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Research Article

Optimizing Extractability, Phytochemistry, Acute Toxicity, and Hemostatic Action of Corn Silk Liquid Extract

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The technological parameters and quality indicators of corn silk were evaluated in this study: specific density, volumetric density, bulk density, porosity, spatial layer, free volume of the layer, the absorption coefficient, weight loss on drying, and extractives. The technology for obtaining a liquid extract of corn silk was developed. The most effective extractant was discovered to be 40% ethanol, with an extraction time of 120 minutes. The qualitative composition and quantitative content of the major groups of biologically active substances (BAS) in the obtained liquid extract were determined. The qualitative composition of the main groups of BAS was determined by conventional chemical reactions. This extract contained free reduced sugars, glycosides (bound reduced sugars), phenols, tannins, flavonoids, saponins, and hydroxycinnamic acids. The quantitative content of phenolic compounds was performed by UV-vis-spectrophotometry. Total phenols, tannins, flavonoids, and hydroxycinnamic acids had quantitative contents of $8.25 \pm 0.33\%$, $1.4 \pm 0.03\%$, $2.20 \pm 0.06\%$, $3.30 \pm 0.13\%$, respectively. The acute toxicity study was carried out with a single intragastric administration to outbred unanesthetized white rats of both sexes. Duration of observation of animals was 14 days. It was revealed that corn silk extract at doses of up to 5.0 ml/kg is safe. A single injection of a liquid extract has no effect on internal organs when compared to a control group. Corn silk liquid extract's hemostatic efficacy was assessed using blood clotting time, prothrombin time, and blood clot retraction index. The corn silk liquid extract reduces blood coagulation time, decreases prothrombin time, and increases the blood clot retraction index. According to these findings, corn silk liquid extract is rich in phytochemicals and possesses a potential therapeutic effect on bleeding disorders. Furthermore, it could be used in the pharmaceutical sciences industry to develop medicines for testing in the treatment of various diseases.

1. Introduction

Bleeding (hemorrhagia) is the outflow of blood from damaged vessels into tissues, body cavities, and external environments [1–3].

The hemostasis system is responsible for halting the bleeding and ensuring that the aggregate state of blood in the body is ideal. The blood artery wall, blood cells (mainly platelets), and both enzymatic and non-enzymatic plasma systems are all part of the hemostasis system. Hemostasis has two mechanisms: vascular platelet and coagulation [1–4].

There is no single approach for the treatment of bleeding. This is due to the different etiology of their occurrence. A fairly common cause of bleeding is a violation of the integrity of the vascular wall that occurs as a result of trauma, vessel erosion in various purulent and pathological processes, increased blood pressure in the vessel, a sharp drop in atmospheric pressure, and increased vessel permeability [1].

Angiospasm is an essential component of the hemostatic system. The main result of its manifestations is the achievement of a persistent spasm of the damaged vessel, which limits blood loss. In addition to vascular spasm, the thrombogenic potential of the vascular wall includes its ability to produce and exhibit, when damaged, molecular activators of platelet adhesion and aggregation, as well as stimulators of fibrin formation. The vascular link of hemostasis depends on the state of the blood vessel [1].

In addition to vascular wall defects, the presence of bleeding, hemorrhage, and a tendency to recur, indicates a decrease in the ability of blood to coagulate—hypocoagulation. The main causes of hypocoagulation are genetic or acquired deficits and/or defects of hemostasis factors [1–3].

Bleeding associated with disorders of the plasma-coagulation link of hemostasis is characterized by hemorrhages in the cavity and soft tissues. Hemorrhages of the mucous membranes are common with defects in the vascular-platelet connection, including nasal, gastrointestinal, uterine hemorrhages, intradermal ecchymosis, and petechiae [1].

