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POLYSACCHARIDE PROFILE, ACUTE TOXICITY AND BILE SECRETION EFFECTS OF THE CHOLERETIC HERBAL PREPARATION 'SAFROART HERBAL TEA'

Khairulla Olimov, Tanzila Mirrakhimova, Gulrano Akhmadova

The aim. *The aim of this study was to determine the polysaccharide composition of the choleretic collection "Safroart herbal tea", focusing on the identification of key carbohydrate fractions and their monosaccharide composition, as well as to evaluate its acute toxicity and choleretic activity. This investigation contributes to understanding the biochemical and pharmacological properties underlying the plant's therapeutic effects.*

Materials and methods. *The herbal collection was subjected to sequential extraction to isolate its polysaccharides, which were fractionated into water-soluble polysaccharides (WSPS), pectin substances (PS), and hemicelluloses (HMC). These fractions were analyzed using paper chromatography, gas chromatography (GC), and infrared (IR) spectroscopy. For pharmacological evaluation, acute toxicity was assessed in mice using a single-dose administration protocol, and the choleretic effect was studied in rats by measuring bile secretion over a 4-hour period following administration of a 10 % aqueous infusion.*

Results. *Alcohol-soluble sugars, including sucrose and fructose, were identified. WSPS were primarily composed of glucose, galactose, and arabinose, while PS and HMC contained rhamnose, arabinose, and xylose. IR spectroscopy confirmed the structural features of the fractions. The herbal collection was classified as practically non-toxic (LD_{50}>4000 mg/kg). In vivo studies revealed a 27.4 % increase in bile secretion in treated rats compared to controls, confirming a significant choleretic effect.*

Conclusions. **'***Safroart herbal tea' contains a complex mixture of polysaccharides with distinct monosaccharide profiles and demonstrates both safety and choleretic efficacy in experimental models. These findings support its potential use as a natural hepatobiliary remedy and contribute to the phytochemical and pharmacological understanding of its therapeutic action*

Keywords: *choleretic collection, Safroart herbal tea, IR spectrum, alcohol-soluble sugars, acute toxicity, bile secretion effect, WSPS, PS, hemicellulose, gas chromatography*

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1. Introduction

Medicinal plant raw materials are widely used in the modern pharmaceutical industry to obtain a range of medicinal plant preparations, in particular collections, which, when properly used, are characterized by high efficacy and are characterized by minimal side effects. In this regard, preparations of medicinal plants are well established for the treatment of chronic diseases requiring long-term therapy [1, 2]. The healing properties of plants are due to the harmonious interaction and optimal ratio of a complex of the different chemical structures of biologically active substances contained in the plant, which have genetic evolutionary affinity with the body; in this regard, medicinal preparations from plants are more actively absorbed by the body and more easily included in biochemical processes [3, 4]. The therapeutic effect of medicinal plants is due to the complex action of different chemical natures of biologically active compounds. Proper composition and selection of the ratio in the creation of a medicinal collection of these plants will be more effective than the drug obtained from a single plant [5, 6].

Diuretics are one of the medicines in the most demand in clinical practice groups. According to statistics, diseases of the gallbladder and biliary tract are second in the structure of diseases of the digestive organs [7–9]. A large share in the group of choleretic agents are plant-based remedies, as they act milder than synthetic drugs and drugs containing bile and bile acids, simultaneously combining choleretic and cholekinetic action, and also have additional therapeutic effects in relation to the gastrointestinal tract improve the secretion of glands of the stomach, pancreas, increasing intestinal peristalsis, having choleretic, epithelializing, anti-inflammatory, styptic, laxative effects [10–12]. The effect of preparations of plant origin is associated with the influence of a complex of biologically active substances included in their composition, such as essential oils, polysaccharides, resins, flavones, phytosterols, phytoncides, some vitamins and other substances from these positions the combination of herbal preparations on the basis of several medicinal plants for the treatment of pathology of the biliary excretory apparatus against the background of concomitant diseases of the digestive system is relevant [13–15].

Pharmacological properties of *Cynara scolymus*, *Calendula officinalis*, *Achillea millefolium*. Provide anti-inflammatory, antispasmodic, and choleretic actions, restore normal peristalsis, and improve the digestive capacity of the gastrointestinal tract. The useful properties

of *Mentha piperita* are largely due to the content of menthol in the essential oil. It improves appetite, increases the secretion of digestive glands and biliary secretion, reduces the tone of the smooth muscles of the intestine, as well as the biliary and urinary tract, relieves spasms, and also gives an aromatic odour to the collection. Proper composition and ratio selection in creating a medicinal collection from these plants will be more effective than a remedy derived from a single plant [16–19].

The article addresses not only the polysaccharide composition of the choleretic herbal collection 'Safroart herbal tea', but also its acute toxicity and choleretic effects, reflecting a comprehensive pharmacognostic and pharmacological evaluation. Polysaccharides are among the key bioactive constituents in many medicinal plants, known for their immunomodulatory, hepatoprotective, and prebiotic properties. Their structural characteristics and monosaccharide composition often correlate with specific biological activities, including gastrointestinal and liver function support. Therefore, identifying and characterizing the polysaccharide fractions provides a chemical foundation for understanding the potential mechanisms of action of the herbal formulation. Simultaneously, the evaluation of acute toxicity is a critical step in establishing the safety profile of any herbal preparation, especially when intended for oral use. This ensures that even at high doses, the preparation does not pose harmful effects, which is essential for its potential therapeutic application. Furthermore, the study of choleretic activity – the ability to stimulate bile secretion – directly relates to the claimed pharmacological action of herbal tea in supporting hepatobiliary function. By combining phytochemical characterization with in vivo safety and efficacy testing, the study offers a well-rounded assessment of 'Safroart herbal tea' as a promising natural remedy for liver and biliary tract disorders.

