The study of some quality parameters and the antioxidant activity of dietary supplements with the pomegranate extract (Punica granatum L.)

Aim. To conduct a qualitative, quantitative analysis and determine the antioxidant activity of dietary supplements with the pomegranate extract.

Materials and methods. Three dietary supplements from different manufacturers – “Extract of pomegranate” (manufactured by Source Naturals), “Extract of pomegranate” (manufactured by Puritan Pride), and “Extract of pomegranate” (manufactured by Vitacost) – were chosen for the study. The thin layer chromatography (TLC) was used to perform the qualitative analysis, the quantity of biologically active substances (BAS) in dietary supplements was determined by the spectrophotometric method, the antioxidant activity – by potentiometric method. The qualitative analysis was performed by thin-layer chromatography (TLC); spectrophotometry was used for the quantitative determination, and the potentiometric method was applied to determine the antioxidant activity.

Results and discussions. The presence of anthocyanins, flavonoids, catechins and phenolic acids were detected in the dietary supplements analyzed. The total content of phenolic compounds was 201.00 ± 6.03, 159.00 ± 4.77, 110.30 ± 3.30 mg, catechins was 16.10 ± 0.50, 11.60 ± 0.50, 7.33 ± 0.50 mg, anthocyanins was 43 ± 1.29, 32.00 ± 0.96, 22.00 ± 0.66 mg, and flavonoids was 30.15 ± 0.90, 29.44 ± 1.00, 18.21 ± 0.55 mg. The antioxidant activity was 266.11 ± 5.32, 212.12 ± 4.24, 150.81 ± 3.02 mmol-equiv./mL for dietary supplements “Extract of pomegranate” (manufactured by Source Naturals), “Extract of pomegranate” (manufactured by Puritan Pride), “Extract of pomegranate” (manufactured by Vitacost) in 1 dosage form, respectively.

Conclusions. The analysis of the qualitative composition, the quantitative content of BAS and the antioxidant activity of dietary supplements with the pomegranate extract allows us to find out that dietary supplements manufactured by Source Naturals and Puritans Pride meet the requirements of the State Pharmacopoeia of Ukraine (SPhU), while a dietary supplement manufactured by Vitacost does not correspond the requirements. The results of the study allow us to state that the problem of compliance of dietary supplements is relevant; therefore, it is necessary to introduce normative documentation for the identification and determination of BAS in dietary supplements.

Keywords: pomegranate; spectrophotometry; analysis; phenolic compounds; dietary supplements; antioxidant activity
**Introduction.** Nowadays, dietary supplements are a promising area for pharmaceutical and food industries as a lot of consumers are interested in their health. Dietary supplements are typically marketed in the form of a capsule, pill, powder or gel and are not presented for use as a conventional food, meal or diet. They contain one or more dietary ingredients (e.g., vitamins, minerals, amino acids, plant extracts) and are intended to supplement the diet. Dietary supplements may offer opportunities to reduce health risk factors and risk of diseases in combination with prescription medicines [1].

Antioxidants take part in the system of the defense mechanisms, which main aim is combating pathological and diseases associated with the attack of free radicals, helping to inhibit oxidative processes. The benefits of antioxidants are their curative counteraction to free radicals in particular, oxidative stress in general, as well as in promoting the prevention and treatment of a number of cancers, improving human health, and increasing expectancy [2, 3].

Pomegranate (Punica granatum L.) is a rich source of polyphenols that have a high potential of antioxidant medicine. It is a fruit-bearing deciduous tree that belongs to the Lythraceae family. The pomegranate was discovered in the North Caucasus and Transcaucasia regions. Today, pomegranate is cultivated in Africa, South Caucasus, South and Central Asia, North and South America and in the Mediterranean region. The fruit is a round berry with a thick reddish husk. The inner part of the husk is a white, thin-walled mesocarp that forms chambers containing edible arils with seeds inside [4].