Bleeding can be a manifestation of self-bleeding diseases such as hemophilia A and B, Willebrand's disease; complication of local vascular tissue damage during infection, inflammation; a complication of another pathology such as cholemic bleeding in patients with jaundice, spontaneous nasal, skin, muscle bleeding during surgery and in the postoperative period. Uterine hemorrhage can also develop as a result of hypotension and atony of the muscles of the uterus. Microcirculation problems cause increased bleeding in people with diabetes. Scurvy causes gum bleeding owing to a lack of vitamin C. Patients suffering from osteoporosis due to an acute calcium deficiency, which is one of the components in blood coagulation, have more bleeding [1, 3, 5].

Based on the above, bleeding refers to both emergencies and systemic diseases. Accordingly, the treatment of bleeding of different etiology requires a different approach.

Hemostatic agents of plant origin, as a rule, accelerate blood clotting and have a moderate hemostatic effect. They are prescribed internally to reduce bleeding in hemorrhagic diathesis, hemorrhoids, nasal, pulmonary, renal, uterine and intestinal bleeding, externally for bleeding gums, after surgical treatment of wounds, etc [4–6].

Bleeding herbal medicine is a set of treatments aimed at eliminating the clinical manifestations and causes of bleeding, as well as preventing and eliminating complications [5, 6].

There is corn among such medicinal plants. Corn is also called maize, with its botanical name–*Zea mays* L. Corn belongs to the cereal family *Poaceae* [7, 8].

Corn plant raw material is a corn silk (*Zeae maydis styli cum stigmatis*). Corn silk is made up of soft silky threads that are gathered in bunches or partially tangled, with bilobed stigmas at the top. Silk is curved and flat, measuring 0.1–0.15 mm in width and 0.5–20.0 cm in length, with stigmas measuring 0.4–3.0 mm in length. Corn silk comes in a variety of colors, including brown, brown-red, and light yellow [8–10].

The most studied BAS in corn silk are phenolic compounds. The presence of tannin and tannin-like polyphenols (approximately 13%) has been established: anthocyanidins and flavan-4-ols luteoforol, apiforol [8, 11]. Furthermore, studies on flavonoids such as luteolin and apigenin glycosides such as orientin, vitexin, isovitexin, homoorientin, and maisin have been conducted [8, 11, 12]. Rutin and formononetin are also present [8, 11, 13]. Corn silk was reported to contain chlorogenic, ferulic, and caffeic acids [9].

According to previous researches, corn silk contains up to 80% carbohydrates, 8% fiber, 3.8% potassium-rich mucus, 10% protein, 3.5% lipophilic substances, 2.5% fatty oil, 3.18% saponins, and sterols (β-sitosterol and stigmasterol) [7–11, 14].

Corn silk is used in traditional medicine as a choleretic, diuretic, and hemostatic agent, as well as to suppress appetite and normalize lipid metabolism [8, 9, 14, 15]. Corn silk is also used in official medicine for medicinal purposes. Corn silk preparations have choleretic, diuretic, anti-inflammatory, hemostatic, and hypoglycemic properties and are used for hypertension prevention and treatment, as well as weight loss [7–9, 14, 16]. Corn silk has been shown to have antioxidant and antibacterial properties [7, 8, 17, 18].

Corn is an agricultural plant. According to the Food and Agriculture Organization of the United Nations (FAO), corn seeds rank seventh among the most widely produced raw materials in the world. According to FAO, the total global corn seeds production in 2018 was 1,147 million 155 tons, with the American continent accounting for slightly more than half of the total (50.4%), followed by Asia (31.5%), Europe (11.2%), Africa (6.9%), and Oceania (0.1%). The United States of America, China, Brazil, *Argentina*, Ukraine, Indonesia, India, Mexico, Romania, and Canada were the top ten maize producers in the world [3] Jordan's corn production is annual production is under 10,000 metric tons. Domestic corn production is largely for human consumption [19].

The development of highly effective and safe medicines based on medicinal herbs with hemostatic action is still extremely important and relevant to humanity. Modern conditions for the development of new pharmaceuticals in the context of the pharmaceutical industry's evolution necessitate not only their efficacy and safety, but also the verification of their quality, economic, social, and medical forecasting of the need for their development.