Aim of the research. To qualitatively and quantitatively determine the polysaccharide content of the choleretic herbal collection 'Safroart herbal tea' and to evaluate its acute toxicity and bile secretion-stimulating effects.

2. Planning (methodology) of research

2. 1. Sample preparation and extraction.
2. 2. Fractionation of polysaccharides.
2. 3. Chromatographic analysis.
2. 4. Infrared (IR) spectroscopy.
2. 5. Pharmacological analysis:
– Acute toxicity;
– Choleretic effect.

3. Materials and methods

This study was conducted from 2023 to 2024, focusing on the comprehensive analysis of a choleretic herbal collection composed of *Cynarae scolymi folia* (40 %), *Calendulae officinalis flores* (20 %), *Menthae piperitae folia* (20 %), and *Millefolii herbae flores* (20 %). The raw plant materials were cultivated and harvested in various agro-ecological zones of Uzbekistan, specifically in the Tashkent and Jizzakh regions, during their optimal vegetative stages. Botanical identification and taxonomic verification were performed by specialists of the Institute of Botany, Academy of Sciences of Uzbekistan, ensuring the authenticity and purity of each plant species. The raw materials were air-dried under controlled conditions and finely ground to a particle size of 1–3 mm in accordance with pharmacopoeial standards before extraction and analysis.

Inactivation of raw materials. 100 g of crushed raw material 'Safroart herbal tea' was treated twice with a boiling mixture of methanol – chloroform (1:1) to remove colouring substances and non-carbohydrate components. Then, the raw material was separated by filtration and dried.

The dried raw material was extracted twice with boiling 82 % ethyl alcohol (1:6) for 1 hour. The alcohol extracts were combined and evaporated, and sucrose and fructose were identified by paper chromatography.

WSPS is isolated by cold extraction WSPS-x and hot extraction WSPS-g. The residue of the raw material was extracted twice with cold water at room temperature for 1.5 h each at a hydromodule of 1:4, respectively. The extracts were separated by filtration, evaporated to a small volume and precipitated with three times the volume of ethyl alcohol. The precipitate was centrifuged (5000 rpm, 10 min), washed and dehydrated with alcohol. The yield of WSPS-x is in Table 1.

Then, the residue was extracted twice with water at 80–85 °C for 1.5 h at hydromodule 1:3, 1:2. The extracts were combined, evaporated and precipitated with alcohol. The precipitate was treated as above. The yield of WSPS-g is in Table 1.

Extraction of PS. After isolation of the sum of PS, the waste was extracted twice with an equal mixture of 0.5 % oxalic acid and ammonium oxalate solutions at 75 °C; extraction was carried out at a hydro module of 1:4 and 1:3. The extract was separated by filtration, dialyzed against running water, evaporated, and precipitated with three times the volume of alcohol. The precipitate was treated similarly as described above. The yield of PS is in Table 1 (from air-dried raw material).

HMC extraction. After isolation, SP was treated twice with 5 % KOH solution at room temperature for 1.5–2 hours, at a hydromodule of 1:3. The extracts were separated by filtration, neutralized with CH_3OH, the solution was evaporated to thickness, and precipitated with three times the volume of alcohol. The precipitated HMC was separated by centrifugation, washed and dried with alcohol; the yield is given in Table 1.

Titrimetric values of PS. For the determination of free carboxyl groups in PS, 25 ml of water was added to 0.25 g of PS. PS was added to 25 ml of water, slightly heated, stirred, kept for 2 h, and titrated with 0.1 M sodium hydroxide solution (indicator - phenolphthalein) until the formation of the pink colour [7–9, 20].

Complete acid hydrolysis of WSPS, PS, and HMC. 100 mg each of isolated polysaccharides were hydrolyzed with 3 ml of 1 H_2SO_4 solution at 100 °C. WSPS for 8 h, PS and HMC for 24 h. After the time elapsed, the hydrolysate was placed in a beaker and neutralized with barium carbonate. The resulting precipitate was filtered, the filtrate was deionized with cationic KU-2, evaporated to a low volume (0.5 ml) and chromatographed on FN-18

paper in the butanol-1-pyridine-water (6:4:3) system with known monosaccharides (witnesses). The chromatograms were dried, shown with acidic aniline phthalate, followed by heating in a desiccator at 110 °C for 1–2 min.

IR spectra of polysaccharides were recorded using a Perkin-Elmer FT-IR/NIR Spectrometer (Spectrum 3, USA) equipped with a Universal ATR Sampling Accessory in the absorption region ranging from 530 to 3600 cm^{-1} [5, 6].

Paper chromatography was performed on Filtrak-FN 13, 18 paper (Germany) using the mobile phase n-butanol-pyridine-water (6:4:3). The test solution was prepared by dissolving the concentrated polysaccharide extract in distilled water, followed by filtration. The reference solution consisted of a standard mixture of monosaccharides (glucose, galactose, arabinose, and rhamnose) prepared in the same solvent. Detection of chromatographic spots was carried out using (1) an acidic aniline phthalate reagent heated at 100 °C for 5 minutes, and (2) a 5 % urea solution to enhance visualization of the polysaccharide components.

