ORIGINAL ARTICLE

Development of the technology for obtaining a thick extract from fruits of milk thistle with the stage of ultrasonic influence

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Abstract

Aim: This article presents the results of the development of technology for obtaining a thick extract of milk thistle fruits using ultrasonic treatment of plant material and extractant in the soaking stage. **Materials and Methods:** For the research, crushed fruits of milk thistle from "Biokor" Ltd, Penza, Russia, series 011216 were used, and the shelf life is 2 years. The particle size of the plant raw material is 0.5–1.0 mm. An aqueous solution of ethanol of 70% by volume was used as an extractant. Ultrasonic influence on the plant material and extractant were carried out under the following conditions: "Bandelin SONOPULS HD 3200" installation, ultrasonic frequency of 20 kHz, sound system of extracting system 5, 10, and 15 min, emitter power 280 W, and temperature 25°C. **Results and its Discussion:** It was found that with the ultrasonic treatment of raw materials and extractant in the soaking stage, the output of the phalavolignan complex almost doubles. To achieve the maximum concentration of flavonolignans in the extract, 5 min of ultrasound exposure to a mixture of raw material and extractant. **Conclusions:** The application of ultrasound at the stage of soaking plant material in the technology of obtaining a thick extract from the fruits of milk thistle is justified. It was shown that under the proposed conditions for extraction of flavonolignans from milk thistle, ultrasound promoted an increase in the yield of flavonolignans from plant raw materials and did not adversely affect these biologically active substances in the extract.

Key words: Milk thistle, flavonolignans, ultrasonic treatment of plant material

INTRODUCTION

At present, there is a steady trend of demographic aging in the Russian Federation. According to the forecasts of the Federal State Statistics Service, by the year 2031, there will be over 37 million people in the country older than the able-bodied, which will constitute 28.7% of the population.[1] The constantly increasing proportion of older people in the population structure determines the tasks of the modern health system aimed at improving the quality of life of elderly patients in preserving and strengthening their health.[2] A feature of geriatric patients is the presence of their combined and chronic pathologies. This necessitates the constant and simultaneous administration of combinations of drugs, which significantly increases the likelihood of side effects of medications taken. A rational solution to this problem is the development of combined medicines based on natural components (vitamins, amino acids, and phytocompositions). Such active components are characterized by low toxicity, and, accordingly, a low probability of overdose and side effects in patients.

Atherosclerosis and fatty liver disease are pathologies, which are most often simultaneously diagnosed in the elderly and senile age.[3] These diseases have similar pathogenesis; however, only one drug is registered in the modern pharmaceutical market, the active components of which combine the effects on pathological changes in the liver parenchyma and atherosclerotic changes in the vessels, but it is of synthetic origin.[4] Proceeding from this, the optimal

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solution to this problem, from our point of view, is the development of a drug based on natural components for the integrated treatment and prevention of such geriatric diseases as atherosclerosis and non-alcoholic steatohepatosis.

Promising plant raw materials used in diseases of the liver are the fruits of milk thistle, containing a complex of flavonolignans (silybin, silychristin, silydianin, and their stereoisomers). The main component of the silymarin complex is silybin.[5] A complex of biologically active substances (BAS) of milk thistle fruits is introduced into the composition of modern preparations mainly in the form of a dry extract. This is due to the fact that to increase the yield of flavonolignans, plants raw materials are degreased using organic solvents such as petroleum ether and carbon tetrachloride or additional technological operations (for example, oil extraction).[6-9] The disadvantage of these methods is the laboriousness of the process, increased production costs due to the use of expensive solvents, as well as the inability to completely remove organic solvent residues from the thick extract and, as a consequence, a possible reduction in the hepatoprotective activity of the health-care product. In addition, it was found that decreasing the milk thistle fruit does not allow to extract the full complex of BAS, providing the hepatoprotective effect. This circumstance is associated with the synergistic effect of flavonolignans and flavonoids, in particular, dihydroquercetin, which has antioxidant properties.[10]

To intensify the extraction process proposed to use ultrasonic treatment of plant material and extractant. When ultrasound is used, the technological processes associated with the mass transfer are substantially accelerated, and the yield of BAS is increased. This is due to the fact that ultrasonic waves create cavitation and turbulent flows in the liquid extractant, as a result, there is a rapid swelling of the plant material and dissolution of the cell's contents, the rate of hydration of the raw material particles increases, turbulent and eddy currents appear in the boundary diffusion layer. Molecular diffusion inside the particles of the plant material and in the boundary diffusion layer is practically replaced by convective, which leads to an intensification of mass transfer.[11] In addition, as a result of cavitation, cellular structures are destroyed, which speeds up the process of transferring the BAS to the extractant by washing them out.

The purpose of the work is to develop a technology for obtaining a thick extract of milk thistle fruits with the use of ultrasound at the stage of soaking plant material.