As a result of the preceding, the current study aimed to determine the technological parameters and quality indicators of corn silk, optimize extractability for obtaining of a liquid extract from corn silk, its' qualitative composition and quantitative content of the major groups of BAS, investigation of its acute toxicity and hemostatic action.

2. Materials and Methods

2.1. Plant Material. Corn silk was purchased at a local market in Amman, Jordan during June and August. The plant material was authenticated by Dr. Khaled Abulaila (Director of Biodiversity Research Department, National Agricultural Research Center, Amman, Jordan). The raw

components were rinsed twice using tap water. They were dried in an air-shadowed environment. The plant sample was ground into a fine powder (3,0 mm) and stored in airtight vials until usage.

2.2. Standards and Chemicals. All chemicals were analytical-reagent grade and the water was distilled. The chemicals included: cholesterol solution (Sigma-Aldrich), vanillin (Sigma-Aldrich), sulfuric acid, chloroform, ferric chloride (Sigma-Aldrich), lead acetate (Sigma-Aldrich), sodium hydroxide (Sigma-Aldrich), iron ammonium alum (Sigma-Aldrich), gelatin, magnesium shavings, hydrochloric acid, aluminum chloride (Sigma-Aldrich), Fehling's reagent, nitrite-molybdenum reagent (Sigma-Aldrich), Folin-Ciocalteu reagent (Sigma-Aldrich), anhydrous sodium carbonate (Synth®), pyrogallol (Fluka), luteolin 7-glucoside (Sigma-Aldrich), and chlorogenic acid (Fluka).

2.3. Technological Parameters and Quality Indicators of Raw Materials. The technological parameters and quality indicators of corn silk of plant raw materials were determined using commonly established methods [20, 21], including: specific density, volumetric density, bulk density, porosity, spatial layer, free volume of the layer, the absorption coefficient, weight loss on drying, and extractives.

2.3.1. Specific Density. A 100 ml pycnometer was filled at 2/3 volume with distilled water and weighed. Then about 5.0 g of the powdered raw material was placed into a dry empty 100 ml pycnometer, distilled water was added at 2/3 volume, and weighted. A pycnometer with the sample was heated in a water bath for 1.5–2 h, occasionally stirring to completely remove from the plant raw material air. Then cooled to a temperature of 20°C, the volume was distilled to the mark with water, and weighted. A specific density (d_n, (g/cm^3)) was calculated according to the formula:

$$d_n = \frac{P \times d_p}{P + G - F}, \tag{1}$$

where P–the mass of the plant raw material, (g); G–the mass of the pycnometer with distilled water, (g); F–the mass of the pycnometer with distilled water and the plant raw materials, (g); d_p–the specific density of water, (g/cm^3), ($d = 0{,}9982$ Г/см^3).

2.3.2. Volumetric Density. About 5.0 g of the powdered raw material was quickly placed into a measuring cylinder (50 ml) with 25 ml of distilled water. The volume of the plant raw material was measured. The volume occupied by the raw material was measured as the difference between measuring a cylinder with distilled water and the raw material and a cylinder with distilled water. A volumetric density (d_0, (g/cm^3)) was calculated according to the formula:

$$d_0 = \frac{P_o}{V_o}, \tag{2}$$

where P_o–the mass of the plant raw material, g; V_o–the volume occupied by the plant raw materials, (cm^3).

2.3.3. Bulk Density. About 5.0 g of the powdered raw material was placed in the measuring cylinder, shaking slightly for alignment. The volume of the plant raw material was measured. A bulk density (d, (g/cm^3)) was calculated according to the formula:

The bulk mass was calculated according to the formula:

$$d = \frac{P_n}{V_n}, \tag{3}$$

where P_n–the mass of the plant raw material, (g); V_n–the volume, which takes the plant raw material, (cm^3).

