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# Comparing phytochemical profile, antimicrobial and antioxidant activities of anthocyanin raspberry fruit thick and catechins green tea leaf extracts

**Abstract:** The aim of the study was investigate and compare phytochemical composition, antimicrobial, and antioxidant potential of raspberry fruit thick and green tea leaf liquid extracts. The quantification of biologically active substances (BAS) was performed using spectrophotometric, titrimetric, and HPLC analysis methods. Antioxidant activity was measured through a potentiometric method, while antimicrobial and antifungal effects were assessed using the well method and determining the minimum inhibition concentration. The total content of phenolic compounds was 0.60 and 10.10%, organic acids – 4.60 and 1.60% for raspberry fruit thick and green tea leaf extract. The total content of catechins in the green tea leaf extract was 10500.0 mg/100 g, where epicatechin-3-O-gallate was dominated (3730.0±74.6 mg/100 g). The total content of anthocyanins in the raspberry fruit thick extract was 109.86 mg/100 g, where cyanidin-3-O-sophoroside was dominated (134.56±2.68 mg/100 g). Both extracts possessed a high antioxidant potential, and effective antimicrobial and anti-fungi effects. The antioxidant, antimicrobial and anti-fungi activity of raspberry fruit extract was higher than green tea leaf extract. In addition, we assumed that anthocyanins had higher antioxidant, antimicrobial and anti-fungi properties than catechins. These findings would promote application of raspberry fruits extract as pharmaceuticals and nutraceuticals.
**Key words:** raspberry, fruit, green tea, comparing analysis, antimicrobial activity, antioxidant. activity

## Introduction

The dangerous of bacterial infections has been evaluated by the international research group. A statistical data has contained about 343 million patient records and pathogen isolates, as result researches have been estimated 13.7 million infection-related deaths at the period of 2019 year. The bacterial infection diseases have been caused 14% of all deaths and 56% of sepsis cases throughout the world in 2019. The mortality level was 100 deaths per 100,000 population. The 55% out 7.7 million deaths have been caused with 3 Gram-negative (-) strains: *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa;* and 2 Gram-positive (+): *Staphylococcus aureus* and *Streptococcus pneumonia*. The number one in causing death among infection diseases was *S. aureus*, around 1.1 million patients were died during 2019. In this year, 225,000 children died due to infection diseases of S. pneumonia, whereas among newborn deaths was K. pneumonia strain (29,000). According to statistical data, bacterial infection easily surpassed by HIV, cancer, self-harm in a number of deaths during a period of 2019 year [1]. Moreover, a 1.0 billion patients are suffered by fungal infections of the skin, nails, and hair, with over 150 million affected by serious fungal diseases that can be fatal [2]. Therefore, the search and elaboration of new antimicrobial medicines against G(+) and G(-), fungi strains is topical for medicine and pharmacy.

Today, medical plants that are rich source of flavan-3-ols and anthocyanins have a high attention from scientific community [3]. Above all, it relates with fact that some resistance pathogens are more sensitive to natural products, secondly, natural compounds have potent antioxidant effect and

moreover, the side effects are rarely happened after application of natural compounds than after synthetic drugs [4].

Raspberry fruit was chosen as a perspective source of anthocyanins, whereas a green tea leaf is the source of catechins. Raspberries (*Rubus idaeus* L.) are cultivated throughout America, Eastern Europe, Russia, Asian as well as raspberry are closely related to blackberries and other brambles or cranberries. A red and black raspberry is the most widespread throughout the world [5]. *R. idaeus* fruits composition is represented by a variety of flavonoid derivatives, is represented by anthocyanin, quercetin derivatives as well as phenolicarboxylic acids, organic acids, vitamin C [6]. The composition of green tea leaf (*Camellia sinensis* L.) contains: catechins (epigallocatehin-3-O-gallate, epicatehin, (+)-catechin, epigallocatechin), organic acids (oxalic acid), flavonoids (rutin) and hydroxycinnamic acids (caffeic acid) [7].

