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ANTIOXIDANT ACTIVITY OF PURPLE CONEFLOWER HERB EXTRACTS, DEPENDING ON THE COMPOSITION OF THE EXTRAGENT

ACTIVITATEA ANTIOXIDANTĂ A EXTRACTELOR DIN PĂRȚI AERIENE DE ECHINACEA PURPURIE, ÎN FUNCȚIE DE COMPOZIȚIA EXTRAGENTULUI

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Rezumat. S-a cercetat dependența efectului antioxidant față de concentrația acizilor hidroxicinamici din extractele din părți aeriene de Echinacea purpurea, obținute la utilizarea extragenților de diferită compoziție. Extractele obținute pe bază de propan-2-ol 20%, etanol 20%, metanol 40%, acetonă 40%, propilenglicol 40% și dimetilsilfoxid 40% în calitate de extragenți, au prezentat o activitate antioxidantă ridicată în combinație cu un conținut ridicat de acizi hidroxicinamici. Acești solvenți pot fi folosiți la elaborarea formelor farmaceutice.
Cuvinte cheie: Echinacea purpurea, activitate antioxidantă, acizilor hidroxicinamici.
Abstract. The dependence of the antioxidant effect on the concentration of hydroxycinnamic acids in extracts from Echinacea purpurea herb obtained with the use of extractants of different composition was investigated. Extracts obtained using 20% propan-2-ol, 20% ethanol, 40% methanol, 40% acetone, 40% propylene glycol and 40% dimethyl sylfoxide as an extractant have high antioxidant activity in combination with a high content of hydroxycinnamic acids. These solvents can be offered for preparing dosage forms.
Keywords: Echinacea purpurea, antioxidant activity, hydroxycinnamic acids.

INTRODUCTION

Echinacea purpurea herb is characterized by the presence in its composition of caffeic, caftaric, chicory, chlorogenic, ferulic, para-hydroxycumaric and other acids belonging to the hydroxycinnamic group [1,2].

This group of substances has a wide spectrum of pharmacological action. There are studies carried out *in vitro*, illustrating antiviral, immunomodulatory [1], anti-inflammatory [3] antitumor [4] and well-expressed antioxidant [1] activity of the hydroxycinnamic acid group in extracts and juices from Echinacea purpureaherb. The spectrum of activity of Echinacea purpurea herb is even wider due to other groups of biologically active substances (polysaccharides, alkylamides, etc.), which determines the widespread use of this type of plant raw material for the production of medicines and biologically active food additives [5].

Now, due to the coronavirus pandemic, interest in this plant has increased, because there are data linking the biologically active substances, namely chlorogenic and caffeic acids, which belong to the group of hydroxycinnamic acids (HCA), with the ability to inhibit the angiotensin-converting enzyme, thereby preventing excessive inflammation, especially in the kidneys and the cardiovascular system, which occurs as a result of binding surface spike S of SARS-CoV-2 virus with angiotensin-converting enzyme 2 [6].

For the most complete processing of the medicinal plant raw materials of Echinacea and maximizing the efficiency of its use in medicine, organic solvents of various nature and their aqueous solutions are used, which provide the most complete extraction of hydroxycinnamic acids from medicinal plant materials. For the production of drugs based on Echinacea, only ethanol and its aqueous solutions are used, although a number of literate sources use methanol, ethyl acetate, acetone and other solvents to obtain dry extracts. Therefore, it is advisable to expand the list of studied extractants: volatile – for obtaining dry extracts, viscous and / or non-volatile – for the development of medicines for local and external use.

Since hydroxycinnamic acids are powerful antioxidants capable of breaking free radical oxidation chain reactions by trapping radicals [7], it is rational to research the antioxidant activity (AOA) of the obtained extracts in the study of extraction and compare them with each other and with the standards of caffeic and ascorbic acids.

MATERIALS AND METHODS

The object of our research was Echinacea purpurea herb produced by «Biotest» - pharmaceutical company of Belarus (series 020718).

The following solvents were selected for the work: methanol, ethanol, propan-1-ol, propan-2-ol, ethylene glycol, propylene glycol, glycerin, dimethyl sulfoxide (DMSO), acetonitrile, acetone and their aqueous solutions with a volume fraction of the organic component 20%, 40%, 60%, 80%, and 100%, water, and standards of caffeic and ascorbic acids.

The analysis was carried out on a Solar PB2201 series spectrophotometer using the built-in computer program for constructing and analyzing absorption spectra.

