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

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Phytomedicines are combined pharmaceuticals containing various groups of phenolic compounds. It is known that phenolic compounds are powerful antioxidants, but their level of antioxidant activity during interactions has not been previously studied.

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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Дослідження антиоксидантної взаємодії аскорбінової кислоти і рутину у лікарському препараті «Аскорутин»

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Фітопрепарати – комбіновані лікарські препарати, що містять різні групи фенольних сполук. Відомо, що фенольні сполуки є потужними антиоксидантами, але раніше не було вивчено рівень антиоксидантної активності при їх взаємодії.

Мета роботи – вивчити взаємодію аскорбінової кислоти та рутину в лікарському препараті «Аскорутин».

Матеріали і методи. Об’єкт дослідження – одна серія таблеток «Аскорутин», рутин та аскорбінова кислота. Рівень антиоксидантної активності вимірювали потенціометричним методом.

Результати. Рівень антиоксидантної активності модельних розчинів рутину (0,38 М) становив 265,51 ± 1,00 ммоль-екв./л, аскорбінової кислоти (0,11 М) – 99,00 ± 1,00 ммоль-екв./л, комбінації рутину та аскорбінової кислоти (0,38 + 0,11 М) – 255,60 ± 1,00 ммоль-екв./л, а препарату «Аскорутин» (0,38 + 0,11 М) – 256,52 ± 1,00 ммоль-екв./л.

Експериментальне значення рівня антиоксидантної активності «Аскорутину», комбінації рутину та аскорбінової кислоти становило на 30 % менше за теоретичні дані.

Висновки. Вивчили антиоксидантну взаємодію аскорбінової кислоти та рутину у препараті «Аскорутин». Встановлено, що комбінація рутину та аскорбінової кислоти має антагоністичний ефект.

Результати цього дослідження будуть стимулом для вивчення взаємодії біоактивних речовин та вибору їх оптимальних комбінацій для розробки лікарських препаратів, спеціальних харчових продуктів, харчових продуктів і косметичних засобів.

Ключові слова: антиоксидантна активність, взаємодія, рутин, аскорбінова кислота, антагонізм.

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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 [5,6]. 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 "Zdorovye", series number AC210323); $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$, $NaHPO_4$, KH_2PO_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:

$$AOA = (C_{OX} - \alpha \times C_{red}) / (1 + \alpha) \times K_{dil} \times 10^3,$$

where, $\alpha = C_{ox}/C_{red} \times 10^{(\Delta E - Eethanol)nF/2.3RT}$; 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; ΔE – 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.

Statistical analysis. The measurements were conducted in five replicates. 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-eq./L, and that of ascorbic acid was 99.00 ± 1.00 mmol-eq./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

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

Sample	Concentration, M	Experimental value of antioxidant activity, mmol-eq./L	Theoretical value of antioxidant activity, mmol-equiv./l	Difference, %
Ascorbic acid	0.11	99.00 ± 1.00	–	–
Rutin	0.38	265.52 ± 1.00	–	–
Ascorbic acid + Rutin	0.11 + 0.38	255.60 ± 1.00	364.52	-30.0
"Ascorutin"	0.11 + 0.38	256.52 ± 1.00	364.52	-30.0

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 ± 1.0 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.

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References

1. Maslov OY, Komisarenko MA, Kolisnyk SV, Golik MY, Tsapko YO, Akhmedov EY. Determination of the extraction frequency of green tea leaves by the antioxidant method. J Org Pharm Chem. 2022;20(1):28-34. doi: 10.24959/ophcj.22.252320
2. Maslov OY, Komisarenko MA, Ponomarenko SV, Kolisnyk SV, Osolodchenko TP, Kostina TA, et al. Antioxidant, antimicrobial and antifungal activity of the obtained "Cachisept" tablets for resorption in the oral cavity for the treatment and prevention of dental caries. Current issues in pharmacy and medicine: science and practice. 2023;16(3):213-6. doi: 10.14739/2409-2932.2023.3.285425
3. Maslov OY, Komisarenko MA, Kolisnyk SV, Antonenko OV, Kolisnyk OV, Kostina TA. The study of the qualitative composition and the quantitative content of phenolic compounds in dietary supplements with lingonberry. J Org Pharm Chem. 2021;19(4):40-6. doi: 10.24959/ophcj.21.243782
4. Maslov O, Komisarenko M, Kolisnyk S, Kostina T, Golik M, Moroz V, et al. Investigation of the extraction dynamic of the biologically active substances of the raspberry (Rubus idaeus L.) shoots. Curr Issues Pharm Med Sci. 2023;36(4):194-8. doi: 10.2478/cipms-2023-0034

