Research Article

Substantiation of the technology for obtaining an extract from the fruit of blessed milk thistle for a geriatric drug creation

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ABSTRACT

Aim: This paper presents the results of studying the effect of ethanol concentration in the water-ethanol mixture on the qualitative and quantitative composition of extracts obtained from crushed fruits of blessed milk thistle with high-performance liquid chromatography analysis. **Materials and Method:** For the extraction, plant materials were used: "Blessed milk thistle additive" made from grinded blessed milk thistle fruits by PJSC "Lektravy," Zhitomir, Ukraine, date 28.03.17, series No. 002, shelf life 24 months. **Result and Discussion:** The optimal concentration of ethanol as an extractant was determined for the maximum extraction of phenolic compounds from plant raw materials. The effect of temperature on the extraction degree of extractives during the filtration extraction method was studied, and optimal parameters for the isolation of biologically active compounds from this plant material were proposed. **Conclusion:** It was found that the optimal concentration of ethanol in the water-ethanol mixture, which simultaneously extracts hydroxycinnamic acids, taxifolin, and flavonolignans and the fruits of blessed milk thistle, falls within the range of 64 ± 6% by weight.

KEY WORDS: Blessed milk thistle fruits, Geriatrics, Water-ethanol extracts

INTRODUCTION

Provision of rational medical aid to persons of the elderly and senile age is an actual problem in medicine. This is due to the process of demographic aging of the population, so in 2016, the population growth in the elderly in the Russian Federation over the past 5 years was about 12% and has a tendency to further growth. At the same time, the number of residents aged 60 years and over is about 20% of the total population.[1]

Pharmacotherapy of elderly people is a serious problem of modern health since it is associated with the appearance of chronic kidney, liver, gastrointestinal, and other organ diseases. We observe a deposit of fat, increase in cholesterol level, development of steatohepatosis, which, together with the need for constant intake of medicines, causes a high risk of toxic and undesirable effects on the liver.[2]

Access this article online	
Website: jprsolutions.info	ISSN: 0975-7619

The low toxicity of drugs from medicinal plant material (MPM) and the possibility of their long-term use makes it possible to use them as an active pharmaceutical substance for a complex geriatric drug and in particular for the pharmacotherapy of toxic and inflammatory liver diseases.

Drugs from the fruits of blessed milk thistle are the most widely used drugs for the treatment of liver disease.[3] At the moment, it is generally accepted that flavonolignanes are the main active substances in the fruits of milk thistle.[4]

Most manufacturers around the world standardize drugs from the fruits of blessed milk thistle by silibinin A and B. According to the literature, the manufacturers use four main types of solvent to extract this group of substances from thistle fruits: Ethanol, methanol, acetone, and ethyl acetate and their aqueous solutions.[5]

Since the elderly, in view of their physiological characteristics and experience difficulties in swallowing solid dosage forms (tablets and capsules),

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Received on: 18-10-2017; Revised on: 26-11-2017; Accepted on: 22-12-2017

the authors decided to develop a complex geriatric drug in the form of granules based on a thick extract of fruits of blessed milk thistle, amino acid methionine, and vitamin B_{12}.[6] At the same time, consumer properties are significantly improved, the technology of obtaining this drug is reduced and simplified, and there is no need to use such toxic solvents as methanol, acetone, and ethyl acetate in the process of isolating flavonolignans from MPM.

The objective of this paper is to justify the technology of obtaining a water-ethanol extract with the maximum content of phenolic compounds from the fruits of blessed milk thistle.

To achieve this goal, it was necessary to solve a number of problems, to study the effect of ethanol concentration in the water-ethanol mixture on the qualitative and quantitative composition of phenolic compounds in extracts, to study the effect of the extraction filtration method on the yield of extractive substances at different temperatures, and on the basis of the data obtained, to propose an optimal isolation technology for the phenolic compounds from the fruits of blessed milk thistle.

MATERIALS AND METHODS

For the extraction, plant materials were used: "Blessed milk thistle additive" made from grinded blessed milk thistle fruits by PJSC "Lektravy," Zhitomir, Ukraine, date 28.03.17, series No. 002, shelf life 24 months.

The grinding was carried out by a shock-cutting method, while a particle fraction with a size of 0.1–0.5 mm was used for the experiments.

The studies on the selection of the optimum ethanol concentration in the water-ethanol mixture used simple maceration during soaking for 24 h and at a weight-to-volume material/extractant ratio - 1:5. The extractant was ethanol with a concentration in the water-ethanol mixture (18, 36, 64, and 92 ± 1% by weight). The extraction temperature was 25 ± 1°C.