The recent literature search has showed that in many researches was estimated anti-inflammatory, anti-radical, cytotoxic and antihypertensive activity of aqueous-ethanolic *R. idaeus* extracts due to presents of high content of anthocyanin compounds [8, 9]. *C. sinensis* leaf catechins are not inferior to derivatives of anthocyanins, and there are a lot of scientific researches that have proofed a variety of pharmacological activity: anti-inflammatory, antimicrobial, anti-hyperglycemic, immune-modulation, and anticancer effects [10, 11, 12]. Moreover, in folk medicine *R. idaeus* shoot and *C. sinensis* leaf are traditionally applied to treat fever, skin infections, diabetes, cancers and liver diseases [13, 14]. In our view, the anthocyanins and catechins are perspective for the development of new antimicrobial, anti-fungi and antioxidant pharmaceuticals. But, according available literature sources indexed in Scopus and Web of Science, there are a low number of researches that evaluate and compare antimicrobial and antioxidant activity of anthocyanins and catechins.

Therefore, the aim of investigation was to assess antimicrobial, anti-fungal and antioxidant potential of *R. idaeus* fruit thick and *C. sinensis* leaf liquid extracts and provide phytochemical analysis of obtained extracts.

## Materials and methods

### *Plant material*

«» The study focused on *R. idaeus* fruits, which were harvested from areas where they are naturally grown. The collection took place in 2021 following the fruiting season, near the village of Ternova in the Kharkiv region (50°19′31″ N, 36°66′93″ E). The study focused on the leaf of *Camellia sinensis* from the Chun Myn variety, which was gathered as raw material in the Anhui province of China during the months of March through May in 2021 (31°03′41″ N, 116°33′25″ E).

### *Reagents*

« Acetonitrile (purchased from «Allchem», Kharkiv), acetic acid (purchased from «Allchem», Kharkiv), phosphoric acid (purchased from «Allchem», Kharkiv), methanol (purchased from «Allchem», Kharkiv), $K_3[Fe(CN)_6]$ was chemical pure (purchased from «Allchem», Kharkiv), $K_4[Fe(CN)_6]$ was chemical pure (purchased from «Allchem», Kharkiv), cyanidine-3-O-glucoside (≥98.0%), cyanidin-3-O-sophoroside (≥98.0%), pelargonidin-3-O-sophoroside (≥98.0%), cyanidin-3-O-rutinoside (≥98.0%), cyaniding-3-rutinoside-5-glucoside (≥98.0%), epicatechin (≥98.0%), epigallocatechin-3-O-gallate (≥98.0%), epigallocatechin (≥98.0%), epicatechin-3-O-gallate were purchased in Sigma Aldrich Company, Lublin, Poland.

### *Equipment*

« Potentiometric measurements were performed on a HANNA 2550 pH meter (FRG) with a glass electrode HI11310 (FRG). The quantitative analysis of biologically active compounds was performed on a UV-1000 spectrophotometer (LabAnalyt, China) with matched 1 cm quartz cells

### *Extraction procedure*

«» A 100.0 g (exact mass) of *R. idaeus* fruits was pressed, then it was added of 96% ethanol in a threefold amount to the extraction, after that filtration, then obtained filtrate was concentrated by a vacuum-evaporator at a temperature of 50-60°C until the humidity of the extract is 25%.

The *C. sinensis* extract was obtained by the following way: the raw material was conducted with 60% ethanol at 80º C within 1 hour with a condenser, ratio raw material/solvent – 1/20. The extraction technique was completed twice to provide totally extract all BAS, then the filtrates were joint and evaporated by vacuum rotary to ratio of extract to raw material 1:2.»

### *Phytochemical analysis*

« The total phenolic compounds were quantified using the Folin-Ciocaltau method, with absorbance readings taken at 760 nm [15]. For the quantification of total anthocyanin content, molecular adsorption analysis was utilized, with measurements of

absorbance at 546 nm. The content of total organic acids was established through acid-base titration, using a potentiometric method to determine the end-point [16]. The total catechins were assessed using the vanillin reagent assay, where absorbance was measured at 505 nm [17].

**HPLC analysis of *C. sinensis* leaf extract and *R. idaeus* thick fruit extract**

For the analysis, a Prominence LC-20 Shimadzu liquid chromatography system with a Thermo Scientific Syncronis aQ C18 column (4.6 × 250) was utilized. All analyses were conducted at a temperature of 40 ºC. The mobile phases consisted of a methanol aqueous solution (A) and a 1.0% solution of phosphoric acid (B). The gradient protocol started with 20–42% A over the first 15 minutes, shifted to 42–43% A from 15 to 25 minutes, changed to 43–90% A from 25 to 45 minutes, maintained 90% A from 45 to 55 minutes, decreased to 20% A from 55 to 60 minutes, and then held at 20% A from 60 to 70 minutes. Prior to use, the mobile phases were filtered using 25mm × 0.45 μm Supelco Iso-Disc Filters PTFE 25-4 and degassed. A flow rate of 0.5 mL/min was maintained, and the injection volume of the samples was 5 μL. Detection wavelengths were set at 255, 286, 350 and 530 nm. Chromatographic peaks of analytes were identified by the following similarity indexes, which were calculated between the test substance and the standard according to the formulas:

$$I_T = 1 - |T_{st} - T_u|$$
$$I_{255} = 1 - |h_{255_{st}} - h_{255_u}|$$
$$I_{286} = 1 - |h_{286_{st}} - h_{286_u}|$$
$$I_{350} = 1 - |h_{350_{st}} - h_{350_u}|$$

where, $I_T$ – retention time similarity index, $T_{st}$ – retention time of standard (min), $T_u$ – test substance retention time (min), $I_{255}$, $I_{286}$ and $I_{350}$ – spectral similarity indices, $h_{255st}$, $h_{286st}$ and $h_{350st}$ – spectral characteristics of the standard, $h_{255u}$, $h_{286u}$ и $h_{350u}$ – spectral characteristics of the test substance.

The least among the three similarity index values of spectral characteristics dictates the similarity level (IL) between substances and standards based on these traits. A higher IL value increases the probability of more precise identification of the substance. Substances whose similarity index with the catechin standard was at least 0.7, and whose peaks on the chromatogram appeared between the catechin peak and the earliest flavonoid peak, were classified as catechins [18].

***Antioxidant activity***

Antioxidant activity of extract was evaluated by potentiometric method [19]. Determination of antioxidant activity was made by following assay: a 2 mmol/L solution of $K_3[Fe(CN)_6]$ was prepared by weighing 0.8232 g into a 25.0 mL volumetric flask, dissolving a compound in a distilled water and filling the flask to volume with the same solvent. A 0.02 mmol/L of $K_4[Fe(CN)_6]$ was prepared by weighing 0.0921 g into a 250.0 mL volumetric flask, dissolving a compound in a distilled water and filling the flask to volume with the same solvent. Than a 5.00 mL aliquot of both prepared solutions was taken and transferred into a 250.0 mL volumetric flask and made up to the mark by 0.067 mol/L phosphate buffer solution. A 50.00 mL of prepared mediator solution was transferred in an electrochemical cell. The initial potential of mediator solution was measured after initial one was established, a 1.00 mL of aliquot of the prepared solutions was added and a final potential was measured. The difference ($\Delta E$) between the initial ($E_0$) and final ($E_1$) potentials was found. Antioxidant activity was calculated according to the following equation and expressed as mmol-equiv./$m_{dry\ res.}$:

$$AOA = \frac{C_{ox} - \alpha \times C_{red}}{1 + \alpha} \times K_{dil} \times 10^3 \times \frac{m_1}{m_2}$$

where, $\alpha = C_{ox}/C_{red} \times 10^{(\Delta E - E\text{ethanol})nF/2.3RT}$; $C_{ox}$ – concentration of $K_3[Fe(CN)_6]$, mol/L; $C_{red}$ – concentration of $K_4[Fe(CN)_6]$, mol/L ; $E_{ethanol}$ – $0.0546 \cdot C_{\%} - 0.0091$; $C_{\%}$ – concentration of ethanol; $\Delta E$ – change of potential; $F$ = 96485.33 C/mol – Faraday constant; n = 1 – number of electrons in electrode reaction; $R$ = 8.314 J/molK – universal gas constant; $T$ – 298 K; $K_{dil}$ – coefficient of dilution, mL.; $m_1$ – mass of dry residue; $m_2$ – mass of dry residue in 1.0 mL of extract or prepared solution of standard.

The standardized *C. sinensis* leaf liquid extract, which was obtained by 60% ethanol and solution of epigallocatechin-3-O-gallate were used as the reference standards.

***Test organisms***

«*S. aureus ATCC 25923, E. coli ATCC 25922, B. subtilis ATCC 6538, C. albicans ATCC 885/653, P. vulgaris NTCS 4636, and P. aeruginosa ATCC 27853* were employed following established

guidelines for evaluating the antimicrobial efficacy of pharmaceuticals.

