Investigation of antioxidant interaction between ascorbic acid and rutin in medicine “Ascorutin”

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The aim of the work was to investigate interaction between ascorbic acid and rutin in medicine “Ascorutin”.

Materials and methods. The objects of the study were one series of tablets “Ascorutin,” rutin, and ascorbic acid. The level of antioxidant activity was measured by the potentiometric method.

Results. The level of antioxidant activity of model solutions of rutin was 265.51 ± 1.00 mmol-eq./L, ascorbic acid – 99.00 ± 1.00 mmol-eq./L, a combination of rutin and ascorbic acid – 255.60 ± 1.00 mmol-eq./L, and medicine “Ascorutin” – 256.52 ± 1.00 mmol-eq./L. An experimental value of the level of antioxidant activity of “Ascorutin”, the combination of rutin and ascorbic acid was 30 % less than theoretical values.

Conclusions. The antioxidant interaction of ascorbic acid and rutin in “Ascorutin” medicine has been studied. It has been established that the combination of rutin and ascorbic acid has an antagonistic effect.

The findings of this research will serve as a stimulus for exploring the interaction of bioactive substances and selecting their optimal combinations for the development of pharmaceuticals, special food products, food, and cosmetic products.

Keywords: antioxidant activity, interaction, rutin, ascorbic acid, antagonism.

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Nowadays, the scientific community has established that the development of cardiovascular, metabolic, neurodegenerative, and oncological diseases is associated with oxidative stress [1,2]. The oxidative stress is a condition of the body in which there is an excess formation of reactive oxygen species (ROS) such as superoxide ($O_2^-$), hydroxyl radical (OH$^-$), and monoxide of nitrogen radical (NO$^-$) [3,4].

The human body has its own antioxidant system, which consists of the following enzymes: superoxide dismutase, catalase, and glutathione peroxidase [3,6]. However, due to endogenous or exogenous influences, the antioxidant system is not always able to completely inactivate free radicals, thereby increasing the risk of developing the diseases above. Therefore, natural antioxidants found in food are consumed, and in addition, special foods and medicines are added to the daily diet to support the antioxidant system [7,8].

In medicine, a wide variety of special food products, pharmaceuticals, and cosmetic products are used, containing extracts with different chemical compositions and biologically active substances with diverse chemical structures and properties. However, in the development of new pharmaceuticals and special food products, there is often a lack of investigation into their pharmacological interactions. The results of these studies have enabled the formulation of optimal compositions for pharmaceuticals and special food products, ensuring only agonistic or synergistic interactions.

Today, in the scientific community of medicine and pharmacy, there is still debate about the creation of a rating of antioxidants, which would be used in the development and creation of medicines, special food products, cosmetics, and food products. Thus, it is difficult to understand which antioxidants should be taken for the treatment and prevention of diseases.

However, in 2020, we approached the solution to this problem; scientists from the Department of Analytical Chemistry of the National University of Pharmacy developed a conditional classification of the “strength” of the antioxidant activity of antioxidants; epigallocatechin-3-O-gallate was taken as the standard; in total, 6 levels were identified: very high, high, medium, below medium, low, very low, and none [9,10].

Since today there is no general rating of antioxidants, the choice of antioxidants was based on their application as drugs in medicine and pharmacy. So, rutin and ascorbic acid were chosen since the drug “Ascorutin” is on the pharmaceutical market and widely applied. The composition of “Ascorutin” contains 50 mg of rutin and ascorbic acid.

In the scientometric databases Scopus and Web of Science, there are a large number of articles on studying the levels of antioxidant activity of rutin and ascorbic acid using different methods [11,12,13], but little attention has been paid to the study of the antioxidant activity of “Ascorutin” and the antioxidant interaction between rutin and ascorbic acid.

**Aim**

The aim of the work was to investigate interaction between ascorbic acid and rutin in medicine “Ascorutin”.

**Materials and methods**

**Chemical reagents.** Ascorbic acid ≥98.0 % (Sigma Aldrich Ltd), rutin ≥98.0 % (Sigma Aldrich Ltd), “Ascorutin” (LLC “Zdrovovye”, series number AC210323); $K_1[Fe(CN)_6]$, $K_2[Fe(CN)_6]$, NaHPO$_4$, KH$_2$PO$_4$ had a chemical grade classification, purchased in Kharkov-Reakhimi.

**Equipment.** Potentiometric determination of antioxidant activity, a Hanna 2550 pH meter (FRG) with a combined platinum electrode EZDO 50PO (Taiwan) was used.

**Antioxidant activity.** The antioxidant activity was assessed using the potentiometric method [14,15,16]. Antioxidant activity was calculated according to the following equation and expressed as mmol-equiv./L:

\[
AOA = \frac{(C_{ox} - \alpha \times C_{red})}{(1 + \alpha) \times K_{dl} \times 10^{9}},
\]

where, $\alpha = \frac{C_{ox}}{C_{red}} \times 10^{0.0546 \cdot \Delta E \cdot 2.3R} \cdot \frac{C_{ox} - \text{concentration of } K_2[Fe(CN)_6] \text{ mol/l}}{C_{red} - \text{concentration of } K_1[Fe(CN)_6] \text{ mol/l}}$; $E_{ethanol} = 0.0546 \cdot C_{ethanol} - 0.0091$; $C_{ethanol}$ – concentration of ethanol; $\Delta E$ – change of potential; $F = 96485.33 \text{ C/mol – Faraday constant}; n = 1 – \text{number of electrons in electrode reaction}; R = 8.314 \text{ J/molK} – \text{universal gas constant}; T = 298 \text{ K}; K_{dl} \text{ – coefficient of dilution}.$

**Statistical analysis.** The measurements were conducted in five replications. The results were expressed as mean values accompanied by standard deviation, reflecting the level of certainty in the measurements. Statistical analysis was performed using MS Excel 7.0 and Statistica 6.0 software, enabling thorough data evaluation and interpretation.

