Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 22 Issue 2 Version 1.0 Year 2022
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

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GJMR-B Classification: DDC Code: 641.4 LCC Code: TX603

Strictly as per the compliance and regulations of:

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I. Introduction

The Decree of the President of the Republic of Uzbekistan Shavkat Mirziyoyev "On measures to further improve the provision of the population with medicines and medical products" dated October 31, 2016 is an important factor in expanding the scope of work in the pharmaceutical industry. This document outlines important tasks for increasing the localization of pharmaceutical production by expanding the use of local raw materials, in particular, medicinal plants, and improving the delivery of quality medicines to the population at affordable prices.

One of the tasks provided for in the Decree of the President of the Republic of Uzbekistan dated April 10, 2020 No. 4668 “On additional measures for the development of traditional medicine in the Republic of Uzbekistan” and No. PP-4670 “On measures for the protection, cultural cultivation, processing of wild medicinal plants and rational use of available resources” is the organization of cultivation and harvesting of plants and raw materials of non-plant species used in traditional medicine, conducting laboratory and scientific research in this direction, increasing the export potential of the industry, as well as integrating education, science and production processes. In this regard, the Tashkent Pharmaceutical Institute is carrying out consistent work to ensure the implementation of the priority tasks outlined in the resolutions.

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One of the promising directions in the field of drug development is the creation of phytopreparations for the treatment and prevention of these diseases. More active usage of phytopreparations, both alone and in combination with synthetic drugs, depending on the severity and nature of diseases, will contribute to the use of drug-saving technologies in clinical practice. Natural biologically active substances of plants are evolutionarily closer to the human body than synthetic ones, they are easily included in metabolic processes and have practically no side effects, and many of them are precursors of physiologically active substances (hormones, mediators). Recent studies have shown that the healing properties of medicinal plants depend on the harmonious interaction of all active substances, which together have a broader effect than individually.

In this regard, studies on the conversion of collections and individual plants used in the form of infusions and decoctions into total preparations-extracts are very promising, since the possibility of their wider use is limited by the imperfection of the dosage form [1,2].

Summarizing the above, we can conclude that the creation of medicines and dietary supplements based on medicinal plant materials using a resource-saving extraction method (due to the maximum depletion of raw materials) is timely and relevant and corresponds to the social order of clinical medicine.

Given the above, the Tashkent Pharmaceutical Institute has developed a choleretic collection “Triflos”, consisting of the following types of herbal medicinal raw materials: flowers of tansy false yarrow, flowers of chamomile, yarrow herb. To date, studies are carried out on its standardization, the establishment of quality standards, as well as the study of specific activity and safety. Cholagogue collection when tested on laboratory rats showed more than 50% increase in bile secretion [3]. The pharmacotherapeutic effects of this collection, which mainly consist in the regulation of phospholipid metabolism in the liver and the normalization of bile flow due to the antispasmodic effect on the sphincter of the gallbladder, according to the opinion prevailing in the literature, is determined by the presence of a number of

phenolcarboxylic acids and flavonoid compounds. So, in the composition of the collection components, along with essential oils, terpenoids, bitter and tannins, resins, organic acids and mineral salts, the presence of chlorogenic, rosmarinic, caffeic, ferulic acids and their corresponding glycosidic derivatives, as well as flavonoids: quercetin, luteolin, acacetin, apigenin and their glycosides. These literature data were confirmed by us experimentally [4].

The purpose of our research is to develop an optimal technology for obtaining a dry extract from this collection using the method of mathematical planning of the experiment.

II. Experiments and its Results

Currently, there are a large number of various extraction schemes designed to increase the yield of active substances, ensure maximum depletion of raw materials and enrichment of the extract with target biologically active substances (BAS). These extraction schemes also include factors that affect the completeness of extraction of biologically active substances, such as the nature and concentration of the extractant, the ratio of raw materials and extractant, temperature, type of extraction, etc. [5-18].

In order to facilitate the laborious process of finding the optimal conditions for extraction, we decided to apply the method of mathematical planning. Based on the characteristics of the technology for obtaining a dry extract, we chose a four-factor experimental plan based on a 5x5 Greek-Latin square. At the same time, the influence of the degree of grinding of raw materials (factor A), the concentration of ethyl alcohol (factor B), the hydromodulus (factor C) and the temperature regime (factor D) on the completeness of the yield of the sum of flavonoids in terms of quercetin in extraction is studied [19].

Factors and their levels are given in Table 1.