***Screening antimicrobial and anti-fungi activity of extracts***

The method of diffusion of the drug into agar carried out using the method of "wells". Preparation of suspensions of microorganisms with a certain concentration of microbial cells (optical density) was carried out using a turbidity standard (0.5 units on the McFarland scale). The Densi-La-Meter device (manufactured by PLIVA-Lachema, Czech Republic; wavelength 540 nm) was used. The suspension was prepared according to the instructions for the device and the information sheet on innovations in the health care system No. 163-2006 "Standardization of the preparation of microbial suspensions", Kyiv [20]. Synchronization of cultures was carried out using low temperature (4°C) [21]. The microbial load was 107 microbial cells per 1 ml of medium and was set according to the McFarland standard. An 18-24-hour culture of microorganisms was used for the work. Mueller-Hinton agar was used for bacteria, whereas Sabouraud agar was used for *Candida albicans*. As reference standards were gentamycin and fluconazole.

**Assay of determination of minimum inhibitory concentration (MIC)**

MIC is defined as the smallest concentration of an antibacterial agent that entirely prevents bacterial growth. The MIC for various extracts was determined through the broth microdilution technique [22].

Statistical analysis

«For all the experiments, two samples were analyzed and all the assays were carried out in 5 times. The results were expressed as mean values with confident interval. The MS EXCEL 7.0 was used to provide statistical analysis.»

**Results and discussion**

According to obtained results shown in Table 1, the *C. sinensis* leaf extract (10.10±0.25%) had higher content of phenolic compounds, than in *R. idaeus* thick fruit extract (0.60±0.02%).

Table 1 demonstrates that the total content of anthocyanin in *R. idaeus* thick fruit extract was 0.10±0.002%, whereas in *C. sinensis* leaf extract anthocyanin was not presence. The percentage of anthocyanin out of total polyphenols was 17% in *R. idaeus* extract.

The highest amount of organic acids was determined in *R. idaeus* thick fruits extract (4.60±0.50%), whereas in the *C. sinensis* leaf extract it was lower 65% (1.60±0.02%). In *R. idaeus* extract, the total organic acids were in 8.5 times higher than polyphenols, whereas in the *C. sinensis* leaf, the total organic acids were in 6.3 times lower than polyphenols. (Table 1)

**Table 1 –** Quantitative content of total phenolic compounds, anthocyanin and organic acids

| Sample | Total phenolic compounds, %±SD | Total anthocyanin, %±SD | Total catechin, %±SD | Total of organic acids, %±SD |
|---|---|---|---|---|
| *R. idaeus* extract | 0.60±0.02 | 0.10±0.002 | NP | 4.60±0.50 |
| *C. sinensis* leaf extract | 10.10±0.25 | NP | 10.47±0.25 | 1.60±0.10 |
| Note: SD – Standard Deviation, n=5;<br>NP – not present | | | | |

The HPLC method was used to carry out a qualitative and quantitative analysis of catechins and anthocyanins in the obtained extracts of *C. sinensis* leaf and *R. idaeus* fruits extract. According to the results of the study, 5 catechins were identified in *C. sinensis* leaf extract, whereas in *R. idaeus* fruits extract 6 anthocyanins (Fig. 1, 2).

The total content of catechins in the obtained *C. sinensis* leaf extract was 10500.0 mg/100 g. Among catechins, epigallocatechin-3-O-gallate dominates – 3730.0±74.6 mg/100 g (35.52% out of the total catechins), whereas the lowest content was (+)-catechin 210 mg/100 g (2.00% out of the total catechins). (Table 2, Figure 2)

**Table 2 –** Chemical composition of catechins in *C. sinensis* leaf extract by HPLC-UV analysis

| № | Catechins | Retention time, min | Similarity index, $I_L$ | Content of catechins in *C. sinensis* leaf extract, mg/100 g of extract ±SD | Part out of total catechins, % |
|---|---|---|---|---|---|
| 1 | Epigallocatechin | 13.013 | 0.875 | 2760.0±55.2 | 26.29 |
| 2 | (+)-catechin | 13.780 | 0.996 | 210.0±4.2 | 2.00 |
| 3 | Epicatechin | 16.494 | 0.851 | 1010.0±20.2 | 9.62 |
| 4 | Epigallocatechin-3-O-gallate | 17.686 | 0.990 | 3730.0±74.6 | 35.52 |
| 5 | Epicatechin-3-O-gallate | 20.754 | 0.814 | 2788.0±55.8 | 26.57 |
| | **Total** | | | **10500.0** | |
| Note: SD – Standard Deviation, n=5 | | | | | |