For extraction, the plant raw material weighing 0.100 ± 0.005 g (accurately weighed) and 5.00 ± 0.01 ml of the given composition solvent was taken. The extraction process was carried out in a water bath at a temperature of 60 °C for 1.5 hours. The resulting extract was filtered through cotton wool.

To determine the antioxidant activity, we used a spec-

trophotometric method with 2,2-diphenyl-1-picrylhydrazyl (DPPH), which is reduced upon contact with an antioxidant, as a result of which the color intensity decreases [8]. The test solution is prepared by adding to 0.600 ± 0.001 ml of the obtained extract 4.20 ± 0.01 ml of 0.01% DPPH solution in methanol. The reaction time is 30 minutes. Methanol is used as a reference solution. Also, for this technique, it is necessary to measure the optical density (A_0) of a solution without antioxidants, prepared by adding 0.600 ± 0.001 ml of methanol to 4.20 ± 0.01 ml of 0.01% DPPH solution.

Measurement of optical density is carried out at a wavelength of 517 nm.

Antioxidant activity of extracts (AOA) in percent is calculated by the formula:

$$AOA=(A_0-A_1)*100/A_0;$$

when A_0 – optical density of DPPH solution without antioxidants;

A_1 – optical density of the test solution.

Data on the efficiency of extraction with aqueous organic solvents of hydroxycinnamic acids were taken from early publications. [9,10].

The results were processed using the Microsoft Office Excel 2015 computer program, the Data Analysis package. The results are presented as mean values **±** half-width of the confidence interval (p≤0,05).

RESULTS AND DISCUSSIONS

In Figure 1 shows the results of measuring the antioxidant activity in the obtained aqueous-organic extracts.

The table 1 below shows the numerical data obtained from the experiment.

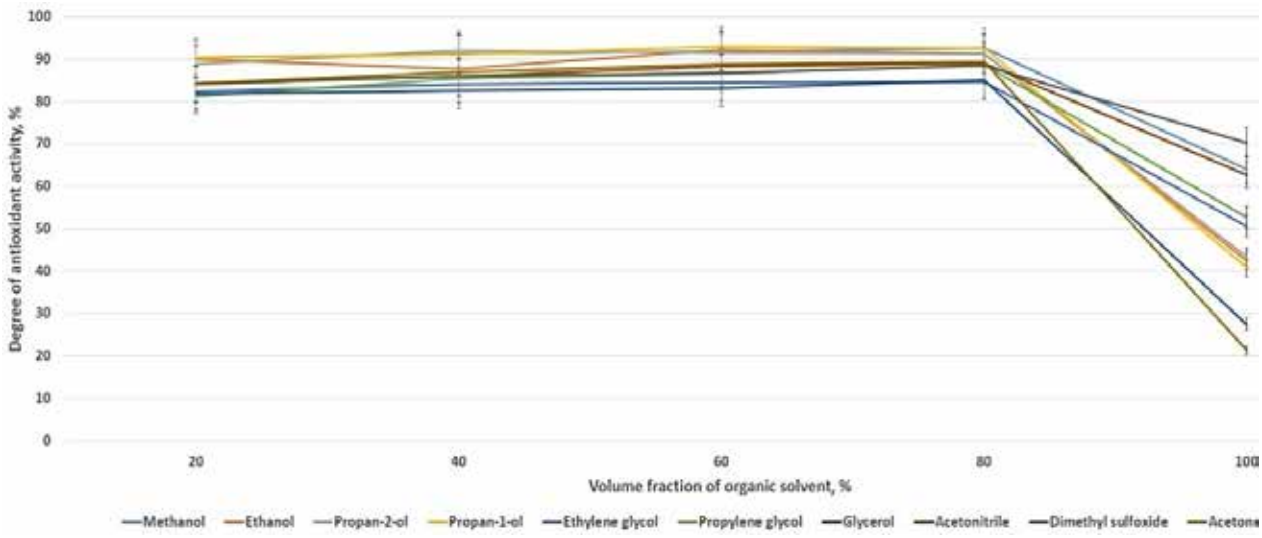


Figura 1. The graph of the dependence of the degree of AOA (%) on the nature and volume fraction of solvents (%)