The arils of pomegranate contain anthocyanins, catechins, flavonoids and derivatives of ellagic and organic acids [5]. Pomegranate peel makes up 40 % of the whole fruit, and it is an inedible part of the fruit [6]. Compared with arils, the peel contains hydrolyzed ellagitannins (e.g., punicalagin), significant higher amount of catechins (e.g., (+)-catechin, epicatechin, gallatechin), phenolic acids (gallic acid, ellagic acid), but unlike arils, the peel does not contain any anthocyanins [7].

There are numerous in vivo and in vitro studies of pharmacological activities of pomegranate extracts, such as antimicrobial [8], anti-inflammatory [9], anticarcinogenic [10], anti-obesity [11] and antioxidant ones [12]. The recent studies have shown that the pomegranate extract can be used for the prevention and treatment of cardiovascular and neurodegenerative diseases, diabetes mellitus [13-15].

Thus, the aim of study was to conduct a qualitative, quantitative analysis and determine the antioxidant activity of dietary supplements with the pomegranate extract.

**Materials and methods.** Three dietary supplements with pomegranate of different manufacturers (USA) were purchased for analysis.

“Extract of pomegranate” contains the extract of the pomegranate pulp, the dosage form is capsule (100 pcs) manufactured by Source Naturals, USA.

“Extract of pomegranate” contains the extract of the pomegranate pulp, the dosage form is tablet (60 pcs) manufactured by Puritan Pride, USA.

“Extract of pomegranate” contains the extract of the pomegranate pulp, the dosage form is capsule (100 pcs) manufactured by Vitacost, USA.

The following substances were used for our research: epicatechin ≥ 98.0 % (Sigma Aldrich), epigallocatechin-3-O-gallate ≥ 99.0 % (Sigma Aldrich), cyaniding-3-glucoside ≥ 99.0 % (Sigma Aldrich), pelargonidin-3-glucoside ≥ 99.5 % (Sigma Aldrich), rutin ≥ 99.0 % (Sigma Aldrich), chlorogenic acid ≥ 98.0 % (Sigma Aldrich), caffeic acid ≥ 99.0 % (Sigma Aldrich).

Toluene, formic acid, ethyl acetate, glacial acetic, hydrochloric acid were of analytical grade and purchased from Kharkiv Reakhim.

“Sorbfil – PTSH – AF – A – UV” plates were used for the TLC analysis.

To determine the amount of biologically active substances (BAS) in dietary supplements, a UV – 1000 UV-spectrophotometer (China) with the corresponding 1 cm quartz cell was used.

**Preparation of standard solutions for the TLC analysis** 0.10 g of cyaniding-3-glucoside, pelargonidin-3-glucoside, chlorogenic acid, epigallocatechin-3-O-gallate, and 0.25 g of epicatechin, rutin were transferred into 100 mL measuring flasks, dissolved in 96 % ethanol and diluted to the volume with the same solvent.

0.01 g of ellagic acid was transferred into a 100 mL measuring flask, dissolved in 96 % ethanol and diluted to the volume with the same solvent.

**Preparation of the standard solution of rutin for the spectrophotometric assay** 0.050 g (accurate weight) of rutin was transferred into a 100.0 mL measuring flask, dissolved in 96 % ethanol and diluted to the volume with the same solvent. 1.0 mL of the solution prepared was taken, transferred into a 25.0 mL measuring flask and diluted to the volume with 96 % ethanol.

**Preparation of the solution with the vanillin reagent** 1.0 g of vanillin was dissolved in 100 mL of 96 % ethanol.
**TLC analysis assay**

The powder of a crushed tablet and the capsule contents of dietary supplements were completely dissolved in 96 % ethanol and filtered into a 25.0 mL volumetric flask, and diluted to the volume with the same solvent. The mobile phase was hydrochloric acid–formic acid–n-butanol (16 : 19 : 65) for detection of anthocyanins [16], toluene–acetone–formic acid (9 : 9 : 2) for catechins [17], ethyl acetate–glacial acetic acid–formic acid–water (100 : 11 : 11 : 26) for flavonoids and phenolic acids [18].