Table 1: Factors and their levels used in the experiment

Factor and its levels	Factor value	Factor and its levels	Factor value
The degree of grinding of raw materials (factor A)		*Ethyl alcohol concentration (factor B)*	
a_1	2-4 mm	b_1	50%
a_2	5-7 mm	b_2	60%
a_3	8-10 mm	b_3	70%
a_4	11-13 mm	b_4	80%
a_5	14-16 mm	b_5	90%
Hydromodulfactor (factor C)		*Temperature factor (D)*	
c_1	1:5	d_1	40^0C
c_2	1:10	d_2	50^0C
c_3	1:15	d_3	60^0C
c_4	1:20	d_4	70^0C
c_5	1:25	d_5	80^0C

The 5x5-experiment plan and the results of determining the quantitative content of biologically active substances (the sum of flavonoids in terms of quercetin) are presented in Table 2.

Table 2: The yield of the sum of flavonoids in terms of quercetin in the extract in a four-factor plan 5x5 with three repeated experiments, %

Factor A	Factor B					Totala_i
	b_1	b_2	b_3	b_4	b_5	
a_1	C_1d_1	c_2d_2	c_3d_3	c_4d_4	c_5d_5	
	1,22	2,15	2,14	1,36	1,04	
	1,28	2,09	2,17	1,41	1,09	
	1,21	2,19	2,11	1,33	0,99	
	3,71	6,43	6,42	4,1	3,12	23,78
a_2	c_2d_3	c_3d_4	c_4d_5	c_5d_1	c_1d_2	
	2,15	1,89	2,02	1,56	1,62	
	2,11	1,93	2,06	1,52	1,59	
	2,17	1,84	1,97	1,6	1,67	
	6,43	5,66	6,05	4,68	4,88	27,7
a_3	c_3d_5	c_4d_1	c_5d_2	c_1d_3	c_2d_4	
	1,3	1,55	1,76	1,67	1,62	
	1,34	1,61	1,71	1,74	1,67	
	1,26	1,52	1,8	1,66	1,59	
	3,9	4,68	5,27	5,07	4,88	23,8
a_4	c_4d_2	c_5d_3	c_1d_4	c_2d_5	c_3d_1	
	1,3	1,69	1,56	1,5	1,24	

		1,34	1,65	1,61	1,52	1,2	
		1,26	1,73	1,51	1,47	1,27	
		3,9	5,07	4,68	4,49	3,71	21,85
a_5		c_5d_4	c_1d_5	c_2d_1	c_3d_2	c_4d_3	
		1,01	1,24	1,76	1,37	1,57	
		1,09	1,29	1,73	1,41	1,59	
		1,02	1,18	1,77	1,32	1,52	
		3,12	3,71	5,26	4,1	4,68	20,87
Total	B_i	21,06	25,55	27,68	22,44	21,27	118
	C_k	22,05	27,49	23,79	23,41	21,26	
	D_l	22,04	24,58	27,67	22,44	21,27	

Before carrying out the analysis of variance, the homogeneity of the variance was checked using the Cochran test. The tabular value of the Cochran test for f1=2 and f=25 is 0.22, i.e., uexp, equal to 0.0709, is less than the tabular one, which confirms the equal accuracy of the experiments. Analysis of variance of the obtained results is presented in Table 3.

Table 3: Dispersion analysis of experimental data to determine the yield of the total flavonoids in the obtained extracts

Source of dispersion	Number of degrees of freedom (f)	Sum of squares (SS)	Mean squares (MS)	F_{expert}	$F_{0,05}$	Hypothesis
Factor A	4	2,245	0,56125	328,0885	2,56	a≠0
Factor B	4	1,82652	0,45663	266,931	2,56	b≠0
Factor C	4	1,538827	0,384707	224,887	2,56	c≠0
Factor D	4	1,782227	0,445557	260,4579	2,56	d≠0
Remainder	8	0,15216	0,01902	11,11847	2,13	res≠0
Errorinsidecell	50	0,085533	0,001711			
Totalamount	74	7,630267				

The obtained values Fexp>Ftabl, which indicates the statistical significance of all four studied factors. The value of Fres.in.cell indicates the presence of an interaction between the factors.

Using Duncan's multiple rank test, the differences in the average values of the data on the yield of the total flavonoids in the obtained extracts are studied. It is established that according to the influence of the degree of dispersion of plant raw materials on the response, they can be arranged in the following row: a1 = a2 = a3> a4> a5, i.e. the optimal degree of grinding of raw materials, providing the maximum yield of the sum of flavonoids, are 2-4 mm, 5-7 mm and 8-10 mm. However, taking into account the fact that the excessive dispersity of plant raw materials will ensure the release of not only biologically active, but also ballast substances, which, accordingly, will lead to a contaminated extract, we decided to use raw materials in further studies, the dimensions of which are in the range of 5-7 mm [20, 21].

