CZU: 615.322.074:582.974+547.972.2

ANTIOXIDANT PROPERTIES OF FLAVONOIDS AFTER PENETRATION OF BLACK ELDERBERRY EXTRACTS THROUGH THE DIALYSIS MEMBRANE

АНТИОКСИДАНТНЫЕ СВОЙСТВА ФЛАВОНОИДОВ ПОСЛЕ ПРОНИКНОВЕНИЯ ЭКСТРАКТОВ ЧЕРНОЙ БУЗИНЫ ЧЕРЕЗ ДИАЛИЗНЫЕ МЕМБРАНЫ

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Резюме. Цель исследования: определить антиоксидантную активность (АОА) экстрактов бузины черной после проникновения через диализные мембраны. Метод: АОА измеряли в среде высвобождения (цитратный буфер) после проникновения извлечений бузины черной через диализные мембраны. Результаты и обсуждение: установлено, что экстракты 20% глицерина с сухим экстрактом 60% ацетонитрила, 20% полипропиленгликоля (ППГ) с сухим экстрактом 40% пропанола и 40% этанола с сухим экстрактом 40% этанола показали высокий уровень АОА в среде высвобождения.

Ключевые слова: Жидкие экстракты, бузина черная (*Sambucus nigra*), степень проникновения, диализные мембраны.

Abstract. Objective: to define the antioxidant activity (AOA) of black elderberry extracts after penetration through dialysis membranes. Method: AOA was measured in the medium of citrate buffer release, when black elderberry extracts penetrated through dialysis membranes. Results and discussion: it was shown that extracts of 20% glycerol with 60% acetonitrile dry extract, 20% polypropylene glycol (PPG) with 40% propanol dry extract, and 40% ethanol with 40% ethanol dry extract showed high level of AOA in the release medium.

Keywords: Liquid extracts, black elderberry (*Sambucus nigra*), the degree of penetration of the dialysis membrane.

Introduction

Black elderberry flowers (*Sambucus nigra flos*) are widely used on the Europe countries as medicinal plant raw material. This plant is a promising source of polyphenolic compounds (in particular, flavonoids) [1], due to which its antioxidant, anti-tyrosine kinase [2], hepatoprotective, and antidiabetic activity is manifested. The ability to inhibit the processes of skin photoaging caused by exposure to ultraviolet B (UV-B) has also been proven [3] and the ability to inhibit virus replication.

It is known that when exposed to UV radiation on human skin, reactive oxygen species (ROS) are produced, which can activate various biological reactions. One of these reactions is the activation of tyrosine kinase through the mobilization of melanocyte-stimulating hormone in the body, in fact the process of melanogenesis. Epidermal melanocytes, when exposed to abnormal UV doses, begin to excessively synthesize melanin, but there is a Keap1-Nrf2/ARE protein pathway that removes ROS at their elevated level, which is facilitated by antioxidant agents [4].

The above facts are the basis for determining the penetrating ability of elderberry extracts through the skin and identifying further prospects for using this plant for the development of external dosage forms with antioxidant properties. To determine the algorithm for such development, it is necessary to first evaluate the hypothetical degree of their penetration through the skin, using in vitro models.

In previous studies [5], the values of the penetration of flavonoids of water-organic and water-alcohol extracts were obtained. If we compare the values of the penetrating power of liquid extracts, they were in the range of 0.5-1.5 mcg/ml for all the studied solvents. The addition of dry extracts to these extracts made it possible to increase the penetration of flavonoids into the release medium through the dialysis membrane. Analyzing these data, combinations of 20% glycerol extract with 60% dry dissolved acetonitrile extract, 20% PPG extract with 40% dry dissolved propanol extract, and 40% liquid ethanol extract with 40% ethanol dry extract were selected to establish AOA.

Materials and methods

Flavonoids of the selected extracts were detected in the release medium in sufficient quantities, so the task was to establish their AOA. This combination of liquid extracts with dry extracts penetrated dynamically through dialysis membranes, in this experiment, the release medium (citrate buffer) was selected every hour for 0.15 ml for 6 hours for the reaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH):

DPPH was dissolved in 100 ml of 96% ethanol to obtain the initial solution. The resulting solution had a pre-measured optical density of no more than 0.9 at 517 nm. To 3.00 ml of the initial DPPH solution, 150 μl of the studied extracts were added, mixed, and the optical density measurement of the system was recorded after one minute at a wavelength of 517 nm on a spectrophotometer. 96% ethanol was used as a compensation solution.

The AOA level was evaluated using formula 1:

$$AOA, \% = \frac{Abase - Ax}{Abase} * 100\%$$

$$AOA, \% = \frac{Abase - Ax}{Abase} * 100\% \quad (1)$$

where $A_{base} A_{base}$ is the optical density of the initial DPPH solution without adding extracts;

A_x – optical density of the system after adding test extracts.