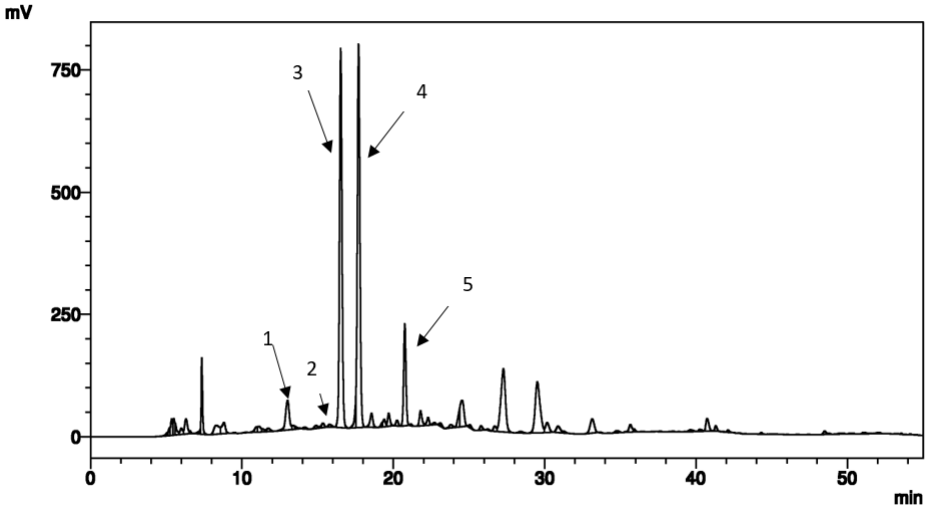


**Figure 2** – HPLC fingerprint (255 nm) of the *C. sinensis* leaf extract

As shown in Table 3, cyanidin-3-O-sophoroside dominated among all anthocyanins (47.4% out of the total anthocyanins), cyanidin-3-rutinoside-5-glucoside (29.0% out of the total anthocyanins) was in second place, and the lowest content was pelargonidin-3-O-sophoroside (0.47% out of the total anthocyanins).

The content of BAS in *R. idaeus* fruit extract was quantified by spectrophotometric, titrimetric and HPLC methods of analysis. The organic acids were present in both extracts, where the highest content of organic acids was determined in *R. idaeus* fruits extract than in *C. sinensis* leaf extract. In our view, it relates with different purpose of organic acids accumulation. The organic acids are precursor for biosynthesis of sugars in fruits, whereas in leaf, organic acids only play a role in photosynthesis as result there is no purpose of high accumulation organic acids in leaf [23]. Ivanovic *et al.* [24] investigated anthocyanin content of *R. idaeus* fruit 80% metanol extract by HPLC method. According to their results, it was detected following anthocyanins (mg/100 g per extract): cyanidin-3-sophoroside (20.4mg /100 g), cyanidin-3-O-rutinoside (3.0 mg/100 g), cyanidin-3-O-glucoside (6.0 mg/100 g), pelargonidin-3-sophoroside (2.85 mg/100 g). Compering with our results, the content of anthocyanins in our research was higher, but cyaniding-3-O-sophoroside was dominated in both extracts.

**Table 3 –** Chemical composition of antocyanins in *R. idaeus* fruit thick extract by HPLC analysis

| № | Antocyanins | Retention time, min | Content of antocyanins in extract, mg/100 g of extract ±SD | Part out of total antocyanins, % |
|---|---|---|---|---|
| 1 | Cyanidin-3-O-sophoroside | 14.170 | 52.14±1.04 | 47.46 |
| 2 | Cyanidin-3-rutinoside-5-glucoside | 14.780 | 31.90±0.64 | 29.03 |
| 3 | Cyanidine-3-O-glucoside | 15.255 | 18.15±0.36 | 16.52 |
| 4 | Cyanidin-3-O-rutinoside | 16.200 | 5.59±0.11 | 5.08 |
| 5 | Cyanidin 3-O-xylosyl-rutinoside | 16.393 | 1.61±0.03 | 1.46 |
| 6 | Pelargonidin-3-O-sophoroside | 19.977 | 0.47±0.01 | 0.45 |
| | **Total** | | **109.86** | |
| Note: SD – Standard Deviation, n=5 | | | | |