The influence of the next studied factor - the concentration of ethanol used as an extractant, can be arranged in the following row: b2 = b3>b4>b5>b1, Thus, ethanol at a concentration of 60% and 70% is the optimal extractant. For the purpose of economic feasibility, our choice was stopped at 60% ethyl alcohol.

It is known that the ratio of vegetable raw materials and extractant has a significant impact on the yield of biologically active substances in the obtained extracts. This was confirmed by the results of our mathematical planning. It was found that almost the same output with a slight difference was obtained with a hydromodulus of 1:10, 1:15, 1:20. In order to save the extractant, we recommend the use of a hydromodule equal to 1:10.

The application of the Duncan rank criterion also helped to reveal the influence of the temperature factor on the yield of the sum of flavonoids, this series can be represented as follows: d2 = d3>d4>d1>d5, thus, the temperature of 500C was chosen as the optimal one.

An analysis of domestic and foreign literary sources indicates that the short-term use of ultrasound in the extraction of plant materials stimulates the release of biologically active substances [22, 23]. Given the above, we carried out extraction from the composition of medicinal raw materials, culminating in 10 minutes of ultrasonic exposure. At the same time, an increase in the yield of the total flavonoids in terms of quercetin is observed from 2.34% to 2.6% (1.11 times).

Thus, the proposed technology for obtaining a dry extract from the Triflos choleretic collection is tested under industrial conditions at BALZAM LLC.

The industrial extractor, on which the proposed technology was tested, consists of:

1) Main tank. This is the extraction tank, which is the main element of this system. It mainly serves to extract biologically active substances from medicinal plant materials;
2) A condenser, which serves to cool and return the condensed liquid to the extraction tank;
3) A vacuum condenser, which serves to concentrate and collect the resulting extract;
4) A tank for collecting the extractant (used as a collection) (Fig. 1).

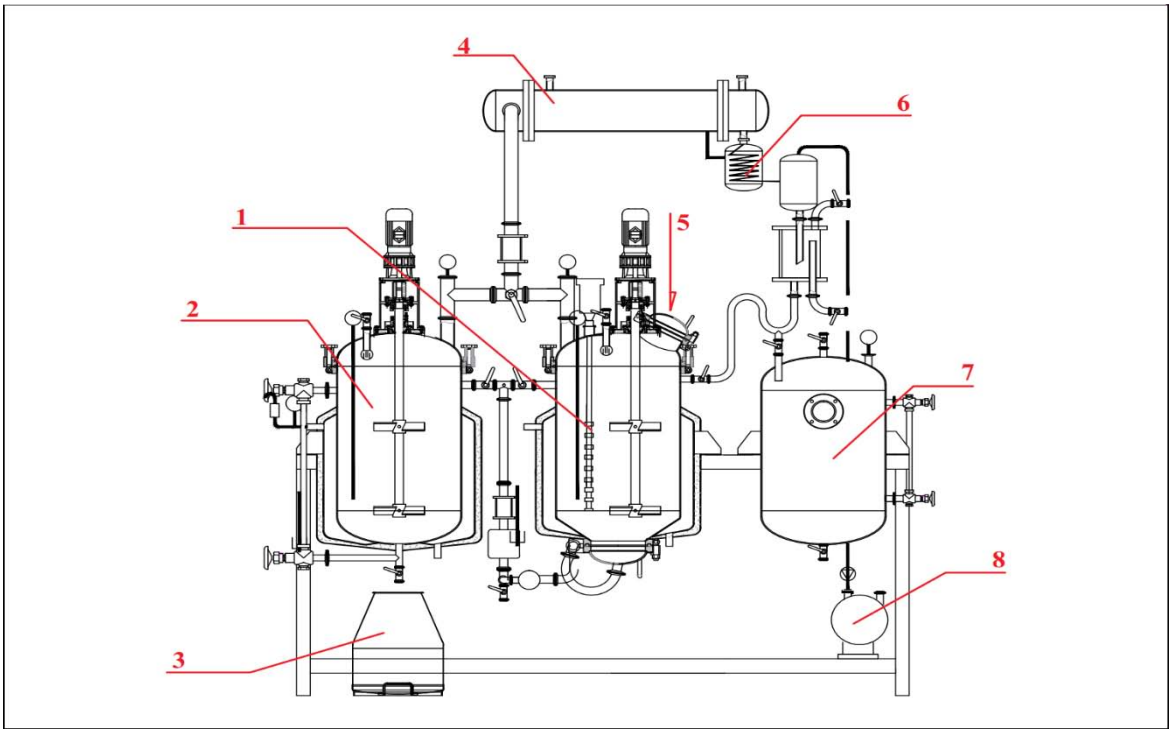

Fig.1: Scheme of the extractor "RUIAN XUANLI MASHINERY TANK". 1-extractor with built-in ultrasonic device; 2-vacuum concentrator; 3-collection; 4- capacitor; 5- loading hopper; 6- refrigerator; 7- tank for alcohol recovery; 8-vacuum pump

This equipment can carry out several operations simultaneously; control and regulation of the temperature regime of the extraction process, use ultrasound, create a vacuum, condense the extractant, recover alcohol, etc.