2.3.4. Porosity. The porosity (P_s) of the plant raw material is the size of the cavities within the cellular tissue. It's calculated as the ratio of the difference between the specific density and the volumetric density to the specific density. The formula is:

$$P_s = \frac{d_n - d_o}{d_n}, \tag{4}$$

where d_n–the specific density of the plant raw material, (g/cm^3); d_o–the volumetric density of the plant raw material, (g/cm^3).

2.3.5. Spatial Layer. The spatial layer (P) characterizes the size of the cavity between parts of powdered plant raw material. It's calculated as the ratio of the difference between volumetric and bulk density to the bulk density. The formula is given as follows:

$$P = \frac{d_o - d}{d}, \tag{5}$$

where d_o–the volumetric density of the plant raw materials, (g/cm^3); d–the bulk density of the plant raw materials, (g/cm^3).

2.3.6. Free Volume of the Layer. The free volume of the layer is calculated as the ratio of the difference between the specific density and bulk density to the specific density. The formula is given as follows:

$$V = \frac{d_n - d}{d_n}, \tag{6}$$

where d_n–the specific density of the plant raw materials, (g/cm^3), d–the bulk density of the plant raw materials, (g/cm^3).

2.3.7. Absorption Coefficient. The absorption coefficient is the amount of solvent that irreversibly permeates air cavities, intracellular pores, and vacuoles in the plant herbal drug. It's calculated as the ratio of the plant raw material mass after percolation with an extractant and squeezing to the plant raw material mass before percolation. Water was used as a

Table 1: Results of corn silk technological parameters and quality indicators determination.

Technological parameters and quality indicators	Results
Specific density, (g/см^3)	1.53 ± 0.07
Volumetric density, (g/см^3)	0.46 ± 0.02
Bulk density, (g/см^3)	0.30 ± 0.01
Porosity	0.69 ± 0.02
Spatial layer	0.34 ± 0.01
Free volume of the layer	0.80 ± 0.03
Absorption coefficient	
Water	5.00 ± 0.25
Ethanol 40%	4.30 ± 0.17
Weight loss on drying, (%)	12.0 ± 0.54
Extractives:	
Water	23.88 ± 0.47
Ethanol 40%	24.70 ± 0.64
Ethanol 50%	23.45 ± 0.40
Ethanol 70%	18.95 ± 0.70
Ethanol 96%	19.38 ± 0.40

common extractant. 40% ethanol solution was used as the most suitable for obtaining of corn silk extract according to the extractives results (Table 1). An absorption coefficient (K) was calculated according to the formula:

$$K = \frac{P_2}{P_1}, \tag{7}$$

where P_1– the mass of the plant raw material before percolation, (g); P_2– the mass of the plant raw material after percolation, (g).

The determination of weight loss on drying and extractives was performed according to the method described in the SPhU (2.2.32) [20, 21].

2.3.8. Loss on Drying. Loss on drying is a widely used test method to determine the residual moisture and any volatile matter content of a plant raw material sample. Loss on drying is the difference in the mass of the sample before and after drying, expressed as a percentage. Flat-bottomed empty weighted dish with a lid was put in the drying cabinet, at 100–105°C for 30 min. Cooled to a temperature of 20°C in a desiccator, weighted and then brought to a constant mass. In a flat-bottomed dish, 1.0 g of the powdered herbal drug was added, dried in drying cabinet at 100–105°C for 30 min. Cooled to a temperature of 20°C in a desiccator, weighted, and then brought to a constant mass. Sample mass is the difference between a mass flat-bottomed dish with a sample and an empty flat-bottomed dish. The total number of measurements was $n = 5$.

2.3.9. Extractives. The distilled water and ethanol solutions (40%–96%) were used as extractants for the determination of extractives (the solvents indicated in Table 1). To 1.0 g of the powdered plant raw material were placed in a flask with 200–250 ml, 50.0 ml of the solvent were added, then heated for 30 min from the moment the solvent boils. Cooled to a temperature of 20°C, diluted to 50.0 ml with a suitable solvent, and filtered. 20.0 ml of the filtrate were dried on a water bath, then the residue was dried in the drying cabinet, at 100–105°C, then weight. The total number of measurements was $n = 5$.