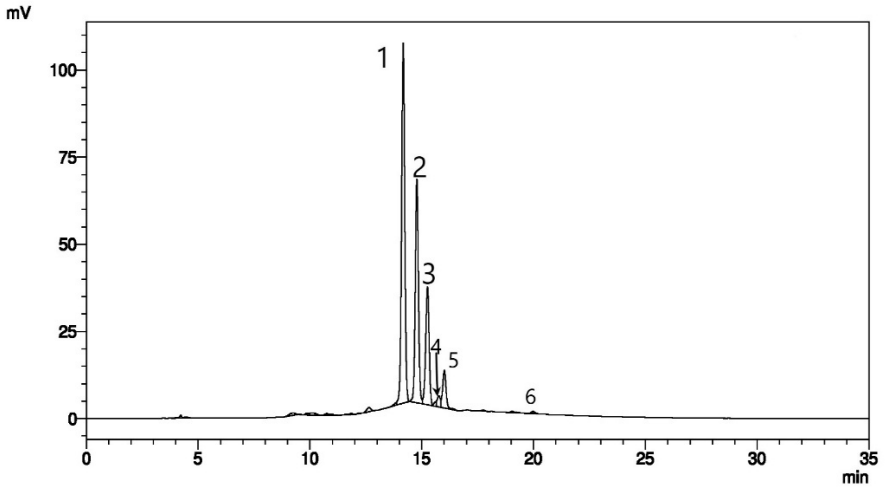


**Figure 3 –** HPLC fingerprint (530 nm) of the *R. idaeus* fruit thick extract

A potentiometric method for determining antioxidant activity was used to evaluate the effect of the obtained extracts. Table 4 shows that the level of antioxidant activity of *R. idaeus* fruits extract significantly interferer to the *C. sinensis* leaf extract. The antioxidant activity of *C. sinensis* leaf higher in 7.7 times of *R. idaeus* extract.

In light of the data obtained, it can be established that the *C. sinensis* leaf extract has the highest level of antioxidant activity. According to the modern classification of antioxidant activity, which was previously developed by us [25], it was found that all extracts obtained have a high level of antioxidant activity. Moreover, a comparative analysis of the "strength" of antioxidant activity was carried out with the gold standard *C. sinensis* leaf. Further, it was prepared solutions (in terms of the amount of polyphenols expressed as gallic acid) of extracts with 0.03 M concentration of *R. idaeus* thick fruits extract, *C. sinensis* leaf and epigallocatechin-3-O-gallate. As a result of the study, it was found that the level of antioxidant activity of *R. idaeus* extract was higher 56% of *C. sinensis* leaf extract and 49% epigallocatechin-3-O-gallate. (Table 5)

**Table 4 –** Level of antioxidant activity of *R. idaeus* thick extract, *C. sinensis* leaf extract

| Sample | Antioxidant activity, mmol-eqv./$m_{dry\ res.}$ ±SD | Conditional term of antioxidant level |
|---|---|---|
| *R. idaeus* thick fruits extract | 70.95±1.42 | High level |
| *C. sinensis* leaf extract | 548.79±10.98 | Very high level |
| SD – standard deviation, n=3 | | |

**Table 5 –** Level of antioxidant activity of *R. idaeus* thick extract, *C. sinensis* leaf extract and standard: epigallocatechin-3-O-gallate at concentration 0.03 mol/L

| Sample | Concentration, mol/L | Antioxidant activity, mmol-eqv./$m_{dry\ res.}$ ±SD |
|---|---|---|
| *R. idaeus* thick fruits extract | 0.03[a] | 60.81±1.22 |
| *C. sinensis* leaf extract | | 27.49±0.54 |
| Epigallocatechin-3-O-gallate | | 30.78±0.62 |
| Note: SD – standard deviation, n=5;<br>a – molar concentration of raspberry thick extract and green tea leaf extract was calculated as total phenolic compounds expressed as gallic acid | | |

