'Odarka'. Raw material was collected during fruiting in the places of its cultivation (Ternova village, Kharkiv region) in 2018. The collected fruits were drupe 5-8 g weigh, the shape was conical, color - red. The pulp was juicy, tender, sweet-sour taste, with a light aroma. The juice was squeezed from the fruits, filtered through a gauze filter

and centrifuged at the centrifugal machine with rotation speed 5000 rpm for 10 minutes. The yield of the juice from the fresh raspberry fruits was 63%.

2.2. HPLC Investigation

The study of ellagitannins of raspberry juice was carried out on the chromatograph Agilent Technologies (model 1100) equipped with vacuum degasser G1379A, 4-channel low pressure gradient pump G1311A, automatic injector G1313A, column oven G13116A and diode array detector G1316A. The chromatographic column ZORBAX-SB C-18 2.1 × 150 mm filled with octadecylsilyl sorbent (the size 3.5 micron) was used for analysis. Mobile phase velocity was 0,25 ml/min, working pressure of the eluent – 110-260 kPa; column thermostat temperature – 45°C. Volume of the injected sample was 2 µl analyzed in triplicate. Gradient regime of chromatography is given in table 1.

Table 1. Gradient regime of chromatography.

Time, min.	A% (0,6% TFA*)	B% 70% - methanol (0,6% TFA)	C% 100% methanol
0	92	8	0
8	62	38	0
24	0	100	0
24.1	0	0	100
29	0	0	100

(* TFA- trifluoroacetic acid)

Parameters of analysis were as follow: the measurement scale – 1.0; scan time – 0.5 seconds; options metering spectrum – each peak from 190 to 600 nm; wavelength – 254 nm. Identification of ellagic acid derivatives was carried out by the retention time of standards and their spectral characteristics.

2.3. Antibacterial Activity Investigation

Study of antibacterial activity of raspberry juice was performed at the I. I. Mechnikov Institute of Microbiology and Immunology of NAMS of Ukraine in the laboratory of Biochemistry and Biotechnology under the supervision of candidate of biological science T. P. Osolodchenko with agar well diffusion method.

Preparation of microbial suspension of microorganisms strains was carried out on the device Densi-La-Meter (PLIVA-Lachema, Czech Republic; wavelength 540 nm). The microbial suspension was prepared according to the devices instruction and the information letter about innovations in health care system No. 163-2006 "Standardization of preparation of microbial suspensions", Kyiv. The synchronization of the microbial strains was performed with low temperature (4°C). The concentration of microorganisms was 10⁷ microbial cells per 1 ml of medium and detected by McFarland standard. 18-24 hour cultures of microorganisms were used in analysis. Mueller-Hinton agar (India «HIMediaLaboratorles Pvt. Ltd India», expiration date December 2018) was used for analysis as well as Agar Saburo (India «HIMediaLaboratorles Pvt. Ltd India», expiration date December 2018) for *Candida albicans*.

Agar diffusion method was used to evaluate the antimicrobial activity of Raspberry juice. The analysis was performed on two

layers of a nutrient medium, poured into Petri dishes. «Hungry medias» (agar-agar, water, salts) were used in the lower layer (10 ml). 3-6 thin-walled stainless steel cylinders with a diameter of 8 mm and a height of 10 mm were set on the medias. The top layer was poured around the cylinders. It consisted of a nutrient medium, molten and cooled to 40°C, in which the corresponding standard of the daily culture of test strains was introduced. Previously, the upper layer was well mixed until a homogeneous mass was formed. After solidification, the cylinders were pulled out with sterile tweezers. Test samples were placed in the formed wells. Before analysis, raspberry juice was applied on sugar in a ratio of 1:1. Chlorophyllipt spray, produced by SRCM (State Research Center of Medicines), was used as a reference drug.

The volume of medium for the top layer was 14-16 ml. The dishes were dried for 30-40 minutes at room temperature. Then they were placed in a thermostat for 18-24 hours.

The following criteria were used in the analysis of antimicrobial and antifungal properties:

1. the absence of inhibition zones around the well as well as presents of inhibition zones up to 10 mm in the diameter, indicates that the strains are not sensitive for the study sample in local action;
2. presents of inhibition zones with the diameter 10-15 mm indicates about low sensitivity of the strains to the study sample in local action;
3. presents of inhibition zones with the diameter 15-25 mm indicates about sensitivity of the strains to the study sample in local action;
4. presents of inhibition zones with the diameter more than 25 mm indicates about high sensitivity of the strains to

the study sample in local action.

Museum strains of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 4636, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633 and *Candida albicans* ATCC 653/885 were used for study antimicrobial and antifungal activity. To obtain reliable data, three independent trials of each sample were carried out.

Table 2. The results of ellagic acid derivatives identification in raspberry juice.