Gas chromatography (GC) analysis of polysaccharide hydrolysates was carried out using a GC Plus2010 chromatograph under the following instrumental conditions: injector temperature – 250 °C; detector temperature – 250 °C; column temperature – 230 °C; total flow rate – 60 mL/min; flow through the column – 0.89 mL/min; carrier gas – nitrogen; column – Rxi-624Sil MS (Restek, USA), column length – 3 m, inner diameter (ID) – 0.25 mm.

Prior to analysis, acid hydrolysis of polysaccharide fractions was performed using 2M trifluoroacetic acid (TFA) at 100 °C for 4 hours, followed by neutralization and evaporation under reduced pressure. The hydrolysates were reconstituted in distilled water and filtered through 0.22 μm membranes prior to injection.

Detection of monosaccharides was based on retention time comparison with authentic standard solutions of glucose, galactose, arabinose, rhamnose, and xylose, all purchased from Sigma-Aldrich (USA). Calibration curves were constructed for each standard to allow for quantitative determination.

The acute toxicity of the medicinal collection 'Safroart herbal tea' was studied using the conventional method of single-dose administration with subsequent determination of LD_{50} and assignment to a toxicity class [18, 19, 21]. The study was conducted in accordance with ethical standards, and the protocol was approved by the Bioethical Committee of Tashkent pharmaceutical institute, laboratory of Innovative Pharmaceutical Compounds, Expert Opinion No. 06/23 dated May 12, 2023.

For the experiment, we used white mice, males and females, in a number of 18 heads, bodyweight 19–21 g, kept in quarantine for 14 days. Before and during the experiments, mice were kept in the vivarium at air temperature +20–22 °C, humidity – not more than 50 %, air exchange volume (exhaust: inflow) – 8:10, and light regime – day-night. Mice were placed in standard plastic cages and kept on a standard diet.

The mice were administered a 10 % aqueous infusion of the herbal collection, prepared at a 1:10 ratio (i.e. 5 g of the dried herbal material in 50 ml of water), as follows:

– group 1 (6 mice) – per os at a dose of 2,000 mg/kg (0.4 ml);

– group 2 (6 mice) – per os at a dose of 3,000 mg/kg (0.6 ml);

– group 3 (6 mice) – per os at a dose of 4,000 mg/kg (0.8 ml).

On the first day of the experiment, the animals were monitored hourly in the laboratory, and parameters of appearance (condition of hair, mucous membranes, etc.), functional state (survival rate during the experiment, general condition, possible convulsions, and death), and behaviour were recorded. Then, daily, during 2 weeks in vivarium conditions, general condition and activity, behavioural features, reaction to tactile, pain, sound, and light stimuli, frequency and depth of respiratory movements, the rhythm of heartbeats, condition of hair and skin cover, tail position, quantity and consistency of fecal masses, frequency of urination, change in body weight, and other parameters were observed in animals of all groups. All experimental animals were kept in the same conditions and on a common diet with free access to water and food.

After completion of the experiment, the LD_{50} and toxicity class of the investigated remedy were determined.

The specific choleretic activity of the medicinal collection 'Safroart herbal tea' was evaluated in an in vivo experiment on 12 sexually mature white rats (weighing 180–200 g), which were randomly divided into 2 groups of 6 animals each. The animals were kept under standard vivarium conditions: 12-hour light/dark cycle, temperature 22 ± 2 °C, relative humidity 55–60 %, with ad libitum access to food and water until 24 hours before the experiment, when they were deprived of food but had free access to water.

On the day of the experiment, the rats were anaesthetized with urethane (40 mg/kg, intraperitoneally). Following a midline laparotomy, the bile duct was isolated and cannulated with a sterile polyethylene tube, which was connected to a calibrated collection vessel. The volume of secreted bile was collected and measured hourly over a 4-hour period.

All experimental procedures were conducted in accordance with ethical standards and approved by the Bioethical Committee of the Tashkent Pharmaceutical Institute, Laboratory of Innovative Pharmaceutical Compounds, Expert Opinion No. 08/23 dated June 5, 2023.

The degree of choleretic activity was judged by the total amount of secreted bile [22, 23].

Statistical calculations were performed using the paired Student's t-test to determine the significance of differences between experimental groups. The results were expressed as mean ± standard deviation (M±SD). A value of $p<0.05$ was considered statistically significant.

4. Results

Galactose, arabinose, and glucose were identified in the monosaccharide composition of the polysaccharides.

Alcohol-soluble sugars were represented by sucrose and fructose according to paper chromatographic analysis.

WSPS were extracted with water: the raw material was extracted with water at room temperature (20–22 °C), and WSPS-x was isolated. After the isolation of WSPS-x,

the rest of the raw material was extracted twice with hot water in a water bath in the ratio of 1:15 and 1:10 at 70–75 °C, stirring constantly, and WSPS-g was obtained. Next, PS and HMC were extracted sequentially. GC determined the ratio of monosaccharide residues. The yield and content and monosaccharide composition of isolated polysaccharides are given in Table 1.

As can be seen from Table 1, the main monosaccharides in WSPS are glucose, galactose, and arabinose, while in PS and HMC, rhamnose, arabinose, and xylose occur most abundantly.