The qualitative and quantitative composition of the extracts was studied by reversed-phase high-performance liquid chromatography (HPLC). Chromatographic studies were performed on a chromatograph by Agilent Technologies 1200 infinity, USA. To record the electronic absorption spectra of the substances to be separated, Agilent 1200 spectrophotometric detector with a diode array was used, with the scanning pitch of 2 nm, scanning range - 190–450 nm; the length of the optical path of the cuvette is 10 mm; the volume of the cuvette is 13 μL. For registration and processing of spectral data and chromatograms, Agilent Chem Station software was used.

The chromatography was carried out under the following conditions: Mobile phase: (a) 1% aqueous solution of formic acid, (b) ethyl alcohol 96% in the gradient elution mode; column: Supelco Ascentis express C_{18}, 2.7 μm × 100 mm × 4.6 mm; the mobile phase speed - 0.5 ml/min; column temperature +35.00 ± 0.01°C; and the volume of the injected sample is 1.000 ± 0.001 μL.

Identification of the components in the extracts was carried out according to the retention time and the absorption spectrum of substances in comparison with standard substances. Qualitative and quantitative analysis of the substances was carried out according to the standard substance of silibinin and taxifolin (Sigma-Aldrich, Germany).

Before the filtration extraction of phenolic compounds with optimally concentrated ethanol, the blessed milk thistle fruits were previously degreased with n-hexane to a depletion degree of ≥98% by lipophilic substances, and then the material was dried at room temperature for 24 h.

In the experiments of filtration extraction, a UN-2/50 infusion pump and a thermostatic cell were used to maintain the constant feed rate of the extractant through the raw material layer. The filtration extraction was carried out at a temperature of 25.0 ± 0.2, 40.0 ± 0.2, and 60.0 ± 0.2°C. In this case, three portions of the extract were poured at a ratio of the extract volume to the weight of the material of 2:1, 2:1, and 5:1 (total discharge was 9:1). The dynamics of depletion of the material was studied on the basis of an analysis of the amount of extractive substances in the drained extracts. The dry residue was determined by the gravimetric method according to the State Pharmacopoeia of the Russian Federation.[7]

RESULTS AND DISCUSSION

The first part of the research is devoted to the study of the qualitative and quantitative content of BAC in extracts obtained with the use of ethanol of different concentrations and the choice of the optimal concentration of ethanol as an extractant.

Figure 1 shows the results of HPLC analysis of the extract obtained from the fruit of blessed milk thistle on the basis of ethanol solution at a concentration of 64% by weight. This ethanol concentration is chosen due to the maximum transition of all groups of phenolic compounds into it, as will be shown below.

As can be seen from the data in Figure 1, among the phenolic compounds in the extract based on ethanol 64% by weight, the compound of hydroxycinnamic acid (I), taxifolin (II), and flavonolignans (III-VII) were identified, with the compounds IV and V being

