**Antimicrobial, antifungal, antioxidant activity and phytochemical investigation of fatty acids by GS/MS of raspberry (Rubus idaeus L.) shoot lipophilic extract**

**Abstract.** The aim of the study was to determine the content of fatty acids using GC-MS in the obtained extract, conduct a study of the antimicrobial, antifungal and antioxidant activity of *Rubus idaeus* shoot lipophilic extract. The quantification of fatty acids was accomplished through GC-MS, antioxidant activity was assessed by potentiometric method, antimicrobial and antifungal activity was determined by well method. The 18 compounds were identified by the GS-MS. The levulenic acid (64.47±0.08 mg/100 g), linoleic acid (8.50±0.08 mg/100 g) and linolenic acid (6.80±0.04 mg/100 g) dominated in the obtained lipophilic *R. idaeus* shoot extract. *Bacillus subtilis* (17.00±0.50 mm) was the most sensitive to lipophilic extract whereas *P. vulgaris* was the most resistant to the lipophilic extract. Moreover, *Candida albicans* was medium sensitive to lipophilic extract (13.50±0.50 mm). The antioxidant activity was 1.00 mmol-equiv./mg dry res, according to Maslov’s antioxidant level classification it has low level. The extract exhibited antimicrobial activity against all tested strains, with the most significant impact observed against *B. subtilis*. However, the obtained lipophilic extract showed a relatively low level of antioxidant activity. Consequently, the derivatives of fatty acids play a substantial role in the antimicrobial and antifungal effects, whereas their contribution to antioxidant activity appears to be limited.

**Key words:** raspberry, fatty acids, lipophilic extract, antimicrobial activity, antioxidant activity.

**Introduction**

The bacterial infection diseases have caused about 14% of all deaths and 56% of sepsis cases throughout the world in 2019. The mortality level was 100 deaths per 100,000 of population. The 55% out of 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*, with over 1.1 million deaths from *S. aureus* [1]. Over a 1.0 billion patients suffer from fungal infections of 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.

The temperate climate is the optimal conditions for genus *Rubus*, this genus is presented by 700 species [3]. Raspberries 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 on the global range [4].

The raspberry leaves and fruits composition is represented by a variety of flavonoid derivatives, is represented by quercetin derivatives as well as phenolicarboxylic acids, organic acids, vitamin C [5]. Phenolicarboxylic acids are represented by ferulic acid, gentisic acid, syringic acid, vanillic acid and ellagic acid in *R. idaeus* leaf and shoot. The recent study showed that main compound in *R. idaeus* leaf and shoot are ellagotannins [6].

The recent literature search has showed that in many researches was estimated antimicrobial...
and antifungal activities against G (+) and G (-) of ethanolic-aqueous and aqueous-ethanolic R. idaeus shoot and leaf extracts [7]. The results showed that all obtained extracts had high level of inhibition growth of bacteria and fungi strains. Moreover, the researches have been concluded that exactly derivatives of ellagotannins and flavan-3-ols responsible for antimicrobial and antifungal actions. There are no doubts that ellagotannins and flavan-3-ols are inhibited the growth of bacteria and fungi strains. However, in our view, the derivatives of fatty acids contribute to antimicrobial and antifungal effect of R. idaeus extracts. Therefore, the aim of study is to determine the content of fatty acids using GC-MS in the obtained extract, conduct a study of the antimicrobial, antifungal and antioxidant activities of R. idaeus shoot lipophilic extract.»

Materials and methods

Plant material. The Rubus idaeus shoots were the objective of the study, which were gathered from places of its native cultivation. The leaves were collected in 2021 after the fruiting phase in the village of Ternova, Kharkiv region, Ukraine (50.19311616222064, 36.66935288403296).

Reagents. Hexane (purchased from Allchem), chloroform (purchased from Allchem), vanillic, benzoic, ferulic, p-oxybenzoic, syringic, gentisic, salicylic and phenylacetic acids from Sigma Aldrich Company.

Extraction technique. A 250.0 (exact mass) g of Rubus idaeus shoots were drudgery in the size 1-2 mm. The leaves were extracted by 60% EtOH at the proportion of leaf: solvent 1:20 (m/v) on water bath with a reflux condenser for 60 minutes at 80°C, the extraction was made two times. Following the cooling process, the solutions were filtered and concentrated to a final volume of 250 mL using a rotatory evaporator at 40°C under vacuity conditions than obtained extract was extracted by a chloroform with volume 125 mL for 15 min two times.