The isolated polysaccharides were light brown in colour with a reddish tinge.

The determination of the viscosity of aqueous solutions of WSPS, PS, and HMC isolated from the collection 'Safroart herbal tea' is presented in Table 2.

WSPS and PS are amorphous powders, cream-colored with a yellowish tinge. In hydrolysates of WSPS, PS and hemicellulose, galacturonic acid was present along with neutral monosaccharides [12, 13].

When determining the main functional groups of pectins, it was found that the isolated PS were characterized by the content of free carboxyl groups, methoxylated carboxyl groups, the total amount of carboxyl groups, methoxyl groups, and a high (λ>50 %) degree of esterification (DE).

Based on its monomeric composition, PS was characterized by a high content of galacturonic acid, along with the neutral sugars arabinose, xylose, and galactose. The DE of PS was determined by titrimetric analysis, and it was found that PS isolated from the collection "Safroart phytotea" belong to highly esterified pectins (Table 3) [14–17].

The IR spectrum of the WSPS-x collection 'Safroart phyto tea' has an intense absorption band at 3288 and 2293 cm^{-1} characterizing the valence vibrations of -OH groups. Intense absorption bands at 1591 cm^{-1} show valence vibrations (C=O) in the groups -$COOCH_3$ and -COOH, i.e. carboxyl groups both free and esterified.

Low-intensity absorption bands at 1408 and 1241 cm^{-1} show valence vibrations of -CH, -CH_2OH, and C-O-C groups since they overlap, and it is difficult to make a clear assignment of the bands. The absorption bands at 1059 cm^{-1} characterize the pyranose ring and its functional groups (Fig. 1).

In the IR spectrum of WSPS-g collection 'Safroart herbal tea', there is an intense absorption band at 3318 and 2976 cm^{-1}, characterizing the valence vibrations of -OH groups. The intense absorption bands at 1607 cm^{-1} show the valence vibrations (C=O) in the groups -$COOCOCH_3$ and -COOH, i.e. carboxyl groups both free and esterified.

Table 1

Monosaccharide composition of polysaccharides contained in the collection 'Safroart herbal tea'

Type of polysaccharide	Yield, %	Ratio of monosaccharide residues						Uronic acids
		galactose	glucose	arabinose	mannose	xylose	rhamnose	
WSPS-x	3.1	2.5	2.0	3.0	–	1.0	–	+
WSPS-g	2.1	2.0	1.0	3.0	–	2.5	–	+
PS	8.5	2.0	1.2	3.5	1.0	2.0	1.5	+
HMC	8.0	2.0	1.0	2.5	1.3	3.0	1.2	+

Table 2

Determination of viscosity of aqueous solutions of WSPS, PS, HMC isolated from the collection 'Safroart herbal tea'

Polysaccharide type	Concentration *C*, %	Flow time *t*, s	Relative viscosity
H_2O	–	30	–
WSPS-x	1	48.0	1.6
WSPS-g	1	66.0	2.2
PS	1	138.0	4.6
HMC-A	1	42.0	1.4

Table 3

Titrimetric parameters of PS contained in the collection 'Safroart phytotea'

–	Cs, %	Ca, %	Co, %	ED, %
Pectin	10.0	16.3	26.7	61.0

Note: Cs – free carboxylic group; Ca – esterified carboxylic group; ED – esterification degree.

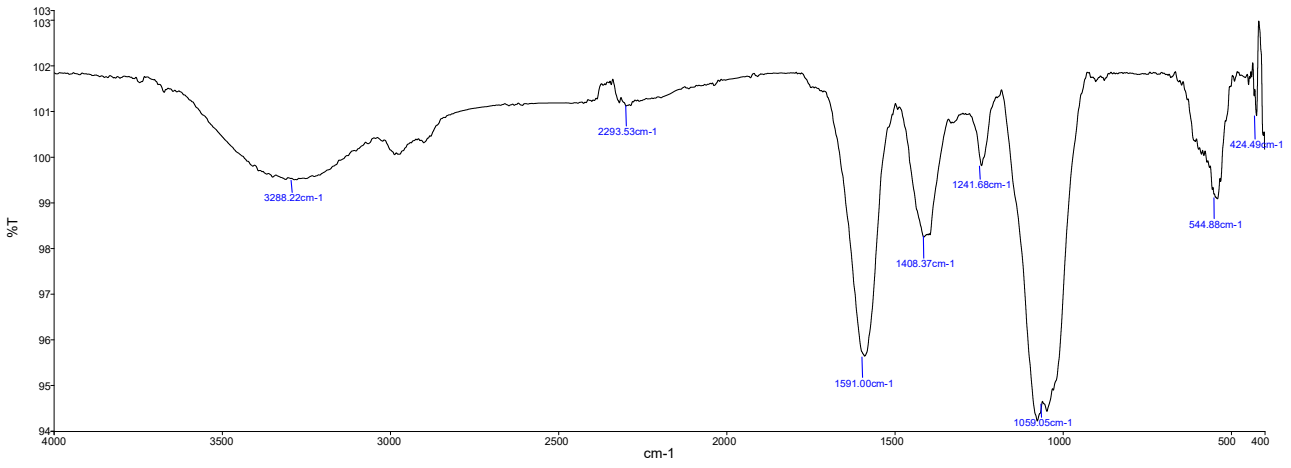

Fig. 1. IR spectrum of WSPS of WSPS-x collection 'Safroart herbal tea'

Low-intensity absorption bands at 1400 and 1237 cm^{-1} show the valence vibrations of -CH, -CH_2OH, C-O-C groups, since they overlap and it is difficult to make a clear assignment of the bands. The absorption bands at 1053 cm^{-1} characterize the pyranose ring and its functional groups (Fig. 2).