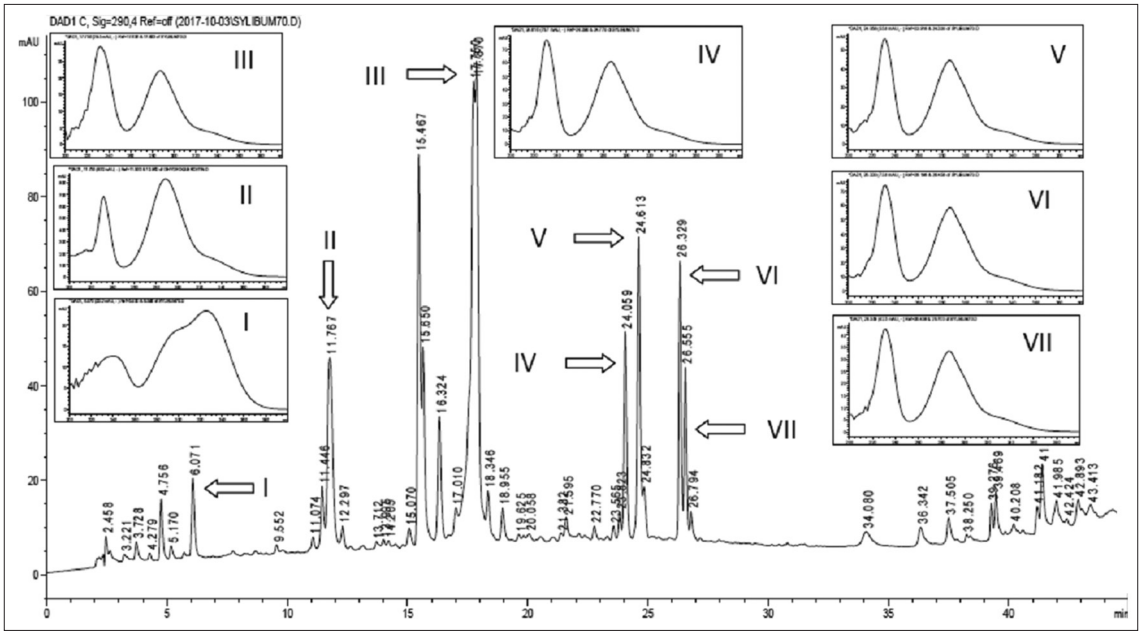

Figure 1: A high-performance liquid chromatography chromatogram of an extract based on 64% ethanol by weight. Identified compounds and their ultraviolet spectrum: I - A derivative of hydroxycinnamic acid, II - taxifolin; III, VI, and VII - unidentified flavonolignans, IV and V - flavonolignans of silibinin

Table 1: Peak areas for phenolic compounds in extracts of blessed milk thistle fruits based on ethanol of different concentrations

Substance	Retention time, min*	Peak area, mAU·s*			
		EtOH 18% by weight	EtOH 36% by weight	EtOH 64% by weight	EtOH 92% by weight
A compound of hydroxycinnamic acid I	6.19±0.30	62.0±1.8	142±4	205±6	13.42±0.40
Taxifolin II	11.74±0.11	285±8	460±13	524±15	161±4
Flavonolignan III	17.83±0.17	190±5	233±7	363±10	187±5
Flavonolignan IV (Silibinin)	24.11±0.24	21.31±0.64	185±5	343±10	231±6
Flavonolignan V (Silibinin)	24.67±0.24	28.35±0.85	241±7	430±12	273±8
Flavonolignan VI	26.38	28.04±0.84	205±6	356±10	198±5
Flavonolignan VII	26.61±0.26	14.85±0.45	113±3	198±5	102±3
Total area for flavonolignans	-	283±8	977±28	1690±47	991±27

*The mean value and mean error (mean±SEM) are given for the number of repetitions n=3 and the significance level P=0.95. SEM: Standard error mean

associated with silibinin. It should be noted that all flavonolignans have an identical ultraviolet spectrum based on a spectrum of taxifolin.

The peak area value for the phenolic compounds found in the extracts from the blessed milk thistle fruits based on ethanol of different concentrations obtained by HPLC analysis is given in Table 1.

As can be seen from the data in Table 1, the maximum peak values for various phenolic compounds are accounted for ethanol with a concentration of 64% by weight. The obtained data of the predominant yield of flavonolignans from plant material to ethanol with a concentration of 64% by weight complie with previous studies.[8] Data on the prevalent yield of a compound of hydroxycinnamic acid and taxifolin from the fruits of blessed milk thistle in ethanol with a concentration of 64% by weight are published for the first time. Furthermore, the data in Table 1 show that flavonolignans dominate among the detected BAC groups in the extract, followed by taxifolin and the hydroxycinnamic acid compound.

For a more graphic representation of the dependence of the transition of these groups of BAC from vegetable material to ethanol with different concentrations, the authors reduced the obtained values of the areas to a relative form. For this purpose, the obtained area values for each ethanol concentration were divided by the largest value among them. The results are shown in Figure 2.

As can be seen from the curves depicted in Figure 2, the dependence of the relative content for the three groups of BAC in the extract on the concentration of ethanol in the extractant has a pronounced maximum near the ethanol concentration of 60% by weight.

Thus, for a compound of hydroxycinnamic acid, the maximum falls within the range of 60 ± 6% by weight, for taxifolin, the maximum falls within the range of 57 ± 6% by weight, and for the sum of flavonolignans, within the range of 64 ± 6%. It is interesting to note that the common (isobestic) point of intersection for the substances under the study falls within the range of the ethanol concentration of 64 ± 6% by weight. Since dihydroquercetin and hydroxycinnamic acids possess a number of valuable pharmacological effects and enhance the antioxidant properties of flavonolignans, the authors decided to use 64% weight. As ethanol extractant, with the help of which an extract with a maximum content of flavonolignans, taxifolin, and hydroxycinnamic acids can be obtained.