The following standard solutions were used: 0.1 % cyaniding-3-glucoside and 0.1 % pelargonidin-3-glucoside (for anthocyanins); 0.25 % epicatechin and 0.1 % epigallocatechin-3-O-gallate (for catechins); 0.25 % rutin, 0.1 % chlorogenic acid, 0.01 % ellagic acid and caffeic acid (for flavonoids and phenolic acids).

The sample plates were air dried, then placed in chromatographic chambers, which were pre-saturated with a developing system and chromatographed in ascending order. When the front of the solvent passed about 8 cm, the plates were removed from the chambers, dried in air for 30 minutes. Anthocyanins were determined in daylight, catechins were detected with the Berlin blue solution, flavonoids and phenolic acids were determined with a 10 % solution of NaOH in 96 % ethanol.

**Total phenolic compounds assay**

The total phenolic compounds were determined by the Folin-Ciocalteu method [18]. 2.0 g (accurate weight) of each dietary supplement were dissolved in 96 % ethanol, filtrated in a 50.0 mL measuring flask, and diluted to the volume with the same solvent. An aliquot of the solution obtained was mixed with 1.0 mL of 1 M Folin-Ciocalteu reagent. The resulting solution was mixed and diluted to the volume with the addition of 20 % NaOH. The optical density was measured at 760 nm in 30 min. The calibration curve was plotted using gallic acid, the calibration equation \( Y = 0.1055X + 0.1745 \) (R² = 0.9951). The total phenolic compounds in dietary supplements in a dosage form calculated with reference to gallic acid was determined with the equation:

\[
X(\%) = \frac{C_x \cdot K_{dil} \cdot m_{\text{aver dos form}} \cdot 100 \cdot 100}{m_s \cdot (100 - W)}
\]

where \( C_x \) – is the concentration of gallic acid according to the calibration curve, \( C : 10^6 \), g/mL; \( m_s \) – is the sample mass, g; \( m_{\text{aver dos form}} \) – is the mass of the average dosage form, g; \( K_{dil} \) – is the dilution coefficient; \( W \) – is the percentage of moisture, %.

**Total anthocyanins assay**

2.0 g (accurate weight) of each dietary supplement were dissolved in 96 % ethanol, filtrated in a 50.0 mL measuring flask, and diluted to the volume with the same solvent. An aliquot of the solution obtained was transferred in a 25.0 mL measuring flask, and diluted to the volume with 0.1 % HCl solution in methanol. The optical density was measured at 528 nm as a compensation liquid was 0.1 % HCl solution in methanol [19].

The total anthocyanins in dietary supplements in a dosage form calculated with reference to cyanidine-3-glucoside was determined with the equation:

\[
X(\%) = \frac{A \cdot K_{dil} \cdot m_{\text{aver dos form}} \cdot 100}{718 \cdot m_s \cdot (100 - W)}
\]

where \( A \) – is the absorbance of the solution analyzed; \( m_s \) – is the sample mass, g; \( m_{\text{aver dos form}} \) – is the mass of the average dosage form, g; \( K_{dil} \) – is the dilution coefficient; \( W \) – is the percentage of moisture, %, 718 – is the absorbance coefficient for cyanidine-3-glucoside at 528 nm.

**Total catechins assay**

2.0 g (accurate weight) of each dietary supplement were dissolved in 96 % ethanol, filtrated in a 50.0 mL measuring flask, and diluted to the volume with the same solvent. An aliquot of the solution obtained was mixed with 1.0 mL of 1 % vanillin solution in 96 % ethanol and added in a 25 mL volumetric flask. Then, the solution was diluted with the addition of 0.5 mol/L HCl in 96 % ethanol solution. The mixture was analyzed at 505 nm after standing for 30 min. The calibration curve was plotted with interval concentrations of 100–400 \( \cdot 10^6 \) g/mL, the calibration equation \( Y = 0.0025X - 0.0851 \) (R² = 0.9951) [20]. The total catechins in dietary supplements in a dosage form calculated with reference to epigallocatechin-3-O-gallate was determined with the equation:

\[
X(\%) = \frac{C_x \cdot K_{dil} \cdot m_{\text{aver dos form}} \cdot 100 \cdot 100}{m_s \cdot (100 - W)}
\]

where \( C_x \) – is the concentration of epigallocatechin-3-O-gallate according to the calibration curve, \( C : 10^6 \) g/mL; \( m_s \) – is the sample mass, g; \( m_{\text{aver dos form}} \) – is the mass of the average dosage form, g; \( K_{dil} \) – is the dilution coefficient; \( W \) – is the percentage of moisture, %.

**Total flavonoids assay**

2.0 g (accurate weight) of each dietary supplement were dissolved in 96 % ethanol, filtrated in a 50.0 mL measuring flask, and diluted to the volume with the same solvent. An aliquot of the solutions obtained was mixed with 1.0 mL of 2 % AlCl₃ solution in 5 % glacial acid in methanol and diluted to 25.0 mL with a 5 % solution of glacial acetic acid in methanol. The solution obtained was kept for 30 min, and optical density was measured at 417 nm as a compensation liquid was an aliquot of the solution obtained, which was diluted to 25.0 mL with a 5 % solution of glacial acetic acid in methanol [13].

The total flavonoids in dietary supplements in a dosage form calculated with reference to rutin was determined with the equation:

\[
X(\%) = \frac{A \cdot K_{dil} \cdot m_{\text{aver dos form}} \cdot 100}{A_{st} \cdot m_s \cdot (100 - W)}
\]

where \( A \) – is the absorbance of the solution analyzed, \( A_{st} \) – is the absorbance of the standard solution of rutin; \( m_s \) – is the sample mass, g; \( m_{\text{aver dos form}} \) – is the mass of the average dosage form, g; \( K_{dil} \) – is the dilution coefficient; \( W \) – is the percentage of moisture, %, \( m_{st} \) – is the mass of the rutin standard substance, g.
Antioxidant assay

The antioxidant activity of the samples analyzed was assessed with the potentiometric method \cite{21, 22}. The antioxidant activity was found using the subsequent formula and presented as mmol-eqv./mL:

\[
AOA = \frac{C_{ox} - \alpha \cdot C_{red}}{1 + \alpha} \cdot K_{dil} \cdot 10^3 \cdot m_{extract}
\]

where \(\Delta E\) is the change of the potential; \(E = 96485.33\) C/mol; \(R = 8.314\) J/molK; \(T = 298\) K; \(n = 1\) (number of electrons); \(K_{dil}\) is the dilution coefficient, mL; \(m_{extract}\) is the mass of the extract in tablets, g; \(\alpha = C_{ox}/C_{red} \cdot 10^{\Delta E \cdot \text{Ethanol} \cdot \text{mol} / 2.3RT}\); \(E_{\text{ethanol}} = 0.0546; C_{\text{ox}} = 0.0091; C_{\text{red}}\) is the ethanol concentration; \(C_{ox}\) is the \(K_{\text{Fe(CN)}_6}\) concentration, mol/L; \(C_{\text{red}}\) is the \(K_{\text{Fe(CN)}_6}\) concentration, mol/L.

Statistical analysis

For each experiment, five samples were subjected to analysis, with each assay conducted five times. The outcomes were presented as average values accompanied by confidence intervals. The statistical analysis was carried out using MS EXCEL 7.0 and STATISTIKA 6.0 software.

Results and discussions. BAS in dietary supplements were determined by the TLC analysis. In the case of catechin identification, the chromatogram showed a predominant band with the value of \(R_f = 0.46\) (epicatechin), after the derivatization with the vanillin reagent, a bright red band with the value of \(R_f = 0.46\) (epicatechin) was also detected.

To detect anthocyanins in dietary supplements, the mobile phase: hydrochloric acid – formic acid – n-butanol (16 : 19 : 65) was used. After chromatographic separation, two red bands were formed with values of \(R_f = 0.60\) (pelargonidin-3-glucose), of \(R_f = 0.80\) (cyanidine-3-glucose).

