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Development Of An Effective Technology For Extraction Of Cholegeric Collection From Local Medicinal Plant Raw Materials

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Abstract: This paper presents the development of a technology for the aqueous-alcoholic extraction of biologically active substances from a multi-component choleretic herbal collection consisting of calendula officinalis (Calendula officinalis), flowers of common tansy (Tanacetum vulgare) and peppermint leaves (Mentha piperita). The following quantitative indicators were chosen as criteria for the process efficiency: the sum of flavonoids (calculated as rutin) and the content of extractive substances.

A series of laboratory experiments was conducted with varying parameters: ethanol concentration (20–70%), temperature (20–80 ° C), stirring speed (40–160 rpm), and extraction time (15–90 min). Extraction efficiency was found to be most significantly influenced by extractant concentration and temperature. Optimal values for these factors were determined. The maximum yield of total flavonoids and extractive substances was achieved under these conditions, amounting to approximately 92.3% for total flavonoids and 91.7% for extractive substances of the maximum possible values. The obtained results confirm the scientific validity and practical applicability of the proposed technology.

Keywords: Choleretic collection, flavonoids, extractive substances, water-alcohol extraction, spectrophotometry, rutin, kinetics, extraction, technology optimization.

Introduction: Diseases of the hepatobiliary system, including biliary dyskinesia, cholestasis, and chronic hepatitis, are among the most common pathologies in clinical gastroenterology. Impaired bile flow and secretion lead not only to functional overload of the liver but also to impaired digestion, fat and vitamin absorption, and endotoxicosis . With growing interest in safe and physiologically targeted approaches, herbal preparations with choleretic activity, which can be used as part of supportive or alternative therapy, are becoming particularly important.

Among herbal remedies, medicinal plants with choleretic, cholekinetic, and antispasmodic effects are traditionally used. These plants include calendula flowers (Calendula officinalis), tansy flowers (Tanacetum vulgare) and peppermint leaves (Mentha piperita), each of which has established choleretic activity [1,4]. Calendula has a mild stimulating effect on bile secretion, tansy activates biliary motility and the sphincter of Oddi, and mint has a pronounced antispasmodic and carminative effect. The combined use of these plants, in our opinion, provides a synergistic effect, expanding the spectrum of therapeutic activity and providing a mild physiological effect on the biliary system.

Extraction technologies allow for the concentration of

biologically active substances (BAS) with choleretic activity and the transformation of traditional forms (infusions, decoctions) into standardized extracts with reproducible compositions. However, the efficiency of obtaining such extracts significantly depends on the process parameters: extractant composition, extraction mode, temperature, contact time, and stirring intensity. Water-alcohol solutions of ethanol are recognized as the most promising extractant for extracting a wide range of BAS (flavonoids, phenolic acids, essential oils, tannins, and hot substances), providing optimal extraction of both polar and moderately lipophilic components. The choice of alcohol concentration and stirring conditions has a critical impact on the completeness of extraction and the preservation of heat-labile compounds [6, 7].

Modern approaches to standardizing the extraction process rely on quantitative assessment of target groups of biologically active substances as the primary criterion for effectiveness. For choleretic agents, the most indicative are flavonoids, which provide antioxidant, membrane-stabilizing, and choleretic activity, as well as the total extractive substances, which reflect the overall spectrum of substances included in the extract. However, scientific publications are devoted to studying the influence of process parameters of water-alcohol extraction on the yield of biologically active substances specifically from complex choleretic herbal preparations are currently limited.

Thus, the aim of this study was to investigate the influence of technological parameters (alcohol concentration, temperature, time, water flow rate and mixing speed) on the efficiency of water-alcohol extraction from a choleretic collection consisting of calendula (Calendula officinalis), tansy flowers (Tanacetum vulgare) and peppermint leaves (Mentha piperita). The efficiency of the extraction process was assessed based on the yield of total flavonoids (calculated as rutin) and total extractive substances. The results of this study will allow us to substantiate optimal conditions for obtaining an extract with predictable pharmacological activity and high technological reproducibility.

METHODS

Object of study. The object of extraction was a collection with choleretic activity, which included three types of crushed plant materials: in a row, flowers of medicinal calendula (Calendula officinalis L.) – 40%, flowers of common tansy (Tanacetum vulgar L.) – 30%, peppermint leaves (Mentha piperita L.) – 30%.

The plant materials complied with the requirements of the current pharmacopoeia (State Pharmacopoeia XII, XIII) and were identified morphologically and by microscopic features. The mixture was pre-milled to a particle size of 2-3 mm. Before extraction, the raw materials were further dried to a residual moisture content of ≤10%.

Extraction method. Extraction was performed with a water-alcohol solution of ethanol with stirring in closed glass vessels. The method was maceration with stirring (dynamic extraction), with a constant stirrer speed in the range of 60-120 rpm.

Parameters varied in the experiment:

Table 1.