To explain the above dependencies, it can be assumed that the matrix of plant material, and in particular cellulose, plays a significant role in keeping these substances on itself with the help of hydrogen bonds and does not allow them to be transferred to an extractant with a high concentration of ethanol, although the substance (in particular, taxolin and flavonolignans) dissolves well in a given solvent. Work on this assumption is beyond the scope of this goal in this article and will be developed in further studies.

It should be noted that although the fatty oil does not dissolve in aqueous solutions of ethanol, it nevertheless transfers from the damaged seed tissue in an insignificant amount to the extract, forming an emulsion, and requires an additional purification of the extract. Therefore, the authors decided to carry out filtration extraction of blessed milk thistle fruits with the help of ethanol 64% by weight, after their preliminary degreasing with n-hexane. This is also confirmed by the materials of patent studies on the methods of obtaining flavonolignan fraction from the fruits of blessed milk thistle.[9,10]

The second part of the research is devoted to the development of an extraction technology for blessed milk thistle fruits using a modified version of the filtration method. This extraction method was proposed, developed, and patented in the 80–90s of the 20th century, by Litvinenko *et al.*[11] In general, the filtration method of extraction is a high-speed version of percolation and makes it possible to speed up the process of separation of BAC from plant materials with the help of organic solvents.

As shown in Figure 3, data on the dynamics of the yield of extractive substances from the grinded fruits of blessed milk thistle with ethanol 64% by weight, at a temperature of 25.0, 40.0, and 60.0 ± 0.2°C, and the ratio of the drained extract volume to the initial mass of the MPM of (9:1) are given. The extraction time was about 3.5 h.

As can be seen from the data in Figure 3, with the help of filtration extraction with the ratio of the extract volume to the MPM mass (9:1), it is possible in 3.5 h to achieve 100% degree of depletion of the MPM at a temperature of 60°C, 92% depletion at 40°C, and 82% of depletion at 25°C.

To simulate the extraction degree of extractives by the temperature within the range studied for ethanol 64% by weight with the volume-weight ratio of the drained extract to the MPM of 9:1, the authors converted the obtained data into the dependence as shown in Figure 4.

As can be seen from Figure 4, the dependence of the extraction degree of extractive substances from the raw material on the process temperature is well approximated by the logarithmic function ($y(\%) = 9.7 + 22.2 \cdot \ln T$), with a very high determination coefficient, which approaches a unity: $R^2 = 0.9992$, which speaks about a successful

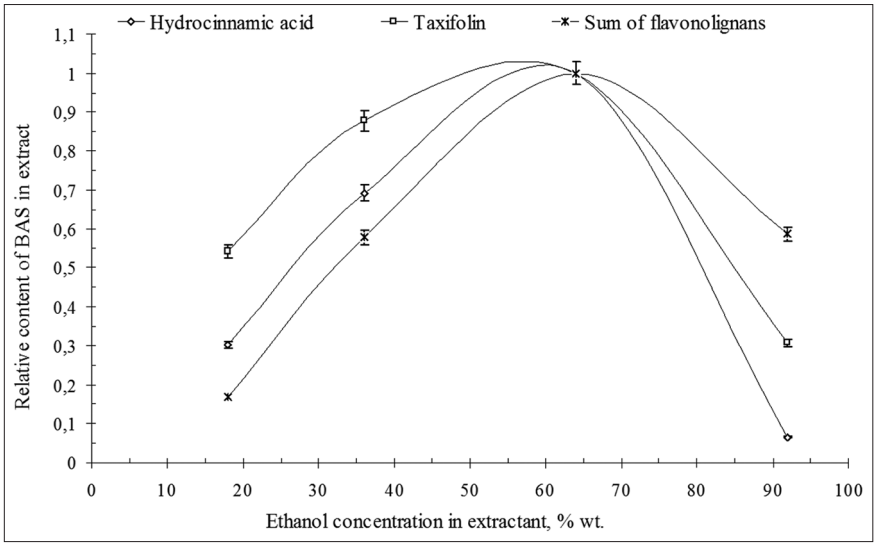

Figure 2: Dependence of the relative content of BAC in the extract on the concentration of ethanol in the extractant