The content of extractives in percentages (X, (%)), calculated on the basis of absolutely dry raw materials, was calculated by the formula:

$$X = \frac{m \times 200 \times 100}{m_1 \times (100 - W)}, \tag{8}$$

where m–the mass of the dry residue, (g). m_1– the mass of the plant raw materials, (g). W–the weight loss on drying, (%).

2.4. Optimizing Extractability of a Corn Silk Liquid Extract (CSLE). The extractant and time for extraction were selected by an experiment based on the yield of extractive compounds (Table 1), cost, and environmental safety.

The raw material-extractant ratio was 1 : 1. A liquid extract is a liquid medicinal form in which one part by weight or volume is equivalent to one part by weight of the dried medicinal plant raw material.

Then the extraction time was selected according to the extractives amount also. 100 g of dried corn silk was grounded, loaded into the extractor, and filled with 40% ethanol (according to the result of extractives determination Table 1) at the ratio of raw material-extractant 1 : 1, taking into account the absorption coefficient of the extractant. Then heated during 15 min, 30 min, 45 min, 60 min, 90 min, 120 min, 180 min, and 240 min from the moment the solvent boils (80–90°C). The extract was cooled to a temperature of 20°C, filtered, and squizzed. 20.0 ml of the filtrate were dried on a water bath, then the residue was dried in the drying cabinet, at 100–105°C, then weight. The content of extractives (%), calculated on the basis of absolutely dry raw materials, was calculated by formula (8). The total number of measurements taken was $n = 5$ [21].

2.5. Phytochemical Investigation of CSLE. The qualitative compositions of the main groups of BAS were determined for the obtained extract by chemical reactions:

(1) *Saponins* [22–26]:

Foam test: 5.0 ml of distilled water was combined with around 0.2 ml of the CSLE. It was violently shacked for five minutes. Foam persistence is used as a saponins indicator.

Salkowski test: 2.0 ml of CSLE were combined with 1.0 ml of chloroform and 5-6- drops of concentrated sulfuric acid. The red ring that appeared between two layers indicates the presence of saponins.

Reaction with 1% alcohol cholesterol solution: 2.0 ml of CSLE were combined with 1.0 ml of 1% cholesterol alcoholic solution. The precipitate indicates the presence of saponins.

Vanillin-sulphate reaction: 2.0 ml of CSLE were combined with 1.0 ml of 0.5% alcohol vanillin

solution, 3-4 drops of concentrated sulfuric acid and heat in water bath at a temperature of 60°C. The red color indicates the presence of saponins.

(2) Phenolic compounds [25, 26]:

Ferric chloride test: 1.0 ml of 3% ferric chloride solution was mixed with 1.0 ml of CSLE. The presence of phenolic compounds is indicated by a greenish-black or a bluish-black color solution.
Reaction with lead acetate solution: the formation of a precipitate following adding 0.5 ml of 1% lead acetate solution to 10.0 ml of CSLE shows the presence of phenolic chemicals.
Reaction with sodium hydroxide solution: 1.0 ml of the CSLE add 1-2 drops of 10% alcohol-aqueous solution of sodium hydroxide. Intense yellow color indicating the presence of phenols.

(3) *Tannins* [25–27]:

Reaction with iron ammonium alum solution: 2.0 ml of analyzed extract were mixed with 4-5 drops of 1% iron ammonium alum solution. The presence of tannins is indicated by a greenish-black (condensed tannins) or a bluish-black (hydrolyzed tannins) color solution.
Gelatin test: a few drops of gelatine solution was added to 2.0 ml of the CSLE. Cloudiness is formed, and disappears after the addition of gelatine surplus. It indicates the presence of tannins.