GC-MS method of analysis. The chromatographic separation of fatty acids was carried out on gas chromatography-mass spectrometer 5973N/6890N MSD/DS «Agilent Technologies» (USA). The mass spectrometer detector is a quadrupole, ionization method is choosen an electron impact, and ionization energy is 70 eV. For the analysis is applied a full ion current. A capillary column was made by HP–INNOWAX (30 m × 250 μm). «The stationary phase was INNOWAX as well as mobile phase was helium, gas flow rate was 1 ml/min; the sample was introduced at 250 °C. The introduction of a sample of 2 μL into the chromatographic column was performed in the splitless mode (without flow distribution), which allows you to do this without loss of separation and significantly (up to 20 times) increase the sensitivity of the chromatography method. Sample injection speed – 1 mL/min, time – 0.2 min.

The research was done according following way: 0.50 mg of the dried extract in a 2 mL vial was added an internal standard (50 μg of tridecane in hexane) and 1.0 mL of a methylating agent – 14% BCl, in methanol, Supelco No. 3-3033. The mixture was kept in a hermetically sealed vial for 8 hours at a temperature of 65 ºC. During this time, fatty acids are completely extracted from the extract and transesterification of acids occurs. The reaction mixture was drained from the sediment and diluted with 1 ml of distilled water. To obtain methyl esters of fatty acids, 0.2 mL of methylene chloride was added, shaken for 1 hour «and subjected to chromatography.

Identification of the methyl esters of the acids was based on the calculation of the equivalent aliphatic chain length using data from the NIST 05 and Willey 2007 mass spectra library. The retention time was also compared with the retention time of standard compounds (“Sigma”). The quantitative content was calculated by a formula:

\[ C(\text{mg/kg}) = K_1 \times K_2 \times 100. \]

where, \( K_1 = S_1 / S_2 \) (S1 – square peak of analyzed substance, S2 – square peak of standard substance); \( K_2 = 50 / M \) (50 – mass of internal standard, that injected with analyzed substance, μg); M – sample mass, mg.

Antioxidant activity. «Antioxidant activity of extract was evaluated by potentiometric method and calculated according to the following equation and expressed as mmol-equiv./m

\[ \text{dry res.} \times 10^3. \]

Test organisms. Test strains of Staphylococcus aureus ATCC 25923, Proteus vulgaris NTCS 4636, Escherichia coli ATCC 25922, Bacillus subtilis ATCC 6538, Candida albicans ATCC 885/653, Pseudomonas aeruginosa ATCC 27853 were applied in accordance with the approvals for the assessment of drugs antimicrobial activity.

Antifungal and antibacterial activities assays. The concentration of lipophilic extract was 1% as a solvent of which were 40% EtOH. The method of diffusion of the drug into agar carried out using the method of “wells” [11].
Results and discussion

The GC-MS method was used to carry out a qualitative and quantitative analysis of fatty acids in the obtained lipophilic extract of *Rubus idaeus* shoot. According to the results of the study, 18 compounds were identified: levulinic, linoleic, linolenic, palmitic, oleic, stearic, arachidic, heneicosanoic, behenic, tetracosanoic, heptadecanoic, 2-hydroxypalmitic, azelaic, palmitoleic acid, myristic, lauric, tricosanoic, pentadecanoic acids (Figure 1).

The sum of the fatty acids in the obtained extract was 92.11 mg/100 g (Table 1).

![GC-MS fingerprint of *Rubus idaeus* shoot lipophilic extract](image)