Results and discussions

The amount of flavonoids in the release medium was evaluated after the reaction of complexation with aluminum chloride (Pharmacopoeia method) [6], after dissolution in the selected dry extracts in the liquid extracts, the amount of flavonoids in the citrate buffer increased by 2 times for ethanol extract (Table 1):

Table 1. The degree of flavonoids penetration in single-propanol, water-isopropanol, and water-ethanol extractions in a combination of liquid extractions and post-dissolution extractions

Solvent / Time	Propanol 40%	Isopropanol 40%	Ethanol 40%
1 h	1,40	1,31	2,11
2 h	1,73	1,72	2,67
3 h	1,83	2,20	2,64
4 h	2,00	1,80	2,20
5 h	2,23	2,47	2,21
6 h	2,30	2,02	2,48

Table 2. The degree of penetration of 20% glycerol extracts when dry extracts of volatile solvents are dissolved in them

Solvent / Time	Ethanol 40%	Methanol 20%	Acetone 40%	Isopropanol 40%	Propanol 40%	Acetonitrile 60%
1 ч	1,81	0,34	0,51	0,51	0,49	1,71
2 ч	1,64	0,34	0,80	0,72	0,97	1,85
3 ч	1,11	0,42	0,80	1,05	0,81	1,87
4 ч	1,02	0,44	1,07	0,79	1,12	2,23
5 ч	1,11	0,44	1,16	1,38	1,34	2,20
6 ч	1,28	0,51	0,90	0,90	1,12	1,94

Table 3. The degree of penetration of 20% PPG extracts when they are dissolved in dry extracts of volatile solvents

Solvent / Time	Ethanol 40%	Methanol 20%	Acetone 40%	Isopropanol 40%	Propanol 40%	Acetonitrile 60%
1 ч	1,41	0,66	0,79	0,66	0,69	1,09
2 ч	1,11	0,67	0,77	0,83	1,14	1,11
3 ч	1,42	0,88	0,88	1,09	0,94	1,06
4 ч	1,51	1,00	1,07	1,53	0,95	1,45
5 ч	1,48	1,36	1,57	1,14	1,59	1,24
6 ч	1,92	1,48	1,48	1,25	1,75	1,99

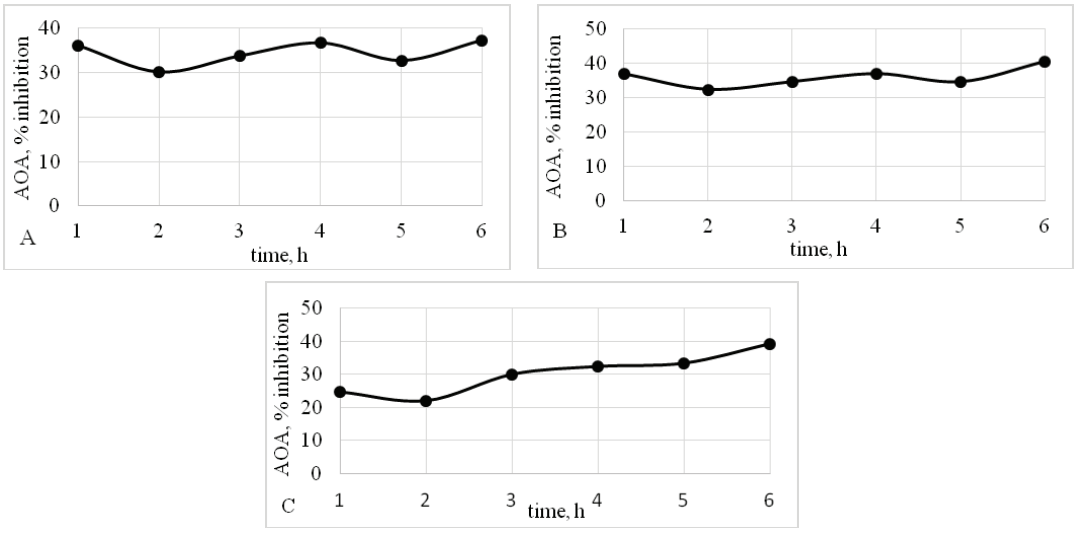

Fig. 1. AOA extracts 20% glycerol with 60% acetonitrile (A), 20% PPG with 40% propanol (B) and 40% ethanol with 40% ethanol extract (C)

From water-organic extracts, PPG and glycerol solvents were selected, since they themselves showed a high penetrating power, they are often used as part of external dosage forms and have a moisturizing and softening effect. Dry extracts of volatile solvents were also dissolved in these solvents to determine which combination would increase the number of flavonoids permeated through the dialysis membrane (Table 2, 3).

Based on these tables, the combinations of solvent and dry extract were selected, the amount of flavonoids in which was released to the greatest extent. For these extracts, the AOA was installed, the data are shown in the next graphs (Fig. 1).

Conclusions

These combinations of extracts showed a good ability to penetrate the dialysis membrane, but it was necessary to check the AOA of flavonoids that penetrated the release medium, i.e. the hypothetical AOA that these flavonoids can create in the skin. By themselves, the extraction data showed high AOA values, about 40%, i.e., given that the solvents themselves showed this activity from 60% to 80% [7], the decrease in its indicator when passing through the membrane was no more than 2 times.

Thus, already at this stage, it is possible to assert a proven high recovery capacity of the spectrum of water-organic and water-alcohol solvents, the ability of these solvents to have a high AOA and to penetrate well through dialysis membranes, which are generally accepted standards for evaluating the release of active compounds by in vitro methods.

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