5. Maslov OY, Komisarenko MA, Golik MY, Kolisnyk SV, Altukhov AA, Baiurka SV, et al. Study of total antioxidant capacity of red raspberry (Rubus idaeous L.) shoots. Rev Vitae. 2023;30(1). doi: 10.17533/udea.vitae.v30n1a351486
6. Maslov O, Kolisnyk S, Komisarenko M, Golik M, Antonenko O. Study of chemical composition and antioxidant activity of tincture, infusion of green tea leaves. Fitoterapiia. Chasopys. 2022;(1):48-52. doi: 10.33617/2522-9680-2022-1-48
7. Maslov OY, Kolisnyk SV, Komisarenko MA, Kostina TA, Dynnyk KV. Development the composition and technology for obtaining a dietary supplement "Cachinol" with the antioxidant activity in the form of granules used in the polycystic ovary syndrome. News of Pharmacy. 2022;103(1):42-7. doi: 10.24959/nphj.22.77
8. Maslov OY, Kolisnyk SV, Hrechana OV, Serbin AH. Study of the qualitative composition and quantitative content of some groups of BAS in dietary supplements with green tea leaf extract. Zaporozhye medical journal. 2021;23(1):132-7. doi: 10.14739/2310-1210.2021.1.224932
9. Maslov OY. [Phytochemical study and standardization medicines with antioxidant activity from green tea leaves] [dissertation]. Kharkiv, Ukraine: National University of Pharmacy; 2022. Available from: https://nrat.ukrintei.ua/en/searchdoc/0823U100107/
10. Maslov OY, Kolisnyk SV, Komisarenko MA, Altukhov AA, Dynnyk KV, Stepanenko VI. Study and evaluation antioxidant activity of dietary supplements with green tea extract. Current issues in pharmacy and medicine: science and practice. 2021;14(2):215-9. doi: 10.14739/2409-2932.2021.2.233306
11. Gęgotek A, Skrzydlewska E. Antioxidative and Anti-Inflammatory Activity of Ascorbic Acid. Antioxidants. 2022;11(10):1993. doi: 10.3390/antiox11101993
12. Yang J, Guo J, Yuan J. In vitro antioxidant properties of rutin. LWT – Food Science and Technology. 2008;41(6):1060-6. doi: 10.1016/j.lwt.2007.06.010
13. Maslov OY, Kolisnyk SV, Komisarenko MA, Akhmedov EY, Koval AO, Kostina TA, et al. Metrological Characteristics of the Potentiometric Assay Developed for Determining the Antioxidant Activity of Ascorbic Acid. J Org Pharm Chem. 2023;21(3):31-7. doi: 10.24959/ophcj.23.276394
14. Maslov O, Komisarenko M, Ponomarenko S, Horopashna D, Osolodchenko T, Kolisnyk S, et al. Investigation the influence of biologically active compounds on the antioxidant, antibacterial and anti-inflammatory activities of red raspberry (Rubus idaeous l.) leaf extract. Curr Issues Pharm Med Sci. 2022;35(4):229-35. doi: 10.2478/cipms-2022-0040
15. Maslov O, Kolisnyk S, Komisarenko M, Komisarenko A, Osolodchenko T, Ponomarenko S. In vitro antioxidant and antibacterial activities of green tea leaves (Camella sinensis l.) liquid extracts. Annals of Mechnikov Institute. 2022;(2):64-7. doi: 10.5281/zenodo.6634846
16. Maslov OY, Kolisnyk SV, Ponomarenko SV, Ahmedov EY, Shovkova ZV. The study of the effect of ethyl alcohol concentrations on the antioxidant activity of ascorbic acid solutions. J Org Pharm Chem. 2021;19(2):44-7. doi: 10.24959/ophcj.21.231947
17. Flaten MA. Drug effects: agonistic and antagonistic processes. Scand J Psychol. 2009;50(6):652-9. doi: 10.1111/j.1467-9450.2009.00776.x
18. Matsui T, Kitagawa Y, Okumura M, Shigeta Y. Accurate standard hydrogen electrode potential and applications to the redox potentials of vitamin C and NAD/NADH. J Phys Chem A. 2015;119(2):369-76. doi: 10.1021/jp508308y
19. Namazian M, Zare HR, Coote ML. Determination of the absolute redox potential of Rutin: experimental and theoretical studies. Biophys Chem. 2008;132(1):64-8. doi: 10.1016/j.bpc.2007.10.010