Table 1. AOA of aqueous-organic extracts

Volume fraction	Methanol	Ethanol	Propan-2-ol	Propan-1-ol	Propyleneglycol
20%	90,39%	89,92%	88,78%	90,36%	81,34%
40%	91,29%	87,75%	92,10±1,66%	91,20%	85,51%
60%	91,84%	92,21±1,06%	91,57%	93,02±0,94%	86,67%
80%	92,67±1,96%	91,37%	91,40%	92,66%	88,63±0,27%
100%	64,06%	42,47%	43,30%	40,84%	52,69%
Volume fraction	**Ethyleneglycol**	**Glycerol**	**Acetonitrile**	**DMSO**	**Acetone**
20%	82,35%	81,81%	84,03%	84,25%	84,23%
40%	84,05%	82,51%	86,05%	85,93%	86,96%
60%	84,58%	83,22%	88,21%	86,79%	88,78%
80%	84,72±0,44%	85,06±1,73%	89,13±1,23%	88,47±0,02%	89,49±0,46%
100%	50,65%	27,51%	62,73%	70,29%	21,32%

From the above data, it follows that water-organic extracts have a high antiradical activity (the degree of antioxidant activity ranges from 81.34% to 93.02%). When using pure organic solvents as an extracting agent, the degree of antioxidant activity decreases by 1.26-4.20 times. Such a sharp decline in activity can be associated with a decrease in the extracting capacity of absolute organic solvents in relation to hydroxycinnamic acids. Below is a graph illustrating the extraction capacity of solvents for hydroxycinnamic acids (the data for the construction were taken from our two earlier publications [9,10]). Despite the poor extracting ability of absolute organic extractants, the antioxidant activity still remains relatively high (probably due to the antioxidant action of other classes of phenolic compounds that fall into the extraction due to the low selectivity of the extraction process). From the above results, it follows that the antioxidant activity of water-organic extracts does not statistically differ from each other, in contrast to the antiradical properties of organic extracts. They differ significantly, and these differences are statistically significant. This allowed us to obtain a number of absolute solvents in order of increasing AOA degree: acetone = glycerin <propan-1-ol = propan-2-ol = ethanol <ethylene glycol = propylene glycol <methanol = acetonitrile <DMSO.

Since the antioxidant activity of aqueous-organic extracts is at the same level, for further research we selected extractants that have the best extraction ability in relation to HCA: DMSO (40%), acetone (40%), methanol 40%, ethanol 20%, propane -2-ol 20%, propylene glycol 40%. For these extractants, we studied the dependence of the antioxidant effect on the dose in order to obtain and compare the EC_{50} (the concentration that creates 50% of the maximum antioxidant effect) with each other and with solutions of antioxidant standards (ascorbic and caffeic acids). The lower the EC_{50}, the more pronounced antiradical properties the antioxidant has. Standardization of extracts for the group of hydroxycinnamic acids (in terms of caffeic acid in μmol / L) was performed and published earlier [9,10] (Figure 2).