To identify flavonoids and phenolic acids in the dietary supplements analyzed the following developing system was used: ethyl acetate : glacial acetic acid : formic acid : water (100 : 11 : 11 : 26). The TLC plate was sprayed with a 10 % NaOH solution, resulting in yellow bands with the value of \(R_f = 0.35\) (rutin), \(R_f = 0.30\) (ellagic acid).

In all dietary supplements studied, we found epicatechin, cyanidine-3-glucose, pelargonidin-3-glucose, rutin and ellagic acid, but did not identify any derivatives of hydroxycinnamic acids.

“Extract of pomegranate” (manufactured by Source Naturals) had the highest content of phenolic compounds (201.00 ± 6.03 mg), whereas other dietary supplements demonstrated a lower content of phenolic compounds. The dietary supplement manufactured by Puritans Pride was 20.90 % lower in content compared to dietary supplements manufactured by Source Naturals, while the dietary supplement manufactured by Vitacost in 45.27 % (Table 1).

The most significant content of catechins was observed in the dietary supplement manufactured by Source Naturals (16.10 ± 0.50 mg), followed by the dietary supplements by Puritans Pride (11.60 ± 0.50 mg) and the dietary supplement manufactured by Vitacost (7.33 ± 0.50 mg) (Table 1).

“Extract of pomegranate” (manufactured by Source Naturals) had the most significant content of anthocyanins (43.00 ± 1.29 mg), while other dietary supplements showed a much lower content of anthocyanins (Table 1).

The greatest content of the total flavonoids was observed in “Extract of pomegranate” (manufactured by Source Naturals) (30.15 ± 0.90 mg), followed by the dietary supplements manufactured by Puritans Pride (29.44 ± 1.00 mg) and dietary supplements manufactured by Vitacost (18.21 ± 0.55 mg) (Table 1).

According to the results obtained, the content of anthocyanins was the highest among phenolic compounds in dietary supplements, flavonoids were in the second place, and catechins were in the last place (Table 1).

The minimum content of each vitamin and/or mineral substance (nutrients) in the recommended daily amounts of dietary supplements should be at least 15 % of the

<table>
<thead>
<tr>
<th>Total phenolic compounds</th>
<th>Total flavonoids</th>
<th>Total anthocyanins</th>
<th>Total catechins</th>
</tr>
</thead>
<tbody>
<tr>
<td>%[^{[a]}]</td>
<td>X[^{[b]}]</td>
<td>%</td>
<td>X</td>
</tr>
<tr>
<td>&quot;Extract of pomegranate&quot; (manufactured by Source Naturals)</td>
<td>57.43 ± 1.72</td>
<td>201.00 ± 6.03</td>
<td>8.61 ± 0.26</td>
</tr>
<tr>
<td>&quot;Extract of pomegranate&quot; (manufactured by Puritans Pride)</td>
<td>44.17 ± 1.26</td>
<td>159.00 ± 4.77</td>
<td>8.18 ± 0.29</td>
</tr>
<tr>
<td>&quot;Extract of pomegranate&quot; (manufactured by Vitacost)</td>
<td>29.73 ± 0.89</td>
<td>110.00 ± 3.30</td>
<td>4.92 ± 0.14</td>
</tr>
</tbody>
</table>

Notes: \[^{[a]}\] the percentage of BAS in 1 tablet or capsule; \[^{[b]}\] the content of BAS in 1 tablet or capsule (mg)
recommended daily intake, and the maximum daily intake should be 3 times higher than the daily recommended intake. The normative document of the daily intake of nutrients “Norms of physiological needs of the population of Ukraine in basic nutrients and energy” [23] states that the daily intake of flavonoids is 250 mg, and catechins is 100 mg. Thus, the minimum daily intake of flavonoids is 37.5 mg, and the maximum – 700.0 mg, while in the case of catechins the minimum daily intake is 15.0 mg, and the maximum – 300.0 mg. The daily intake of flavonoids and catechins of dietary supplements manufactured by Source Naturals was 60.3 and 32.3 mg, whereas in the case of dietary supplements manufactured by Puritans Pride – 117.8 and 46.4 mg, and dietary supplements manufactured by Vitacost – 18.2 and 7.33 mg, respectively (Table 2, 3).