(4) *Flavonoids* [25, 26]:

Cyanidin test (Shinoda's test): 10.0 ml of CSLE was handled with magnesium ribbons, and a few drops of concentrated hydrochloric acid were added. Magenta coloration is a sign of the presence of flavonoids.
Reaction with aluminum chloride solution: 2.0 ml of CSLE were combined with 2.0 ml of 3% aluminum chloride solution. The lemon yellow color indicates the presence of flavonoids.
Reaction with vanillin in concentrated hydrochloric acid: few drops of 1% solution of vanillin in concentrated hydrochloric acid were added to 1.0 ml of CLSE. Bright red color indicates the presence of flavonoids.

(5) Free and bound reducing sugars [26, 27]:

Fehling's test before and after hydrolysis: Fehling A and Fehling B reagents were combined in equal proportions and 2.0 ml of this mixture was added to the CSLE. Then gently boiled. The same reaction was done after hydrolysis of CLSE. A brick red precipitate indicated the presence of reducing sugars.

(6) Hydroxycinnamic acids [28]:

Reaction with nitrite-molybdenum reagent: 1,0 ml of CSLE was mixed with 2.0 ml of 0.5 M hydrochloric acid, 2,0 ml of a mixture of 10% sodium molybdate solution and 10% sodium nitrite solution, 2.0 ml of diluted sodium hydroxide solution in the specified order. A brick red color indicated the presence of hydroxycinnamic acids.

2.6. Determination of Total Flavonoid Content in CSLE. Spectrophotometry was used to determine the quantitative amount of flavonoids in liquid extract, according to the SPhU 2.0 Vol.3 monograph "Matricariae flos^{N}" in terms of luteolin-7-glucoside, wavelength 410 nm [29]. Spectrophotometer Shimadzu UV-1900 (Japan) was used for the experiment.

2.7. Determination of Total Tannin Content in CSLE. The quantitative content of tannins was determined using the spectrophotometry method indicated in section 2.8.14 of the SPhU 2.0 Vol. 1 in terms of pyrogallol and dry raw materials, wavelength 760 nm [30]. Spectrophotometer Shimadzu UV-1900 (Japan) was used for the experiment.

2.8. Determination of Total Phenolic Compounds Content in CLSE. The content of polyphenolic compounds was determined using the Folin–Ciocalteu method and spectrophotometry method (Shimadzu UV-1900 (Japan)), in terms of pyrogallol and dry raw materials, wavelength of 765 nm [24, 31].

2.9. Determination of Total Hydroxycinnamic Acids Content in CSLE. The studies of the quantitative content of hydroxycinnamic acids were carried out in accordance with the methodology given in the SPhU 2.0 Vol.3 in the monograph "Urticae folium," in terms of chlorogenic acid, wavelength 330 nm [30]. The spectrophotometry method was used. The spectrophotometer was Shimadzu UV-1900 (Japan).

2.10. Pharmacological Studies of CSLE

2.10.1. Experimental Animals. Pharmacological studies of CSLE were conducted on mature white (nonlinear) Wister albino rats. The rats were obtained from the vivarium at Isra University's Faculty of Pharmacy in Amman, Jordan. The protocol (SREC/22/09/053) of the study was approved by the Ethical Committee of the Faculty of Pharmacy, Isra University. Rats of both sexes were kept on a standard diet in a vivarium at a temperature of 22-23°C, with free access to food and water, according to the "day-night" retention regimen. All animals were kept in plastic cages with bedding as required [32]; animals of each sex were kept separately. Before the start of each experiment, the animals were transferred to the laboratory, where they were quarantined for 14 days under similar conditions [32].

The experiments were conducted in accordance with the principles outlined in the Convention for the Protection of Vertebrate Animals, which was used in the experiment [32].

2.10.2. Acute Toxicity Study. The acute toxicity study of the liquid extract was carried out with a single intragastric administration in dosage of 2.0–10.0 ml/kg to outbred unanesthetized 32 white rats of both sexes with an initial average weight of 162.2 ± 4.2 g. Duration of observation of animals - 14 days.