To obtain a dry extract, medicinal plant raw materials, crushed to a size of 5-7 mm, are weighed and mixed in the ratio: tansy flowers - 15 parts, chamomile flowers - 10 parts, yarrow herb - 10 parts. The mixture of vegetable raw materials was loaded into a special container and soaked with half the amount of 60% ethyl alcohol until a "mirror surface" is formed, left for 24 hours. After soaking, the soaked raw material is transferred to the extractor and 60% ethanol was added, bringing the raw material-extractant ratio to 1:10, the mixture was heated to 500C, and circulation extraction was carried out in the RuianXuanli Machinery Tank extractor. Next, ultrasonic extraction is performed for 10 minutes. After the vacuum extraction process is completed, the liquid extract is pumped through the filter into the reactor and left for 24 hours to settle. Purification of the distillation residue of the water-alcohol extract is carried out by filtration.

Then, the extractant was distilled off in the reactor using vacuum for 3 hours. The extract remaining after distillation of the extractant was spray dried in a high-speed spray dryer "LPG-15 Spray Dryer" (manufactured by RuianXuanli Machinery Co., LTD). (Fig. 2).

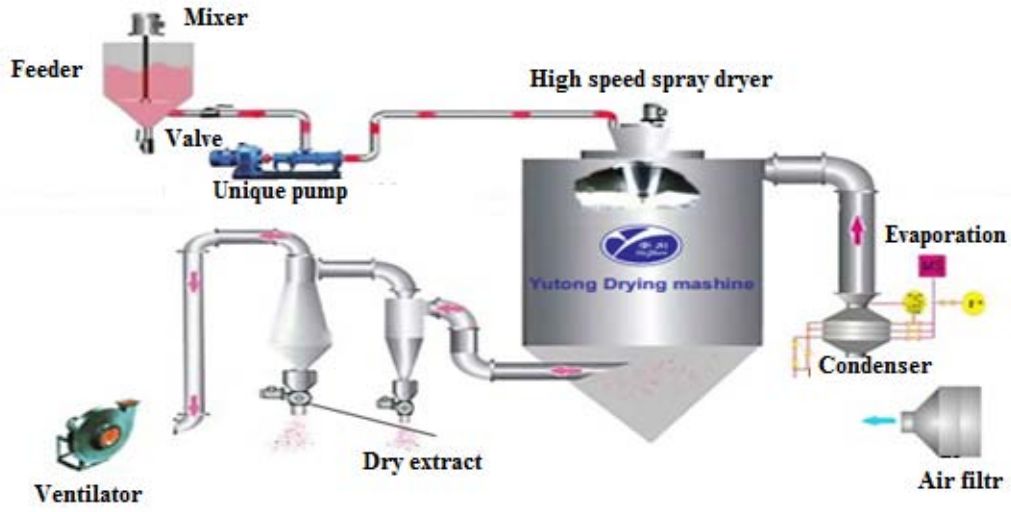

Fig. 2: Spray dryer "LPG-15 HIGH SPEED SPRAY DRYER"

As a result of the research, a light brown dry extract with a weak specific odor and a bitter taste is obtained. The dry extract yield is 21±1.53%.

III. Conclusions

1. Using the method of a four-factor experimental plan based on a 5x5 Greek-Latin square, the choice of the degree of grinding of raw materials, the concentration of ethanol, the ratio of raw materials and extractant, the temperature regime in the development of the technology of dry extract of the choleretic collection "Triflos" is carried out. The results of dispersion analysis shows that the most complete yield of the sum of flavonoids is observed with the following indicators of the above factors: dispersion of raw materials -5-7 mm, 60% ethyl alcohol, hydromodulus 1:10, temperature 500C.
2. The expediency of using ultrasonic treatment for a more complete recovery of target biologically active substances is scientifically substantiated.
3. This technology of circulating extraction using ultrasound is tested in industrial conditions. A dry extract is obtained, the yield of which was 21%.

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