Retention time, min	Compound	Content mg/L
10.08	Sanguin H-10 (isomer 1)	0,8
12.91	Lambertianin C	0,6
13.38	Sanguin H-6	4,9
21.20	Ellagic acid	12,6
22.75	Derivative of ellagic acid	3,7

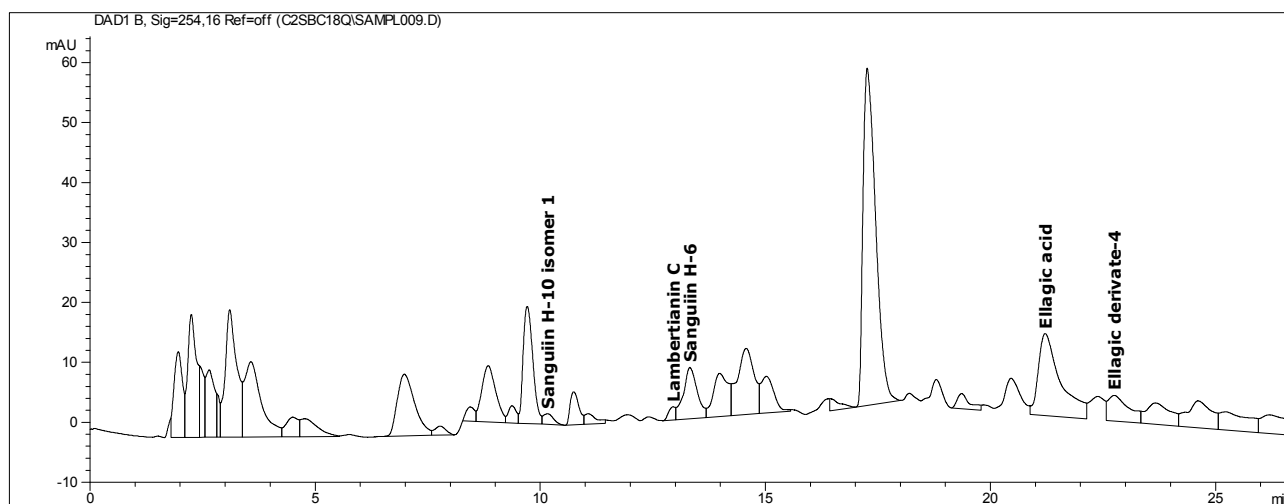


Figure 1. Chromatogram of ellagitannins investigation in raspberry juice.

The content of ellagitannins in raspberry juice was 22.6 mg / L when analyzed by HPLC. The dominant substances were ellagic acid and Sanguin H-6 with their contents of 55 and 21%, respectively, relative to the sum of ellagitannins. In the 'Phenomenon' raspberry juice (Ukraine) one isomer of Sanguin H-10 (isomer 1) was identified, while in Polish

varieties two isomers of Sanguin H-10 were identified. This indicates that in Polish raspberry juice ellagic acid was in the form of ellagitannins, while in Ukrainian raspberry juice unbound ellagic acid dominated. The content of free ellagic acid in the Ukrainian variety exceeded more than three times its content in Polish varieties [14].

3.2. Results of Study of Antibacterial Activity

Table 3. Antimicrobial and antifungal activity of raspberry juice and reference drug.

Museum strains	Inhibition zones in mm, n=3	
	Raspberry juice	Chlorophyllipt SRCM
<i>Staphylococcus aureus</i> ATCC 25923	23, 23, 23	15, 14, 14
<i>Escherichia coli</i> ATCC 25922	20, 20, 20	15, 14, 14
<i>Proteus vulgaris</i> ATCC 4636	18, 18, 17	growth
<i>Pseudomonas aeruginosa</i> ATCC 27853	19, 19, 19	growth
<i>Bacillus subtilis</i> ATCC 6633	23, 23, 22	15, 14, 14
<i>Candida albicans</i> ATCC 653/885	20, 21, 20	growth

The antimicrobial activity of the raspberry juice was determined. It was established antimicrobial and antifungal activity against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 4636, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis*, ATCC 6633 and *Candida albicans* ATCC 653/885.

The antimicrobial activity of the juice was higher than the

comparison drug "Chlorophyllipt" for more than twice in relation to all strains. The greatest effect was manifested relative to *Staphylococcus aureus*, which can be the cause of purulent-inflammatory processes in almost all human organs with severe consequences. This is especially true in connection with the increasing resistance of strains not only to traditional antibiotics, but also to new groups [15].

4. Conclusion

Search for sources of natural antimicrobial substances is extremely important due to increasing number of people suffering from inflammatory and immunological diseases. Ellagotannins are a promising group of biologically active substances for research. Raspberries are one among rich source of ellagotannins. The content and composition of ellagotannins may vary depending on the variety and geographical location of the procurement of plant materials.

The present study demonstrated that raspberry juice obtained from fruits variety 'Phenomenon' grown in Ukraine contained ellagic acid and three ellagic acid derivatives. The content of the components was determined by HPLC. The raspberry juice showed antimicrobial activity in relation to all strains while the greatest effect to *Staphylococcus aureus*.

Obtained results of the antimicrobial activity of raspberry juice indicate the prospect of a further search and development of new antimicrobial agents based on it.

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