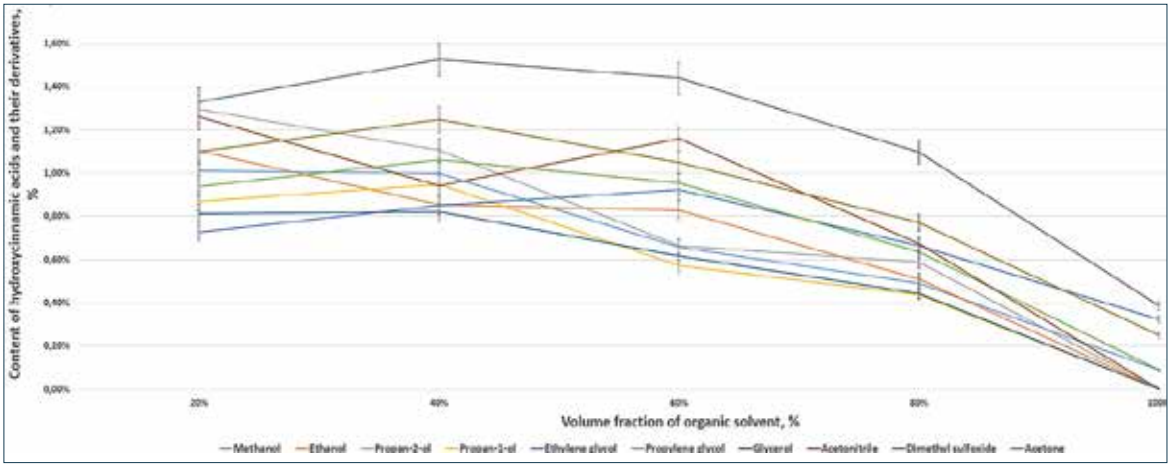
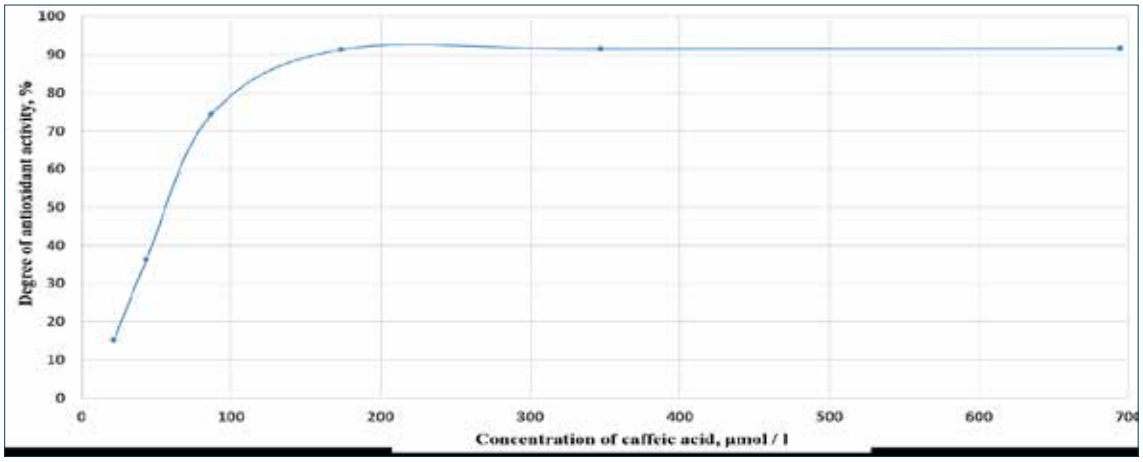


Figure 2. Graph of dependence of HCA content (%) on the nature and volume fraction of the organic component of the extracting agent (%)



Figures 3 and 4 show graphs of the dependence of the antioxidant effect on the concentration of caffeic acid.

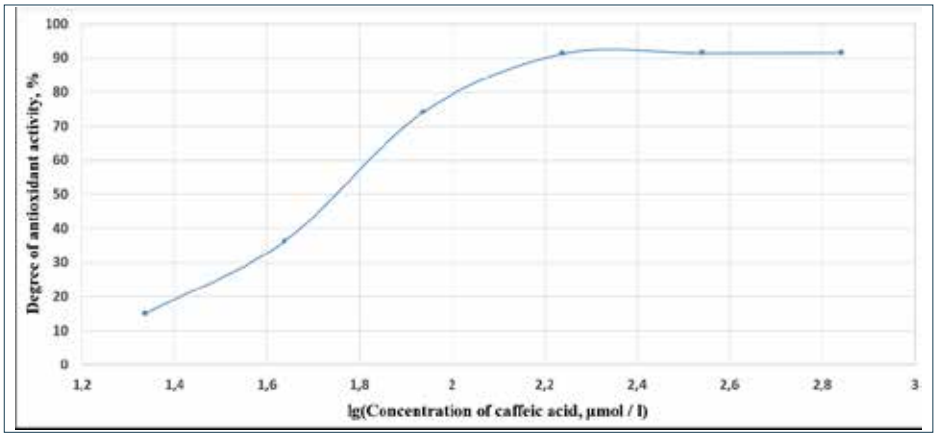


Figure 3. Graph of the dependence of the antioxidant effect on the concentration of caffeic acid (hyperbolic dependence)