According to the mentioned above requirements for the daily intake of flavonoids and catechins, the dietary supplements manufactured by Source Naturals and by Puritans Pride meet the requirements of the SPhU 2.0. Thus, these two dietary supplements can be recommended for the daily intake of nutrients.

Dietary supplements with pomegranate are recommended as antioxidant food additives since the pulp of pomegranate fruits contains a large number of derivatives of ellagotannins and catechins. However, there is no any information about the level of the antioxidant activity in pack inserts to dietary supplements. Therefore, we determined the level of the antioxidant activity of the dietary supplements analyzed by the potentiometric assay developed [24]. According to the results obtained, the dietary supplement manufactured by Source Naturals demonstrated the highest level of the antioxidant activity (266.11 ± 5.32 mmol-equiv./mL) than others. Comparing the results of the antioxidant activity with the reference drug “Ascorutin” we saw that “Ascorutin” was significantly inferior to dietary supplements with pomegranate by 368, 273, and 165 % for dietary supplements manufactured by Source Naturals, Puritans Pride and Vitacost, respectively (Table 4).

In our recent studies [25] we have developed and described a new conditional classification of the antioxidant activity according to Maslov since the standard substance is epigallocatechin-3-O-gallocate. According to

<table>
<thead>
<tr>
<th>Dietary supplements</th>
<th>Daily intake of flavonoids by dietary supplements, mg</th>
<th>Min daily intake of flavonoids, mg</th>
<th>Max daily intake of flavonoids, mg</th>
<th>Compliance requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Extract of pomegranate” (manufactured by Source Naturals)</td>
<td>60.3</td>
<td>37.5</td>
<td>750.0</td>
<td>Satisfy</td>
</tr>
<tr>
<td>“Extract of pomegranate” (manufactured by Puritans Pride)</td>
<td>117.8</td>
<td></td>
<td></td>
<td>Satisfy</td>
</tr>
<tr>
<td>“Extract of pomegranate” (manufactured by Vitacost)</td>
<td>18.2</td>
<td></td>
<td></td>
<td>Not satisfy</td>
</tr>
</tbody>
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<table>
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<tr>
<th>Dietary supplements</th>
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<th>Compliance requirement</th>
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<tbody>
<tr>
<td>“Extract of pomegranate” (manufactured by Source Naturals)</td>
<td>32.3</td>
<td>15.0</td>
<td>300.0</td>
<td>Satisfy</td>
</tr>
<tr>
<td>“Extract of pomegranate” (manufactured by Puritans Pride)</td>
<td>46.4</td>
<td></td>
<td></td>
<td>Satisfy</td>
</tr>
<tr>
<td>“Extract of pomegranate” (manufactured by Vitacost)</td>
<td>7.3</td>
<td></td>
<td></td>
<td>Not satisfy</td>
</tr>
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</table>
the above classification all the dietary supplements analyzed had a high level of the antioxidant activity, whereas the reference drug “Ascorutin” had the mean level of this activity. Thus, the dietary supplements analyzed have antioxidant properties and can be recommended to reduce the level of free radicals in the human body (Table 4).

**Conclusions.** The analysis of the qualitative composition, the quantitative content of BAS and the antioxidant activity of dietary supplements with the pomegranate extract allows us to find out that dietary supplements manufactured by Source Naturals and Puritans Pride meet the requirements of the SPhU, while the dietary supplement manufactured by Vitacost does not correspond the requirements. The results of the study allow us to state that the problem of compliance of dietary supplements is relevant; therefore, it is necessary to introduce normative documentation for the identification and determination of BAS in dietary supplements.

**Conflict of interests:** authors have no conflict of interests to declare.

**REFERENCES**


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