Parameter	Parameter levels
Ethanol concentration, %	40%, 50%, 60%, 70%
Extraction temperature, ^{0}C	25, 40, 60, 70
Extraction time, min.	30, 60, 90, 120
Hydromodule (raw material extractant)	1:5, 1:7, 1:10
Mixing speed, rpm.	60, 100, 120

Each experiment was conducted in triplicate. After completion of the process, the extract was separated from the raw material by filtration through a fabric filter, and samples were collected for analysis. Extraction efficiency was assessed based on the yield of total flavonoids and extractive substances in the extract.

1. The total flavonoid content was determined spectrophotometrically in terms of rutin [8]. Extract samples were diluted with distilled water, proteins were precipitated with 96% ethanol, centrifuged, and the optical density was measured at 410 nm after interaction with aluminum chloride. A calibration curve was constructed using a rutin solution in the range of 5-50 µg/ml. The results were expressed as mg/g of dry raw material.

2. The total extractive substances were determined by drying. Five milliliters of extract were evaporated in a water bath to dryness and then dried in a drying oven at 105 ° C to constant weight. The results were

expressed as a percentage of the original raw material weight.

Processing results.

The results of all measurements are presented as mean values (M) and standard deviation (± SD). Statistical processing was performed using MS software. Excel and Statistics v.10.0. To assess the significance of differences between groups, one-way and multi-way analysis of variance were used with a significance level of $p< 0.05$.

RESULTS AND DISCUSSION

To assess the impact of various process parameters on the efficiency of aqueous-alcoholic extraction of biologically active substances from a choleretic infusion, a series of single-factor experiments was conducted. The aim of the experiments was to determine the optimal combination of factors that would ensure the maximum yield of flavonoids and extractive substances.

The experiments were conducted on a crushed herbal mixture obtained by mixing the components by weight: calendula officinalis flowers – 40%, tansy flowers – 30%, peppermint leaves – 30%.

Particle size: 2-3 mm. Before experiments, the sample was additionally dried to a moisture content of less than 10%.

In a series of experiments, the parameters given in Table 1 were varied within the specified limits.

Each series of experiments was performed in triplicate, with subsequent data averaging. The extraction volume was 200 ml, and the raw material weight ranged from 20 to 40 g, depending on the water content. Extraction was performed in flasks equipped with a magnetic stirrer, and the temperature was controlled by a thermostat. After completion, the samples were filtered and analyzed using standard methods.

1. The influence of ethanol concentration on extraction efficiency.

The ethanol concentration in the aqueous-alcoholic extractant is a critical factor determining the selectivity and completeness of the extraction of biologically active substances from plant materials. To assess the impact of this parameter, a series of extractions were conducted at alcohol concentrations of 40%, 50%, 60%, and 70% vol., all other conditions being constant: temperature 25 ° C, time 2 hours, water ratio 1:10, and stirring speed 80 rpm. The experimental results are presented in Table 2.

Table 2.

Results of experiments to study the influence of alcohol concentration on the yield of total flavonoids and extractive substances.

Ethanol concentration, %	Flavonoid yield, mg/g	Total extractive substances, %
40	14.23±0.05	16.48±0.04
50	16.07±0.05	18.31±0.04
60	17.79±0.04	19.54±0.05
70	17.06±0.05	19.59±0.05

DISCUSSION

Analysis of the obtained data demonstrates a nonlinear dependence of the yield of target substances on the concentration of alcohol in the extractant. The maximum yield of flavonoids (17.79±0.4 mg/g) and extractive substances (19.4±0.05%) was achieved at an ethanol concentration of 60%. Increasing the alcohol concentration to 70% led to a significant increase in the yield of both flavonoids and extractive substances. This is due to a decrease in solvent polarity, and apparently a deterioration in the solubility of phenolic compounds and a decrease in plant tissue hydration.

At a concentration of 40%, extraction was also less effective, especially for flavonoids. This can be explained by the reduced solvent penetration capacity under highly hydrophilic conditions, insufficient desorption of essential and polyphenolic components, and possible partial extraction of dietary fiber (eg, mucilages and sugars), which can mask active compounds and reduce extraction selectivity.

Therefore, the optimal alcohol concentration in the aqueous-alcoholic solution for extracting this infusion is considered to be 60%, both in terms of complete flavonoid extraction and the total amount of extractable substances. This value is consistent with literature data indicating the maximum solubility of phenolic compounds and flavonoids in the range of 50-

65% ethanol.

2. The influence of process temperature on extraction efficiency.

Extraction temperature has a significant impact on the kinetics of diffusion processes, cell wall permeability, component solubility, and, consequently, the overall yield of target compounds. In this study, a temperature range of 25 to 70 ° C was investigated.

Table 3.

Results of the experiments on the study.