MATERIALS AND METHODS

For the research crushed fruits of milk thistle from "Biokor" Ltd, Penza, Russia, series 011216 were used, and the shelf life is 2 years. The particle size of the plant raw material is 0.5–1.0 mm. An aqueous solution of ethanol of 70% by volume was used as an extractant. Plant materials weighing 10 g (exact weight) were poured with part of the extractant to a "mirror" (about 30 ml), insisted for 5 min and worked on the resulting mixture by ultrasound under the following conditions: "Bandelin SONOPULS HD 3200", ultrasound frequency 20 kHz, the time of sounding of the extraction system is 5, 10, and 15 min, the emitter power is 280 W, and the temperature is 25°C. Next, the impregnated raw material was transferred to a percolator, insisted for 24 h, and the raw material was percolated to an extract of 80.0 ml in volume for 1 h. The extract of the comparison was obtained under similar conditions except for the process of exposure to ultrasound. The extracts obtained were evaporated with a rotary evaporator RV-10 at a temperature of 60°C and a vacuum of 600–650 mmHg up to a residual humidity of 25%.

For qualitative analysis of the extracts reversed phase high performance liquid chromatography (RP HPLC). As substances of the standards, taxifolin and silibinin (Merck Aldrich) were used. The analysis technique is described in detail in a previously published paper.[12]

As a reference drug, "Carsil" tablets from Sopharma Pharmaceuticals company were used, the active substance: A dry extract of the milk thistle fruit (Silybi mariani fructus extractum siccum) (35–50:1), (Extractant: methanol ≥99.0%) - 40.9–56.3 mg, which is equivalent to 22.5 mg of silymarin. Series No. 2190416, expiration date 04/2018.

RP HPLC of a solution of a sample of crushed tablets "Karsil" was carried out according to the following procedure: One tablet of the preparation was thoroughly crushed, and a sample of 0.03 g (exact weight) was taken, dissolved in 5.0 ml (exact weight) of an aqueous solution of ethanol of 70% by volume. The solution was stirred for several minutes, allowed to stand for 1 h, centrifuged at 13,000 rpm for 5 min, and HPLC analysis.

RESULTS AND DISCUSSION

At the first stage of the study, the optimal time of ultrasound exposure to a mixture of raw material and extractant was

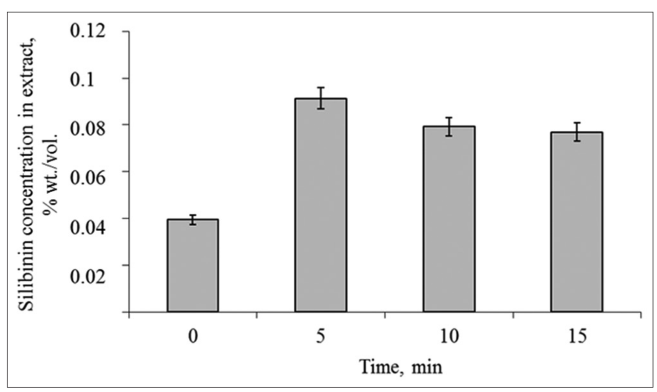

Figure 1: Dependence of the concentration of the sum of flavonolignans in the extract as a function of the time of exposure to ultrasound