Thus, the analysis of IR spectra of polysaccharides gives information about the presence of ester groups, metals, and types of glycosidic bonds. All this complements the data from the chemical analysis of polysaccharides.

In the IR spectrum of PS collection 'Safroart herbal tea,' there is a characteristic broad absorption band of OH groups in the region of 3236 cm^{-1}. The absorption bands of –2977 and 2866 cm^{-1} correspond to CH groups with insignificant intensity. And the bands of symmetric and asymmetric CH groups.

The following absorption bands are characteristic of carboxy polysaccharides: 1898, 1702, 1590, and 1426 cm^{-1} (COO^-) correspond to the absorption bands of the ionized carboxyl group bound to metals.

The presence of esterified -CH_3- groups is shown by the absorption band at 1145 cm^{-1}.

The pyranose ring fragments -C-C-O, C-OH, etc. show absorption bands at 1086, 1045, and 1017 cm^{-1}.

PS is characterized by α-glycosidic linkage between uronic acid residues, which is well shown by the intense absorption band at 955 and 879 cm^{-1}.

Other absorption bands that are present in the IR spectrum, in its low-frequency region at 717 cm^{-1}, indicate the presence of β-glycosidic linkage for side branches in the macromolecules of SP.

Thus, the analysis of IR spectra of isolated polysaccharides provides information about the type of polysaccharide (acidic or neutral type) and the presence of glycosidic bonds (Fig. 3).

Analyzing the IR spectrum of hemicellulose (HMC) of the collection 'Safroart herbal tea', we note a wide, intense absorption band at 3276 cm^{-1}, corresponding to deformation symmetric and asymmetric vibrations of OH groups. In the region of 1621 cm^{-1}, the absorption of crystalline water is manifested.

The absorption band in the region of 1557, 1400 cm^{-1} shows ionized carboxyl (COO-). Usually, uronic acids are almost always present in the hydrolysate of HMC.

The next band at 1338, 1078 cm^{-1} is associated with vibrations of OH hydroxyl groups. The presence of pyranose monosaccharides constituting the HMC is reflected by absorption bands in the region of 1045 and 1016 cm^{-1}. The absorption bands in the low-frequency region of 883 and 790 cm^{-1} indicate the presence of α- and β-glycosidic bonds in the polysaccharide molecule (Fig. 4).

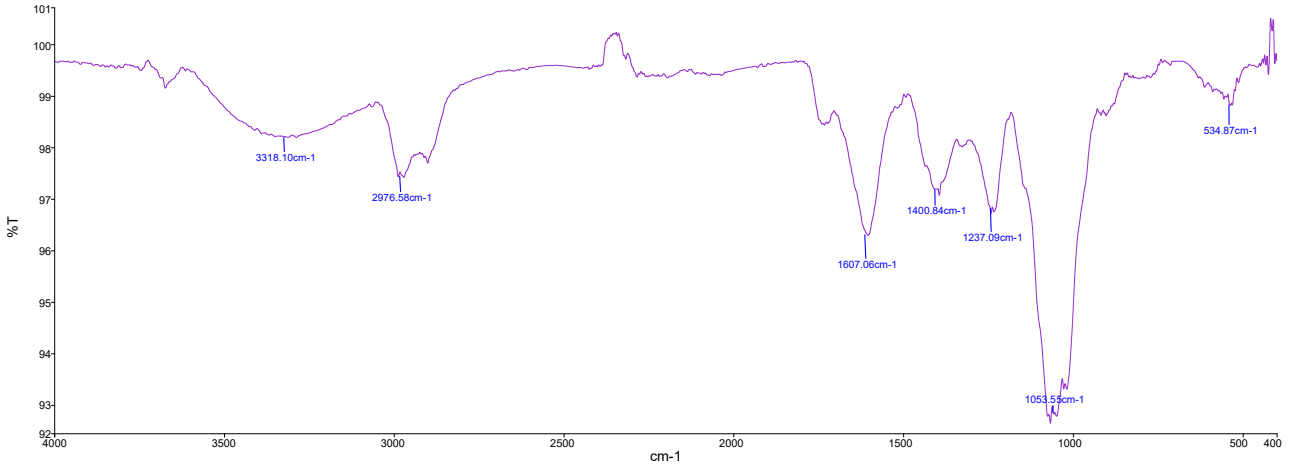

Fig. 2. IR spectrum of WSPS of WSPS-g collection 'Safroart herbal tea'