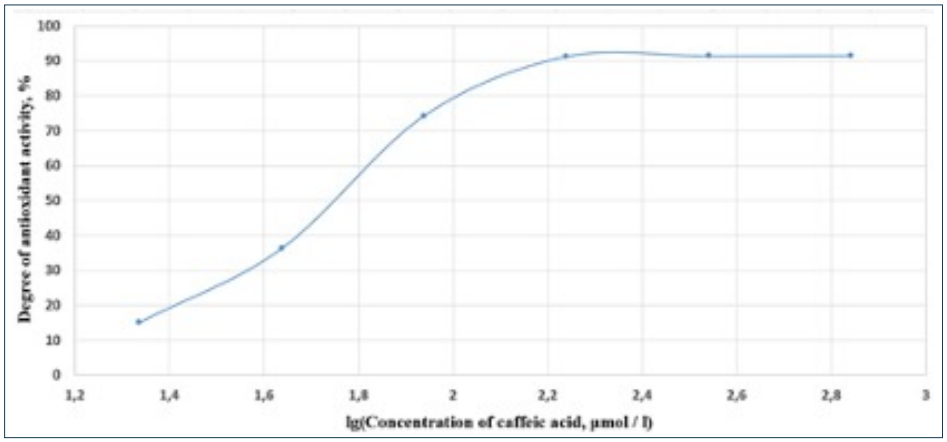


Figure 4. Graph of the dependence of the antioxidant effecton the concentration of caffeic acid (S-shaped dependence)

In standard coordinates, the dependence is hyperbolic; at high values of the concentration of caffeic acid, the antioxidant effect reaches a plateau, taking on a maximum value. In semi-logarithmic coordinates, we observe an S-shaped relationship through which we find the concentration of antioxidant that creates 50% of the maximum effect. Similar calculations were carried out for solutions of the ascorbic acid standard and extracts obtained using the extractants selected by us (tables 3 and 4).

Table 2. Concentrations of standard solutions creating 50% of the maximum antioxidant effect

Antioxidant Standards Solutions	EC_{50}, µmol / l
Ascorbicacid	20,23
Caffeicacid	51,52

Table3. Concentrations of extracts producing 50% of maximum antioxidant effect

Extractant	EC_{50} (in terms of caffeic acid in µmol / l)
Methanol (40%)	17,29
Ethanol (20%)	18,95
Propan-2-ol (20%)	20,95
Propyleneglycol(40%)	19,23
DMSO (40%)	26,86
Acetone (40%)	27,76

From the data given in Tables 2, 3 and the results of checking the statistical significance of differences in antioxidant activity using a two-sample t-test with different dispersions, we see that ascorbic acid has 2.55 times stronger antioxidant effect compared to caffeic acid, but

the same as the obtained by us aqueous-alcoholic extracts. Extracts, for which 40% DMSO and 40% acetone were used, have a slightly less strong antioxidant effect compared to ascorbic acid (EC_{50} is significantly higher in extracts), but stronger than caffeic acid. They do not differ from each other.

Thus, we obtained a series of antioxidant activity of the extraction in comparison with the antioxidant standards: ascorbic acid = methanol (40%) = ethanol (20%) = propan-2-ol (20%) = propylene glycol (40%)> DMSO (40%) = acetone (40%) >> caffeic acid.

The stronger antioxidant activity of the extracts in comparison with caffeic acid can be explained by the nonselectivity of the extraction, as a result of which other classes of compounds (for example, other phenolic compounds), also possessing AOA, got into the extract.

Thus, taking into account the high antioxidant activity of the extracts and the good extracting ability (the HAC content is more than 1%), we can offer 20% propan-2-ol, 20% ethanol, 40% methanol and 40% acetone, 40% propylene glycol and 40% DMSO for obtaining dosage forms. In this case, the choice of a solvent will depend on the resulting dosage form. For example, for the manufacture of tablets, volatile extractants (propan-2-ol, ethanol, methanol, acetone) are suitable, since a dry extract is needed as the main component. Viscous propylene glycol or low volatility DMSO can be used for the outer molds. The choice of solvent will also depend on the penetration of HCA through biological barriers. Therefore, further research will focus not only on increasing the level of extraction, including through pretreatment of medicinal plant raw materials, but also on the study of the penetration of HCA.

CONCLUSIONS

The antioxidant activity of water-organic extracts of Echinacea purpurea herb ranges from 81.34% to 93.02%, and when using absolute organic extractants, the antioxidant activity decreases by 1.26–4.20 times. The antioxidant properties of 100% organic extracts differ significantly in contrast to hydroalcoholic ones. We have obtained a number of absolute solvents in order of increasing AOA degree: acetone = glycerol <propan-1-ol = propan-2-ol = ethanol <ethylene glycol = propylene glycol <methanol = acetonitrile <DMSO.

Based on the calculation of EC_{50} (concentration creating 50% of the maximum antioxidant effect), a number of antioxidant activities of the extracts we selected were obtained: ascorbic acid = methanol (40%) = ethanol (20%) = propan-2-ol (20%) = propylene glycol (40%)> DMSO (40%) = acetone (40%) >> caffeic acid. That is, the antioxidant properties of the extracts are on a par with the classic antioxidant (ascorbic acid).

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