At first glance, the *C. sinensis* leaf extract had significantly higher antioxidant potential than *R. idaeus* fruit extract. However, comparing extracts at the same molar concentration, it was found that *R. idaeus* fruit extract had 2 times higher the level of antioxidant activity than *C. sinensis* leaf extract. In our view, it relates with the fact that anthocyanins more potent antioxidants than catechins. Lapidot *et al.* [26] determined antioxidant activity of malvidin-3-glucoside, catechin, malvidin and resveratrol by the method of oxidation myoglobin with $H_2O_2$. It was shown that inhibition efficiency of the antioxidant decreased in following order: malvidin-3-glucoside > catechin > malvidin > resveratrol. In research of Muselik *et al.* [27], it was carried out evaluation the level of antioxidant activity of derivatives of catechins: epicatechin, (+)-catechin, epicatehin, epicatechin-3-O-gallate, gallocatechin; and anthocyanins: cyanidin-galactoside, malvidin-3-glucoside and delphinidin-3-glucoside by ferric reducing antioxidant power assay. It was found the level of antioxidant activity decreased in the following order: epicatechin-3-O-gallate > delphinidin-3-glucoside > cyanidin-galactoside > gallocatechin > malvidin-3-glucoside > epicatechin > catechin. The antioxidant activity of epicatechin-3-O-gallate had the highest antioxidant power whereas the catechin – the lowest one, where cyanidin-3-galactoside interfere to epicatechin-3-O-gallate, but greater than other derivatives of catehins. The major part of composition of *C. sinensis* leaf is presented by epicatechin-3-O-gallate and low amount – epicatechin and (+)-catechin. However, it is quite difficult to evaluate the contribution of each compound on total antioxidant power of extract as well as it is unknown whether catechins interact by synergistic way or antagonistic one. Thus, the level of antioxidant activity of extract depends not only on composition of extract, but also, on ration and interaction of compounds.

In this research work, the antimicrobial and antifungal activity of the obtained *R. idaeus* thick fruits and *C. sinensis* leaf extract was investigated against the following strains of *S. aureus*, *B. subtilis*, *E. coli*, *P. vulgaris*, *P. aeruginosa*, as well as a strain of the fungus *C. albicans*. According to the obtained results, extracts obtained from the *R. idaeus* fruit and *C. sinensis* leaf had an effective antimicrobial and antifungal effect.

Among pathogens strains, *R. idaeus* fruits extract was the most inhibits *S. aureus* strains (35.00±0.20 mm), whereas at the second place were *B. subtilis* (33.0±0.2 mm) Gramm-positive strains *P. vulgaris* was the most resistance bacteria to the action of *R. idaeus* fruits extract (21.0±0.2 mm). Comparing results with *C. sinensis* leaf extract, it was determined that *R. idaeus* fruits extract was 17, 21, 30 and 13% better inhibit bacterial strains of *S. aureus*, *B. subtilis, E. coli* and fungi *C. albicans* than *C. sinensis* leaf extract, respectively. Comparing obtained results with reference standard gentamycin, it was found that *S. aureus*, *B. subtilis* and *E. coli* were 37, 27 and 46% more sensitive to *R. idaeus* fruits extract than gentamycin. Whereas, *P. vulgaris* and *P. aeroginosa* was 16 and 3% more sensitive to gentamycin.

Anti-fungal investigation against *C. albicans* showed that *R. idaeus* fruits extract 20 and 13% more actively inhibit the growth of fungi than *C. sinensis* leaf and fluconazole, respectively.

The investigated extracts significantly inhibit the bacterial and fungi strains with MIC. In the previously above conducted antimicrobial study, the extract of *R. idaeus* fruits was the most active independently of the tested strains. Table 7 shows, the *R. idaeus* fruits extract with MIC value of 0.14 μM was the most active against *S. aureus*, whereas *C. sinensis* leaf extract MIC values was 80% lower. The highest MIC value of *C. sinensis* leaf extract was against fungi pathogens *C. albicans*. The MIC value of *R. idaeus* fruits extract against pathogens *E. coli*, *P. vulgaris* and *B. subtilis* was significantly lower than in the case of *C. sinensis* leaf extract.

**Table 6 –** Retardation zone (mm) resulting from the screening of antimicrobial activity of *R. idaeus thick extract, C. sinensis* leaf extract and standards: gentamycin, fluconazole

| Sample | Concentration, mM | Diameter of the growth retardation zone, mm±SD | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gramm-positive | | Gramm-negative | | | Fungi |
| | | *S. aureus* ATCC 25923 | *B. subtilis* ATCC 6633 | *E. coli* ATCC 25922 | *P. vulgaris* ATCC 4636 | *P.aeruginosa* ATCC 27853 | *C.albicans* ATCC 653/885 |
| *R. idaeus* thick extract | 0.009[a] | 35.0±0.2 | 33.0±0.2 | 30.0±0.2 | 21.0±0.4 | 25.0±0.3 | 23.0±0.3 |
| *C. sinensis* leaf extract | 0.009[a] | 29.0±0.2 | 26.0±0.3 | 21.0±0.4 | 24.0±0.3 | 28.0±0.2 | 21.0±0.4 |
| Gentamycin | 0.003 | 22.0±0.2 | 24.0±0.3 | 25.3±0.3 | 25.0±0.3 | 25.6±0.3 | 12.0±0.6 |
| Fluconazole | 0.003 | 18.0±0.2 | 12.0±0.6 | 14.3±0.5 | 12.3±0.6 | 10.0±0.7 | 20.0± 0.5 |