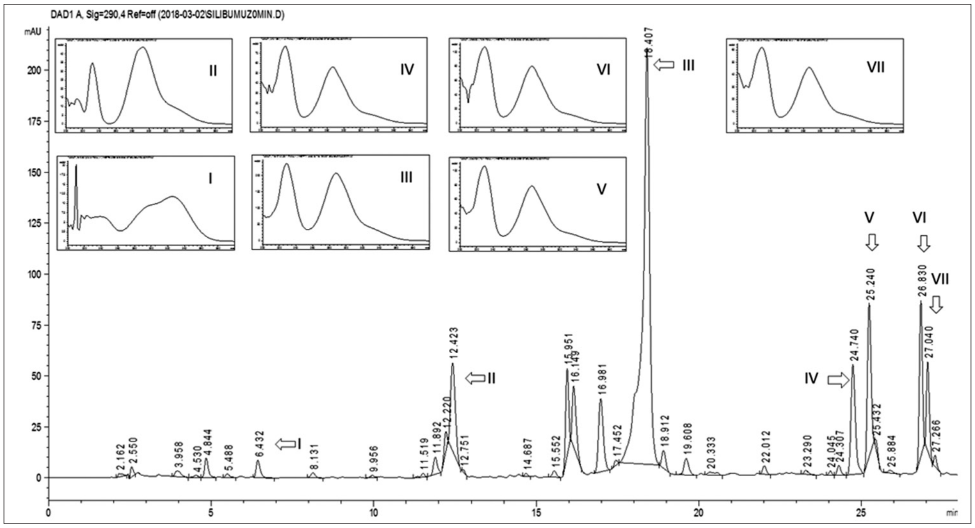

Figure 2: Chromatogram of the extract obtained without using ultrasound

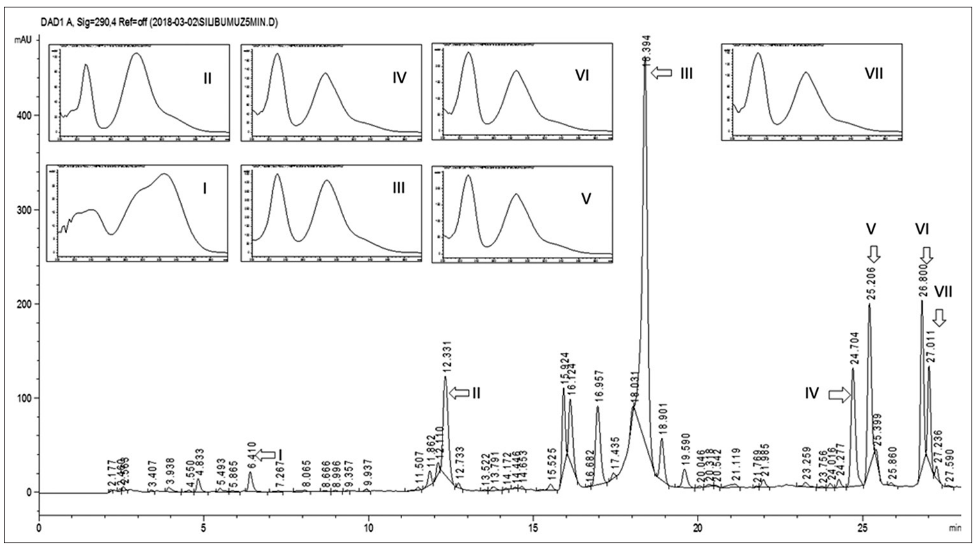

Figure 3: Chromatogram of the extract obtained using ultrasound

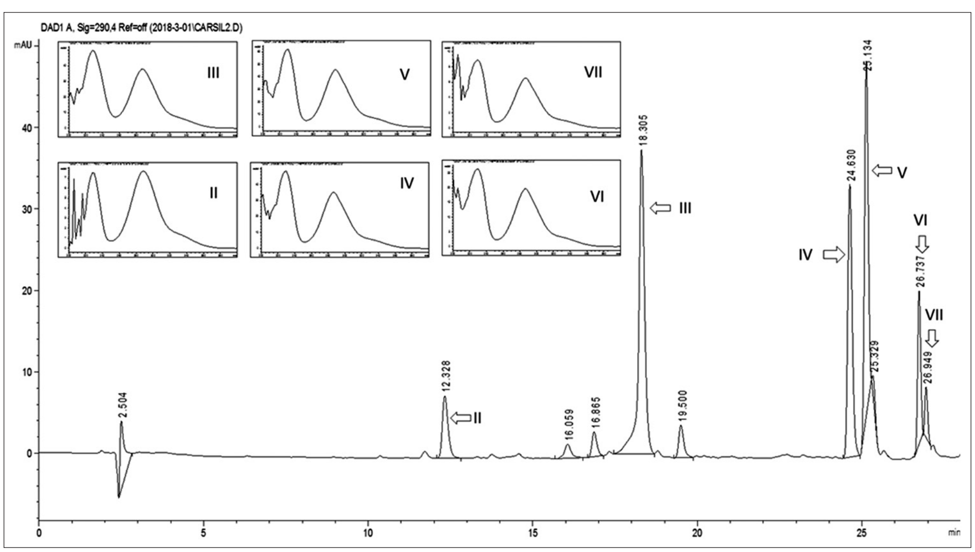

Figure 4: Chromatogram of the drug "Karsil"

determined to achieve the maximum degree of the yield of the sum of flavonolignans from plant material. The results are shown in Figure 1.

As can be seen from the data in Figure 1, to achieve the maximum concentration of the amount of flavonolignans in the extract, it is necessary to apply 5 min of ultrasound to a mixture of raw material and extractant. Thus, the obtained results show the validity of ultrasound application at the soaking stage of raw materials and demonstrate the advantage of this technology in the traditional percolation extraction of the sum of flavonolignans from milk thistle.

At the next stage of the study, we compared the qualitative and quantitative parameters of the extract obtained with the help of ultrasound, without ultrasound and the "Karsil" comparison preparation to identify possible negative effects of ultrasonic technology on BAS.

Results of RP HPLC analysis of extracts obtained with ultrasound, without it, and the drug "Karsil" are shown in Figures 2-4.

As can be seen from the chromatograms in Figures 2 and 3, the area of the silibinin peak in the extract obtained using ultrasonic impact is almost 2 times greater than in the extract without processing. In addition, it can be seen from the chromatograms that the extract obtained using ultrasound in the soaking stage in qualitative composition does not differ from the extract obtained without ultrasound and practically does not differ from the "Karsil" preparation, which indicates the possible identity of the pharmacological effects of the obtained extracts and the standard medicine. The obtained results indicate that under the given conditions, ultrasonic treatment of the fruits of milk thistle and extractant in the soaking stage allows to increase the yield of silibinin and does not adversely affect the released BAS.

CONCLUSIONS

The application of ultrasound at the stage of soaking plant material in the technology of obtaining a thick extract from the milk thistle fruit is substantiated.

It is shown that the application of ultrasound at the stage of soaking plant material in the technology of obtaining a thick extract from the fruit of milk thistle leads to a significant increase in the concentration of the sum of flavonolignans in the extract.

It was found that under the proposed conditions for the extraction of flavonolignans from milk thistle, ultrasound did not adversely affect these BAS in the extract.

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