Figure 1 – GC-MS fingerprint of *Rubus idaeus* shoot lipophilic extract

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Rt, min</th>
<th>Quantitative content in extract, mg/100 g ±SD</th>
<th>% out of sum fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Levulinic acid</td>
<td>12.689</td>
<td>64.47±1.00</td>
<td>69.99</td>
</tr>
<tr>
<td>2</td>
<td>Linoleic acid</td>
<td>30.294</td>
<td>8.50±0.08</td>
<td>9.23</td>
</tr>
<tr>
<td>3</td>
<td>Linolenic acid</td>
<td>31.580</td>
<td>6.80±0.08</td>
<td>7.38</td>
</tr>
<tr>
<td>4</td>
<td>Palmitic acid</td>
<td>25.433</td>
<td>4.06±0.08</td>
<td>4.41</td>
</tr>
<tr>
<td>5</td>
<td>Oleic acid</td>
<td>30.183</td>
<td>1.91±0.04</td>
<td>2.07</td>
</tr>
<tr>
<td>6</td>
<td>Stearic acid</td>
<td>30.061</td>
<td>1.68±0.04</td>
<td>1.82</td>
</tr>
<tr>
<td>7</td>
<td>Arachidic acid</td>
<td>32.659</td>
<td>0.64±0.04</td>
<td>0.69</td>
</tr>
<tr>
<td>8</td>
<td>Heneicosanoic acid</td>
<td>34.236</td>
<td>0.58±0.04</td>
<td>0.63</td>
</tr>
<tr>
<td>9</td>
<td>Behenic acid</td>
<td>35.601</td>
<td>0.58±0.04</td>
<td>0.63</td>
</tr>
<tr>
<td>10</td>
<td>Tetracosanoic acid</td>
<td>38.494</td>
<td>0.55±0.04</td>
<td>0.60</td>
</tr>
<tr>
<td>11</td>
<td>Heptadecanoic acid</td>
<td>26.410</td>
<td>0.54±0.04</td>
<td>0.59</td>
</tr>
<tr>
<td>12</td>
<td>2-hydroxypalmitic acid</td>
<td>32.043</td>
<td>0.43±0.04</td>
<td>0.47</td>
</tr>
<tr>
<td>13</td>
<td>Azelaic acid</td>
<td>24.764</td>
<td>0.41±0.04</td>
<td>0.45</td>
</tr>
<tr>
<td>14</td>
<td>Palmitoleic acid</td>
<td>25.776</td>
<td>0.34±0.04</td>
<td>0.37</td>
</tr>
<tr>
<td>15</td>
<td>Myristic acid</td>
<td>21.738</td>
<td>0.33±0.04</td>
<td>0.36</td>
</tr>
</tbody>
</table>
As shown in Table 1, levulinic acid dominates among all fatty acids (69.99% out of the total fatty acids), linoleic acid is in second place (7.38% out of the total fatty acids), and linolenic acid is in the third place, and the lowest content was pentadecanoic acid (4.41% out of the total fatty acids). As can be seen from the above results, the content of levulinic, linoleic and linolenic acids is 81.78% out of total fatty acids (Table 1).

Analyzed extract showed the antimicrobial and antifungal against *S. aureus*, *E. coli*, *P. vulgaris*, *P. aeruginosa*, *B. subtilis*, *C. albicans* strains.

According to the conducted research, it was found that extract strongly inhibited the growth of *B. subtilis* (17.00±0.50 mm). In the case of G(-) bacteria, it was found that strongly inhibited the growth of *E. coli* (15.00±0.50 mm) and *P. aeruginosa* (17.00±0.50 mm). *P. vulgaris* strain was the most resistant among bacteria turned out to be. *C. albicans* was medium sensitive for lipophilic extract (13.50±0.50 mm). In addition, it was found that *R. idaeous* shoot lipophilic extract had higher antimicrobial activity against G(+) than G(-) bacteria strains. (Table 2)

### Table 2 – Antimicrobial and antifungal activities of *R. idaeous* shoot lipophilic extract

<table>
<thead>
<tr>
<th>Sample</th>
<th>The diameter growth retardation zone, mm±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em> ATCC 25923</td>
</tr>
<tr>
<td>Lipophilic extract</td>
<td>16.50±0.50</td>
</tr>
<tr>
<td>40% EtOH</td>
<td>Growth</td>
</tr>
</tbody>
</table>

SD –standard deviation, n=3

The antioxidant effect was determined with the potentiometric method. Table 3 shows that the antioxidant activity of lipophilic extract was 1.00±0.01 mmol-equiv./m<sub>dry res.</sub>. Comparing with standard «Ascorutin» the antioxidant effect level of analyzed extract lower in 98%. According to developed a novel conditional classification of antioxidant action by Maslov [12] the lipophilic extract had a low level of scavenging activity of free radicals, whereas the standard «Ascorutin» – medium level (Table 3).