Note: SD – standard deviation, n=5;
a – molar concentration of raspberry thick extract and green tea leaf extract was calculated as total phenolic compounds expressed as gallic acid

**Table 7 –** Minimal inhibitory concentration of the different R. idaeus thick extract, and C. sinensis leaf extract against the 6 references pathogens

| Sample | MIC, μM | | | | | |
|---|---|---|---|---|---|---|
| | ***S. aureus* ATCC 25923** | ***B. subtilis* ATCC 6633** | ***E. coli* ATCC 25922** | ***P. vulgaris* ATCC 25922** | ***P.aeruginosa* ATCC 27853** | ***C.albicans* ATCC 653/885** |
| *R. idaeus* thick extract | 0.14 | 0.28 | 1.13 | 0.56 | 0.28 | 0.56 |
| **Green tea leaf extract** | **0.70** | **0.70** | **2.80** | **2.80** | **2.80** | **5.60** |

The analyzed *R. idaeus* fruit and *C. sinensis* leaf extracts showed high antimicrobial and antifungal activity against the following strains of *S. aureus*, *P. aeruginosa*, *P. vulgaris, B. subtillis* and *C. albicans*. According to the obtained data, at first glance it can be considered that the antimicrobial and antifungal activity of *R. idaeus* fruit and *C. sinensis* leaf extracts is significantly inferior to the action of gentamicin and fluconazole, because their concentration of solutions was in 3 times lower than the content of polyphenols in the extract. However, we would like to note that gentamicin has serious toxicity to the auditory nerve, kidneys and liver, which can lead to serious complications of the disease. Comparing the antifungal effects of fluconazole and *R. idaeus* fruit and *C. sinensis* leaf extracts, it was found that they inhibited the growth of the fungal strain at the same level, while the concentration of fluconazole was also lower, like gentamicin. We can declare that fluconazole is a leader as anti-fungi medicine, but at the same time it weakly inhibits the growth of gram-negative and gram-positive bacteria, but to *R. idaeus* fruit and *C. sinensis* leaf extracts both strains of bacteria and fungus are sensitive. Thus, *R. idaeus*

fruit and *C. sinensis* leaf extracts is a combined pharmaceutical that affects different mechanisms of vital activity of bacteria and fungi, thereby having a wide spectrum of action against different strains of bacteria and fungi, and at the same time not possessing serious toxicity.

The *R. idaeus* fruit is a rich source of anthocyanins, whereas *C. sinensis* leaf of catechins. It is well known that anthocyanins biosynthesis pathway is based on chemical conversion of catechins. Li *et al.* [28] declared that at the beginning of ripping period the content of anthocyanin start increasing, whereas the content of catechins decreasing. However, a question which of this group of flavonoids possess higher antimicrobial and anti-fungi activity is still actually for today. In our research, it was comparing antimicrobial potential of *R. idaeus* fruit and *C. sinensis* leaf extracts. In the antimicrobial and anti-fungi tests, which carried out by method of well, it was shown the *R. idaeus* fruit extract was more active against pathogens *P. aeruginosa, E. coli*, *B. subtilis*, *C. albicans* whereas *P. vulgaris* were sensitive to both extracts practically at the same level. Furthermore, it was determined MIC values for both extracts, as result *R. idaeus* fruit extract had better results than *C. sinensis* leaf extract. Therefore, based on mentioned results, it was assumed that antimicrobial and anti-fungi activity of anthocyanins higher than catechins. However, both extracts had a high content of organic acids and its presence should not be neglected. Further in our research, we planned to answer on question whether impact organic acids on antimicrobial potential or not.

## Conclusion

It was found that total phenolic compounds were higher in *C. sinensis* leaf extract, whereas total organic acids were in *R. idaeus* fruit thick extract. Both extracts possessed a high antioxidant potential, and effective antimicrobial and anti-fungi effects. Although we assumed that anthocyanins had higher antioxidant, antimicrobial and anti-fungi properties than catechins. In future studies, the hypothesized impact of organic acids on antimicrobial and anti-fungi effects should be verified by isolation of organic acids from both extracts. These findings would promote application of *R. idaeus* fruits extract as pharmaceuticals and nutraceuticals.

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## Conflict of interest

All authors are aware of the article’s content and declare no conflict of interest.

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