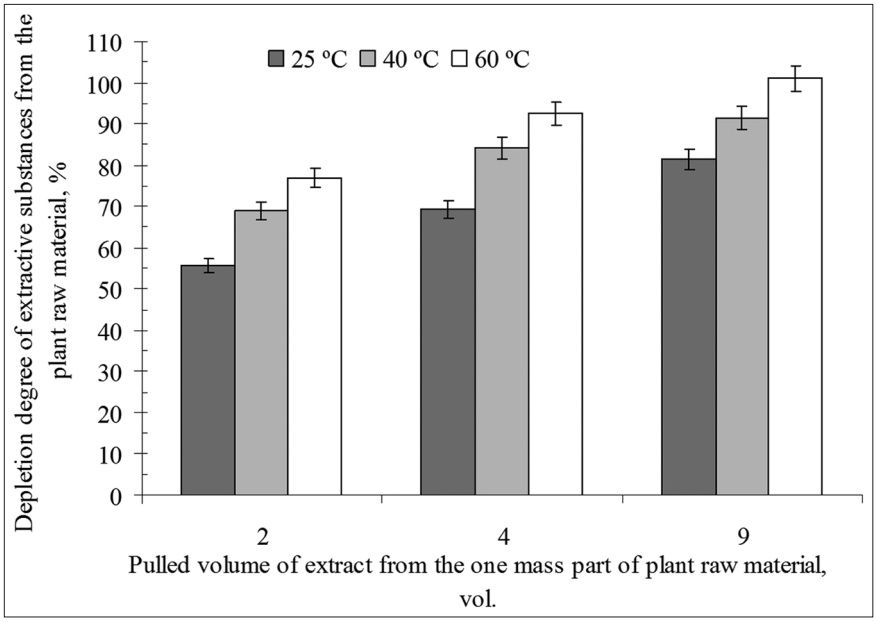

Figure 3: Dynamics of the yield of extractive substances from the fruit of blessed milk thistle at different temperatures

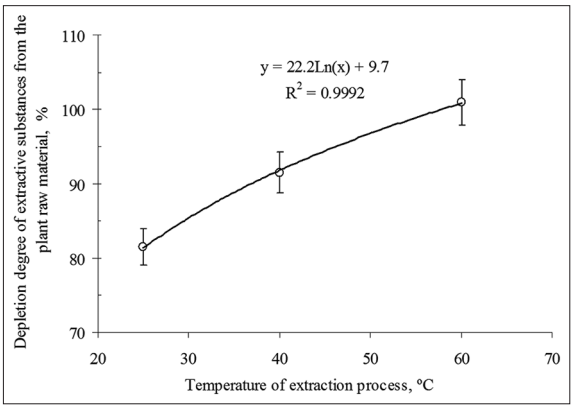

Figure 4: Dependence of the yield of extractive substances from the fruits of blessed milk thistle on temperature at a ratio of 9:1

choice of the regression model and allows describing the behavior of the function from the parameter in the range of its change quite accurately. For example, according to this model, the yield at temperatures of 45, 50, and 55°C will be 94, 97, and 99%, respectively. Thus, it can be concluded that, to achieve a raw material depletion rate ≥95%, it is necessary to carry out the extraction process at a temperature of ≥50°C, which will be the most optimal option.

When comparing the obtained data with literature data,[5,9,10] this extraction method has a number of advantages in time, the amount of extractant and energy costs. For example, in our studies, using a filtration extraction method at a temperature of 50°C, a ratio of the volume of extract/mass of the material of (9:1), within 3.5 h, we achieved a 97% degree of depletion of the material in terms of hydrophilic substances.

This technology for the isolation of hydrophilic BAC from the fruit of blessed milk thistle is much superior to the methods of extraction described in the scientific literature and undoubtedly requires further development. At the same time, it is very promising to use this extraction method in the technology of preliminary extraction of oil, which will allow creating a complex technology for processing blessed milk thistle fruits.

SUMMARY

The technology of obtaining a water-ethanol extract with the maximum content of phenolic compounds from the fruits of blessed milk thistle was justified.

In the course of the study, the effect of ethanol concentration in the water-ethanol mixture on the qualitative and quantitative composition of extracts obtained from the grinded fruits of blessed milk thistle was studied.

The optimal concentration of ethanol in the extractant was determined to maximize the extraction of phenolic compounds from the fruit of blessed milk thistle.

It was found that the optimal concentration of ethanol in the water-ethanol mixture, which simultaneously extracts hydroxycinnamic acids, taxifolin, and flavonolignans and the fruits of blessed milk thistle, falls within the range of 64 ± 6% by weight.

The influence of the filtration extraction method on the yield of extractive substances was studied and an optimal technology for obtaining biologically active compounds from this plant material was proposed.

The research was carried out with the financial support of the Ministry of Education and Science within the framework of the State task of the National Research University "BelSU" for 2017–2019, project No. 12.6429.2017/БЧ.

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Source of support: Nil; Conflict of interest: None Declared