### Table 3 – Result of antioxidant activity of *R. idaeous* shoot lipophilic extract

<table>
<thead>
<tr>
<th>Sample</th>
<th>AOA, mmol-equiv./m&lt;sub&gt;dry res.&lt;/sub&gt;±SD</th>
<th>The conditional level of antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipophilic extract</td>
<td>1.00±0.01</td>
<td>Low level</td>
</tr>
<tr>
<td>«Ascorutin»</td>
<td>51.10±1.05</td>
<td>Medium level</td>
</tr>
</tbody>
</table>

SD –standard deviation, n=3
Fatty acids, whether free or part of complex lipids, play crucial roles in metabolism. They serve as a major source of metabolic fuel by storing and transporting energy, are essential components of all cell membranes, and act as gene regulators. Fatty acids are carbon chains that have a methyl group at one end (designated as omega, ω) and a carboxyl group at the other end. They are divided in two subgroups: saturated and unsaturated acids, it dependence on the presence of double bonds in the structure [13]. In a recent research of Celik et al. [14], it has been evaluated the extract obtained with chloroform from *R. idaeus* fruit from cultivated fruit. It was found that total fatty acids content was 290.0 mg/100 g in extract, the 10 fatty acids were identified: linoleic, linolenic, palmitic, oleic, stearic, arachidic, lauric, azelaic, behenic, and myristic acids. The main fatty acids were represented by unsaturated acids: linoleic (35%) and linolenic (20%) acids. Compared with our results, the total content of fatty acids was 68% lower, the content of linolenic acid was lower in 50%, and the content of linoleic acid in 45%. In our view, levulinic acid is a specific marker of *R. idaeus* shoot, whereas in fruit are linolenic and linoleic acid. According to compared results, the sum content of fatty acids in *R. idaeus* fruit extract is higher than in the obtained lipophilic extract from shoot. In our view, it relates with the biometabolism of flavonols in plant. According to the theory of growing plant, derivatives of fatty acid involved in responses to stress, growth, and development that is quite important for fruit, whereas for shoot fatty acids play only role of transporters.

In our recent studies, it has been demonstrated that the primary groups of biologically active compounds in *R. idaeus* shoots are derivatives of catechins and ellagitannins. Due to the presence of these compound groups, the extracted *R. idaeus* shoot extract exhibits powerful antioxidant, antimicrobial, and antifungal effects. However, other phenolic compounds, such as fatty acids (levulinic acid, linoleic acid, linolenic acid, etc.), are also present in *R. idaeus* shoots but in minor quantities. We were interested in understanding whether these fatty acids possess antioxidant, antimicrobial, and antifungal properties, or if they are merely inert compounds with no pharmacological value.

To investigate this hypothesis, we conducted the following extraction method: initially, a dual extraction of the raw material was performed using 60% ethanol, followed by ethanol evaporation and liquid-liquid extraction with chloroform. This is because fatty acids acid derivatives have high solubility in nonpolar solvents. The obtained extract was evaporated to a 1:2 mass ratio relatively to the raw material, and subsequent analysis was conducted to evaluate antimicrobial, antifungal, and antioxidant activities.

Our study revealed that the obtained lipophilic extract demonstrates antimicrobial activity against both G(+) and G(-) bacterial strains and exhibits antifungal activity against *Candida albicans*. However, it shows low level of antioxidant activity. Consequently, derivatives of fatty acids contribute significantly to the antimicrobial and antifungal actions, while catechin and ellagitannins derivatives are responsible for the antioxidant activity. Based on these findings, it can be concluded that in the development of a pharmaceutical product with antioxidant properties, fatty acid derivatives should be eliminated.

**Conclusion**

The lipophilic extract from *R. idaeus* shoots contains various fatty acids, including levulinic, linoleic, linolenic, palmitic, oleic, stearic, arachidic, heneicosanoic, behenic, tetracosanoic, heptadecanoic, 2-hydroxypalmitic, azelaic, palmitoleic, myristic, lauric, tricosanoic, pentadecanoic acids with the highest concentrations observed for levulinic, linoleic, and linolenic acids. This study highlights the antimicrobial and antifungal properties of the *R. idaeus* shoot lipophilic extract. The extract exhibited antimicrobial activity against all tested strains, with the most significant impact observed against *Bacillus subtilis*. However, the obtained lipophilic extract showed a relatively low level of antioxidant activity. Consequently, the derivatives of fatty acids play a substantial role in the antimicrobial and antifungal effects, whereas their contribution to antioxidant activity appears to be limited.

**References**


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