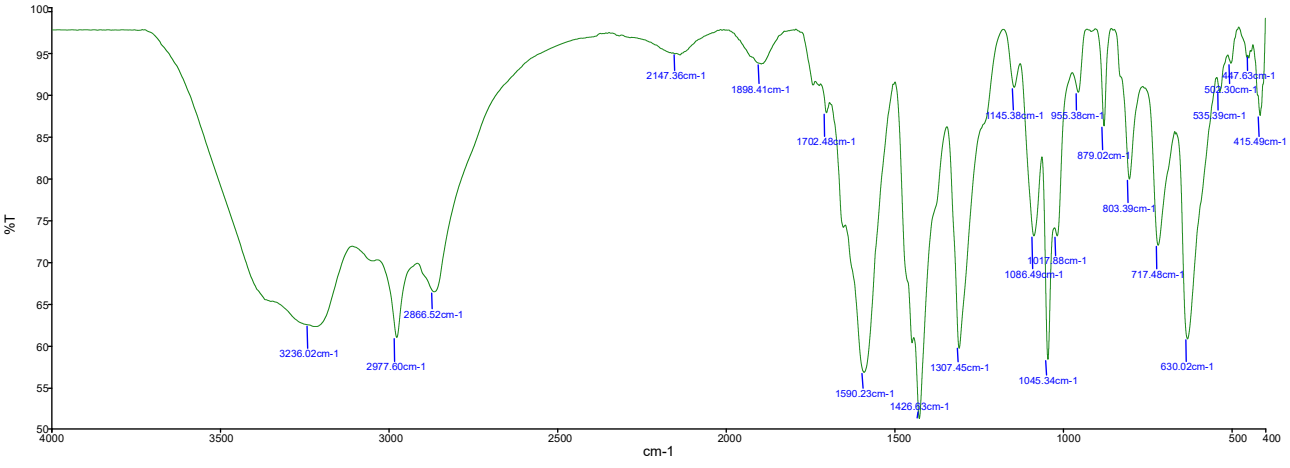

Fig. 3. IR spectrum of PS of the collection 'Safroart herbal tea'

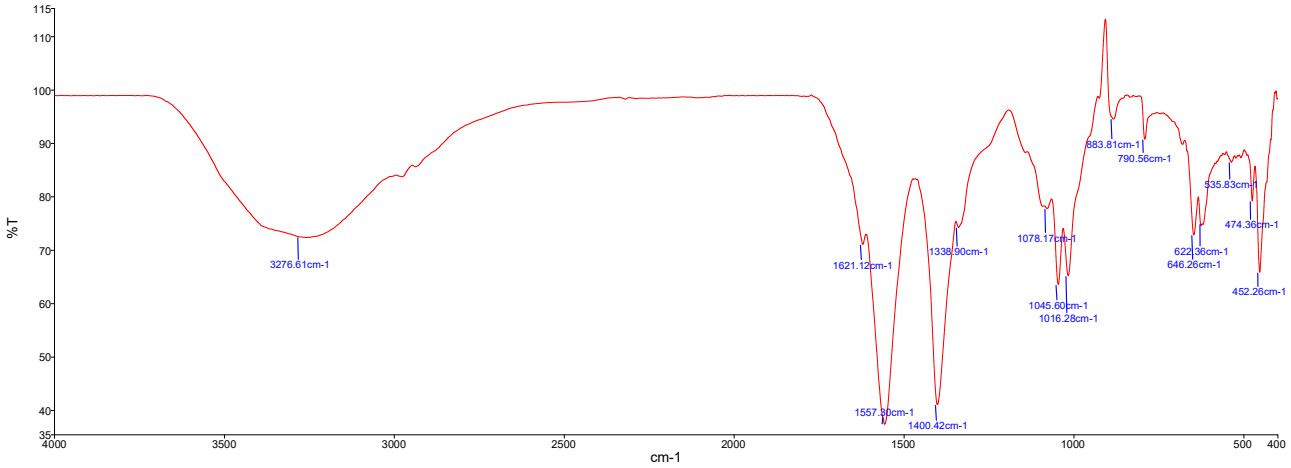

Fig. 4. IR spectrum of HMCs of the collection "Safroart herbal tea"

Acute toxicity of the collection 'Safroart herbal tea'.

When studying the acute toxicity of the concentrated infusion of the medicinal collection 'Safroart herbal tea' the following data were obtained:

1. Group 1 (2000 mg/kg): after administration of the drug during the day, the mice remained active; no visible changes in behaviour and functional state were observed. The condition of hair and skin was normal without changes; food and water were not refused, and no death of mice was observed. On the second day and during the subsequent observation period, there were no pathological changes in the behaviour and physiological parameters of the mice. Water and feed intake were normal; no lag in growth and development was observed. There was no death of mice during 14 days.

2. Group 2 (3000 mg/kg): after administration of the drug during the day, mice are active; no changes in behaviour and functional state were observed. The condition of hair and skin was normal without changes; food and water were not refused, and no death of mice was observed. On the second day and during the subsequent observation period, there were no pathological changes in the behaviour and physiological parameters of the mice. Water and feed intake were normal; no lag in growth and development was observed. There were no deaths of mice during 14 days (Table 4).

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Table 4

Determination of acute toxicity (LD_{50}) of aqueous infusion of the medicinal collection 'Safroart herbal tea'

No. of mice group	Dose, ml		Route of administration	Result
	mg/kg	ml		
1	2000	0.4	Per os	0/6
2	3000	0.6	Per os	0/6
3	4000	0.8	Per os	0/6
LD_{50}	>4000 mg/kg			

3. Group 3 (4000 mg/kg): after drug administration during the day, mice were active; no visible changes in behaviour and functional state were observed. The condition of hair and skin was normal without changes; food and water were not refused, and no death of mice was observed. On the second day and during the subsequent observation period, there were no pathological changes in the behaviour and physiological parameters of the mice. Water and feed intake were normal; no lag in growth and development was observed. There was no death of mice during 14 days.