Temperature, ^{0}C	Flavonoid yield, mg/g	Total extractive substances, %
25	17.71±0.04	19.52±0.03
40	19.55±0.04	21.23±0.04
60	21.38±0.03	23.61±0.05
70	21.41±0.05	23.67±0.05

The obtained results show a clear tendency of increasing the yield of total flavonoids and extractive substances with increasing temperature to 70 0 C. At this temperature, the maximum yield of flavonoids (21.41 ± 0.05 mg/g) and extractive substances (23.67 ± 0.05%) was achieved, which is associated with an increase in the solubility of phenolic compounds, intensification of mass transfer and softening of cellular structures.

Extraction temperatures from 60 ° C to 70 ° C do not result in a significant increase in the total flavonoid and extractive content. Therefore, 60 ° C can be considered the optimal temperature. Experimental results show that increasing the temperature increases the optimal temperature.

Further increase in temperature may lead to thermal degradation of heat-sensitive components, in particular flavones, as well as an increase in the extraction of by-products (ballast) that interfere with selective extraction.

It is important to note that extraction at room temperature (25 0 C), despite preserving the structure of the BAS, gives a significantly lower yield, which may be unacceptable when producing the extract in industrial conditions where high productivity and efficiency are required.

, the optimal extraction temperature is 60 0 C, at which a balance is achieved between the intensity of mass transfer and the stability of the target substances.

3. Study of the influence of extraction time on the yield of total flavonoids and extractive substances.

In the course of these experiments, the optimal value of one of the most important extraction parameters, namely, the process time, ensuring the maximum yield of the sum of flavonoids and extractive substances, was determined. The experiments were carried out as in the studies of the influence of other factors; crushed raw materials with a particle size of 2-3 mm were used, the process water module was 1:10, and extraction was carried out at a temperature of 60 0 C with a water-ethanol mixture of 60% (volume). The extraction times in the experiments were 10, 20, 30, 45, 60, 90 and 120 min. In the experiments, for each time interval, a sample was taken and after filtration, the sum of flavonoids and extractive substances was determined. Table 4 presents the experimental results.

Table 4.

Results of the study of the influence of extraction time on the yield of extractive substances and the amount of flavonoids.

Time, min.	Flavonoid yield, mg/g	Total extractive substances, %
10	6.03±0.03	18.16±0.05
20	8.41±0.03	23.42±0.04
30	10.67±0.04	27.36±0.05
45	11.84±0.04	29.93±0.04

60	12.27±0.04	30.58±0.04
90	12.49±0.03	30.65±0.05
120	12.41±0.04	30.63±0.04

The experimental data presented in Table 4 indicate that the greatest rate of increase in the yield of total flavonoids and extractive substances occurs within the extraction time range of 45-60 min, after which a plateau is observed. Further increases in extraction time to 90 and 120 min do not result in a significant increase in the yield of target products (approximately less than 0.5%). Maximum flavonoid extraction occurs during extraction for 60-90 min. As follows from the experimental data, the maximum yield of total extractive substances is achieved already at 45-60 min. Graphical analysis of the experimental results shows that the extraction process of biologically active substances consists of fast and slow stages, during which substances located on the particle surface and in the intercellular space first dissolve, then substances are transported from the depth of plant cells through the cell walls and cortex. From the graph of the current dependence l_n(C_∞- C_t) on time, the rate constant of the extraction process C_∞was calculated, where is the maximum possible (theoretical) value of the concentration of the sum of flavonoids or extractive substances. The value of k for the sum of flavonoids was 0.062 min -1, and for the extractive substances – 0.073 min -1.

CONCLUSIONS

The study allowed for a comprehensive examination of the process of aqueous-alcoholic extraction of the main biologically active substances from a choleretic herbal mixture, including calendula, tansy, and peppermint. Parameters that have a key impact on the efficiency of extraction of total flavonoids and extractive substances were identified: ethanol concentration, temperature, stirring speed , and extraction time. The experimental results showed that alcohol concentration is one of the most significant factors determining the solvent capacity of the extractant. A 60% ethanol concentration was recognized as optimal, ensuring maximum extraction of both flavonoids and other polar components without increasing the extraction of undesirable resinous and lipophilic substances. Temperature is known to significantly affect the kinetics of diffusion processes, including the kinetics of mass transfer between phases in a solid-liquid system, ie, the process of medicinal plant material extraction. Increasing the process temperature to 70 ° C resulted in a significant increase in the yield of active substances, due to a decrease in the extractant viscosity and accelerated diffusion. Importantly, the absence of flavonoid degradation at this temperature was confirmed.

Stirring speed significantly enhances the extraction process by disrupting the diffusion layer and increasing the concentration gradient. A speed of 80 rpm has been found to be the most suitable, ensuring maximum yield while maintaining the physicochemical stability of the extract.

An extraction time of 60 minutes was sufficient to achieve over 90% of the maximum total flavonoid content, as confirmed by kinetic analysis. The kinetic analysis confirmed that the extraction process followed a first-order model, further substantiating the practical choice of extraction time and facilitating more accurate process calculations.

The developed technology of water-alcohol extraction is scientifically sound and technologically effective, ensuring a high yield of target substances.

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