**Results**

The treatment and prevention of cardiovascular, metabolic, and neurodegenerative diseases often involve the use of a variety of individual and combination plant-based medications, such as “Cratal”, “Ascorutin,” hawthorn tincture, motherwort tincture, etc. These medicinal preparations are complex agents, and their chemical composition includes various derivatives of flavonoids and phenolic compounds, which play a crucial role in their pharmacological actions.

In pharmacology, the following terms are used to describe the type of interaction between pharmaceuticals: antagonism ($1 + 1 = 0$), agonism ($1 + 1 = 2$), and synergy ($1 + 1 = 5$) [17]. In our opinion, when combining different antioxidants, various types of interactions will also occur, primarily related to the chemical properties of antioxidants.

In our experiment, four model solutions were prepared: rutin (0.38 M); ascorbic acid (0.11 M); a mixture of rutin and ascorbic acid (0.38 and 0.11 M); “Ascorutin” solution (0.38 and 0.11 M). Then, the level of antioxidant activity of the aforementioned solutions was studied using the potentiometric method.

According to the results of the study, the antioxidant activity of rutin was 265.52 ± 1.00 mmol-equiv./L, and that of ascorbic acid was 99.00 ± 1.00 mmol-equiv./L. Subsequently, the theoretical antioxidant activity of a mixture of ascorbic acid and rutin was calculated, amounting to 364.52 mmol-equiv./L. The next stage of the experiment involved investigating the
antioxidant effect of a mixture of ascorbic acid with rutin and the drug “Ascorutin”. Table 1 shows that the level of antioxidant activity of the mixture of ascorbic acid and rutin was 255.60 ± 1.00 mmol-eq./L, and “Ascorutin” exhibited an antioxidant activity of 256.52 ± 1.00 mmol-eq./L.

Discussion

The experimental results showed that the level of antioxidant activity from the experimental data was 30 % less for the two research objects when compared to the theoretical data. Additionally, it’s worth noting that the sum of the antioxidant effect of the mixture of ascorbic acid with rutin and the drug “Ascorutin” was less by 3.7 % and 3.0 %, respectively, than the antioxidant effect of rutin alone. This indicates an antagonistic interaction, rather than agonism or synergism, between rutin and ascorbic acid. These findings underscore the importance of studying the interactions of biological active substances with each other. It also raises the question of whether antagonism observed in antioxidant interactions could extend to other pharmacological activities, such as anti-inflammatory or cardioprotective effects.

We can assume that the antagonism of rutin and ascorbic acid is because the standard electrode potential of ascorbic acid is -58 mV [18], which indicates high reduction properties, and rutin – 88 mV [19].

As you can see, the standard electrode potential of rutin is much higher than that of ascorbic acid, which is associated with lower reducing abilities. In the potentiometric method, the oxidizing agent \( K_3[Fe(CN)_6] \) is used; when adding a solution of ascorbic acid with rutin to the mediator system, first of all, the redox reaction will occur with ascorbic acid, since its potential is lower, and then it will react with rutin. However, after oxidation, ascorbic acid is converted into a pro-oxidant, which will also oxidize rutin, leading to a decrease in the antioxidant properties of rutin.

Conclusions

1. The antioxidant interaction between ascorbic acid and rutin in “Ascorutin” medicine has been studied.
2. It has been established that the combination of rutin and ascorbic has an antagonistic effect.
3. The findings of this research will serve as a stimulus for exploring the interaction of bioactive substances and selecting their optimal combinations for the development of pharmaceuticals, special food products, food, and cosmetic products.

Prospects for further research: the next stage of our research will involve studying the antioxidant interaction of phenolic compounds, such as catechin, gallic acid, and caffeic acid; and developing a medicinal product with an optimal composition of phenolic compounds that exhibit only agonistic or synergistic antioxidant interactions.

Conflict of interest: authors have no conflict of interest to declare.

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References

Table 1. Theoretical and practical results of antioxidant activity of rutin, ascorbic acid, and medicine “Ascorutin”

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration, M</th>
<th>Experimental value of antioxidant activity, mmol-eq./L</th>
<th>Theoretical value of antioxidant activity, mmol-equiv./L</th>
<th>Difference, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>0.11</td>
<td>99.00 ± 1.00</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rutin</td>
<td>0.38</td>
<td>265.52 ± 1.00</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ascorbic acid + Rutin</td>
<td>0.11 + 0.38</td>
<td>256.52 ± 1.00</td>
<td>364.52</td>
<td>-30.0</td>
</tr>
<tr>
<td>“Ascorutin”</td>
<td>0.11 + 0.38</td>
<td>256.52 ± 1.00</td>
<td>364.52</td>
<td>-30.0</td>
</tr>
</tbody>
</table>