Since, according to literature data [24], the volume of administered liquid at a single intragastric administration is not more than 0.8 ml, the introduction of a larger dose of aqueous infusion was not possible. In this regard, the LD_{50} of the medicinal collection 'Safroart herbal tea' is proposed to be considered as a dose exceeding>4000 mg/kg.

According to the classification of toxicity of substances, the studied medicinal collection 'Safroart herbal tea' belongs to the V class of toxicity – practically non-toxic.

Study of specific activity of the collection 'Safroart herbal tea'. A 10 % aqueous infusion of the medicinal collection 'Safroart herbal tea' was prepared at a 1:10 ratio (5 g of herbal material per 50 ml of water). The infusion was filtered through gauze. The studied infusion was administered to experimental rats in the amount of 500 mg/kg (1 ml per 200 g/animal). The amount of bile was determined within 4 hours. The obtained results were compared with the data obtained from the control group of animals, which in parallel received an adequate volume of water.

The study of the effect of the infusion of the medicinal collection 'Safroart herbal tea' on bile secretion showed that the collection caused an increase in bile secretion, compared with the control group after 1 hour by 34 %, which was 0.55±0.03 ml (P<0.05) with the control 0.41±0.03 ml (Table 5).

Table 5

Effect of the medicinal collection 'Safroart herbal tea' on biliary excretion (*M*±*m*)

Weight of rats, g	Volume of excreted bile (ml/100g), via				
	1 hour	2 hour	3 hour	4 hour	Total
Control group					
185.5±3.8	0.41±0.03	0.43±0.04	0.42±0.02	0.42±0.03	1.68±0.1
Infusion of the medicinal collection 'Safroart herbal tea'					
186.6±6.5	0.55±0.03, *p*<0.05	0.54±0.02, *p*<0.05	0.52±0.03, *p*<0.05	0.53±0.03, *p*<0.05	2.14±0.09, *p*<0.05

As can be seen from the table, after 4 hours, the amount of excreted bile in rats receiving the choleretic collection 'Safroart herbal tea' was increased by 26 % and amounted to 0.53±0.03 ml (*P*<0.05) compared to the control of 0.42±0.03 ml.

The average increase of bile for 4 hours in rats receiving the medicinal collection 'Safroart herbal tea' was increased by 27.4 % compared to the control and was 2.14±0.09 ml (*P*<0.05) with the control 1.68±0.1 ml, indicating a reliable choleretic effect of the medicinal collection 'Safroart herbal tea'.

5. Discussion

The study confirmed that 'Safroart herbal tea' contains a complex of biologically relevant polysaccharides, including (WSPS), (PS), and (HMC). The fractions were successfully isolated through sequential aqueous and alkaline extraction methods, yielding 3.1 % (WSPS-x), 2.1 % (WSPS-g), 8.5 % (PS), and 8.0 % (HMC). Monosaccharide composition analysis showed that WSPS fractions were rich in glucose, galactose, and arabinose, while PS and HMC were characterized by higher proportions of rhamnose, xylose, and arabinose, with PS also containing significant amounts of galacturonic acid. The high degree of esterification in PS (61 %) indicated that it belongs to the group of highly esterified pectins, a class known for its functional and biological activity.

IR spectroscopy supported the structural identification of the isolated fractions, revealing characteristic absorption bands for hydroxyl (-OH), carboxyl (-COOH and $COOCH_3$), and glycosidic linkages. The presence of both α- and β-glycosidic bonds in PS and HMC was confirmed, and the pyranose ring structures typical of plant polysaccharides were evident. The viscosity data further differentiated the fractions, with PS exhibiting the highest viscosity, correlating with its complex branching and high molecular weight. These findings confirm that the polysaccharide components of 'Safroart herbal tea' possess well-defined structural features and suggest their potential contribution to the formulation's observed choleretic activity and its future pharmacological applications.

The results of the conducted study demonstrate that the 10 % aqueous infusion of the medicinal herbal blend "Safroart Herbal tea" does not exhibit toxic effects even when administered at high doses. The findings on the acute toxicity of the concentrated infusion indicate that, even at a dose of 4000 mg/kg in mice, the survival rate remained 100 % with no observed pathological changes. According to international toxicological standards, these results classify "Safroart Herbal tea" in the toxicity class V – substances with practically no toxic effects. These findings are consistent with previous studies on other medicinal plant-based phytopreparations. For instance, existing data from Uzbek and international sources indicate that several herbal extracts used in medicinal formulations exhibit similarly low toxicity levels [14].

The results of this study demonstrate that 'Safroart herbal tea' exerts a pronounced choleretic effect when administered orally as a 10 % aqueous infusion. A statistically significant increase in bile secretion was observed in the experimental group compared to the control, particularly within the first hour of administration, indicating a rapid onset of action. Over the 4-hour observation period, cumulative bile output in the treated group increased by 27.4 %, confirming the functional efficacy of the formulation.

The enhanced bile flow can be attributed to the synergistic action of bioactive constituents present in the individual components of the herbal blend. *Cynara scolymus* is known for its cynarin and sesquiterpene lactones content, which have documented hepatobiliary stimulatory properties. *Calendula officinalis* and *Achillea millefolium* possess flavonoids and triterpenoids with choleretic and anti-inflammatory effects. *Mentha piperita* contributes menthol and essential oils that support bile flow and smooth muscle relaxation within the biliary tract.

The observed increase in bile secretion supports previous pharmacological findings on individual plant components and validates the hypothesis that a polyherbal approach can yield additive or synergistic therapeutic effects. This is particularly relevant for phytopreparations intended for the management of hepatobiliary disorders such as cholestasis, biliary dyskinesia, and sluggish bile flow associated with digestive dysfunction.

Importantly, the experimental animals tolerated the infusion well, with no adverse effects observed

during the 4-hour test period, which aligns with the findings from the acute toxicity study confirming the safety profile of the formulation.

While these findings are promising, it is important to note that only acute choleretic activity was assessed. Long-term studies evaluating chronic administration, effects on liver enzymes, histopathological changes in hepatic tissue, and possible interactions with conventional hepatoprotective drugs would provide a more comprehensive understanding of the clinical potential of 'Safroart herbal tea'.

Practical relevance. Regarding practical application, several points should be emphasized. The high safety profile of "Safroart Herbal tea" allows it to be used in pharmaceutical and clinical practice. Specifically, due to its choleretic properties, this herbal tea may be beneficial in treating liver and biliary tract disorders, particularly cholestasis and hepatobiliary dysfunction. Furthermore, its low toxicity profile suggests that this preparation can be used for extended periods, giving it a potential advantage over other herbal choleretic agents available in the international pharmaceutical market.

Limitations of the study. This research focused only on acute toxicity and bile secretion effects. However, the long-term toxicological safety and potential effects on other physiological systems have not yet been fully explored. Additionally, further research is needed to determine the efficacy of the herbal blend in different clinical settings.

Prospects for further research. Several aspects should be considered for future studies:

1. Long-term toxicity and pharmacokinetic studies – investigation of the effects of prolonged use of the preparation, including metabolism and elimination mechanisms.

2. Clinical trials – conducting clinical studies to assess the efficacy of "Safroart Herbal tea" in patients.

3. Pharmacological combinations – evaluating the effectiveness of the preparation when used in combination with other medicinal agents.

4. International comparisons – comparing the effects and advantages of "Safroart Herbal tea" with other choleretic preparations available in different countries.

This study has demonstrated that the "Safroart Herbal tea" herbal blend is non-toxic and possesses choleretic activity. Further in-depth studies will help establish the scientific basis for its practical application and widespread use in medical practice.

6. Conclusions

The study of the carbohydrate composition of the choleretic collection 'Safroart herbal tea' led to the successful isolation of three main polysaccharide fractions: water-soluble polysaccharides (WSPS), pectin substances (PS), and hemicelluloses (HMC).

The yields of the fractions were as follows: WSPS-x – 3.1 %, WSPS-g – 2.1 %, PS – 8.5 %, and HMC – 8.0 %. The monosaccharide composition of WSPS was dominated by glucose, galactose, and arabinose, while PS and HMC were rich in rhamnose, arabinose, and xylose. Additionally, PS showed a high content of galacturonic acid and a DE greater than 58 %, classifying them as highly esterified pectins.

WSPS and PS are amorphous powders, cream-coloured with a yellowish tint. In hydrolysates of WSPS, PS and hemicellulose, along with neutral monosaccharides and galacturonic acid, were present.

When determining the main functional groups of pectins, it was found that the isolated PS is characterized by the content of free carboxyl groups, methoxylated carboxyl groups, the total amount of carboxyl groups, methoxyl groups, and a high (λ>50 %) DE.

The monomeric composition of PS was characterized by a high content of galacturonic acid, along with the presence of neutral sugars, predominantly arabinose, xylose, and galactose. The DE of the PS was determined by titrimetric analysis, revealing that the PS isolated from the 'Safroart herbal tea' collection belongs to the group of highly esterified pectins.

In WSPS, the main monosaccharides are glucose, galactose, and arabinose, and in PS and hemicellulose, there are rhamnose, arabinose, and xylose.

The study of the acute toxicity of the medicinal collection 'Safroart herbal tea' showed that a single intragastric administration of 1:10 aqueous infusion in doses of 2000 mg/kg, 3000 mg/kg, and 4000 mg/kg did not cause the death of mice, as well as visible changes in the behaviour and functional state of animals.

The obtained data show that the investigated choleretic collection, 'Safroart herbal tea,' at a single administration of aqueous infusion to white rats, has a reliable choleretic effect.

The study findings confirm that 'Safroart herbal tea' is a practically non-toxic medicinal herbal infusion with significant choleretic properties. These results support its potential use as a natural hepatoprotective agent and warrant further investigation into its clinical applications. Expanding research on this herbal collection could lead to its development as a standardized, evidence-based phytomedicine for liver and gallbladder health.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data availability

The manuscript has no associated data.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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Khairulla Olimov, PhD, Associate Professor, Head of Department, Department of Pharmaceutical Chemistry, Tashkent Pharmaceutical Institute, Oybek str.,45, Tashkent, Uzbekistan, 100015

Tanzila Mirrakhimova, Doctor of Pharmaceutical Sciences, Associate Professor**,** Tashkent Pharmaceutical Institute, Oybek str.,45, Tashkent, Uzbekistan, 100015

Gulrano Akhmadova*, PhD**,** Tashkent Pharmaceutical Institute**,** Oybek str.,45, Tashkent, Uzbekistan, 100015
E-mail: bmg919218@gmail.com

***Corresponding author:** Gulrano Akhmadova, e-mail: bmg919218@gmail.com*