The registration of external signs of damage, disorders of autonomic functions, reactions to external stimuli, and the ability to consume food and water were carried out by visual inspection. To exclude possible sexual sensitivity to drugs, the analysis of the results was performed separately for females and males.

The criterion points for determining the toxic effect of liquid extract were dead animals, that registered for 14 days [33]. Animals of different groups were administered the appropriate doses of liquid extract recommended by this method. The toxic effect of the extract was assessed by the mean lethal dose of LD_{50}. The animals had free access to food and water 30 minutes after drug administration. Mass and body temperature were measured at 3, 7 and 14 days; the reaction of animals to external stimuli was evaluated at the same time. At the end of this period, the euthanasia of surviving animals was performed, and a macroscopic examination of the internal organs was performed, and their mass coefficients were determined [34].

2.10.3. Investigation of the Liquid Extract's Specific Hemostatic Activity in terms of Blood Coagulation. The trials were conducted on sexually mature white rats of both sexes (21 individuals). Random sampling was used to divide the animals into three groups: the first group (8 rats) received intact animals (control); the second group (8 rats) received a corn silk liquid extract orally at a dose of 1 ml/kg once a day for 5 days; and the third group (5 rats) received the reference preparation—water pepper liquid extract (WPLE), orally at a dose of 1 ml/kg once a day for 5 days.

It's important to compare the pharmacological action of the new extract and existing plant medicine with proven effectiveness. Water pepper extract is registered as a medicine in the State Register of Medicinal Products of Ukraine with a hemostatic activity which is produced by PJSC Pharmaceutical Factory "Viola" (Ukraine). Therefore, WPLE was chosen as a comparison medicine for CSLE-specific hemostatic activity investigation.

The blood clotting time and prothrombin time (PT) terms were used to evaluate the hemostatic activity. The blood clotting time of the studied extract on the process of blood coagulation was studied by the appearance of fibrin threads in the second drop of blood from the tail vein of rats. The drops of blood in free fall fell on the spherical (temporary) glass. The temperature in the laboratory was 22–25°C.

A stopwatch was used to record the time required to draw a drop of blood. Every 20–30 seconds, a thin glass rod touched a drop of blood from its center to its perimeter until the first fibrin strands emerged. The start of blood clotting was recorded at this time.

The stopwatch stopped once a blood clot had formed. The start of blood coagulation (the development of fibrin strands, sec) and the completion of blood coagulation (the formation of a clot, sec) was thus determined [35].

The prothrombin time was calculated using the Owren method. Noncontact blood was drawn from rats and collected in vials containing EDTA before being centrifuged at 13,000 rpm for 10 minutes at 4°C. For 2 minutes, the clear platelet-free plasma was placed in a 37°C water bath. Each sample received 100l (100 g/ml) suspended in 3% v/v Tween 85, which was mixed with 1 ml of plasma. Coagulation time was measured three times. The vehicle served as the control.

The retraction of the blood clot in the same rats which were injected with the examined preparations (CSLE and WPLE) into the stomach for 5 days, including the rats in the control group, was also evaluated. In the process of euthanasia of sedated (urethane, 1.5 mg/kg, intraperitoneally) rats, mixed blood in a volume of 3 ml was taken from each rat and placed into measured graduated tubes. All tubes were left at room temperature for 24 hours. The degree of peeling of the blood clot and the volume of serum was assessed a day later. The retraction index was determined by dividing the volume of serum separated by the total volume of blood drawn for the trial (3 ml) [36].

2.11. Statistical Analysis. The results were mean ± SD of three parallel measurements. All statistical comparisons and reliability were made by Student's criterion to determine the standard deviation with a level of significance of 95%.

Statistical analysis of the results obtained was carried out by the method of the smallest squares according to the SPhU monograph "5. 3. N. 1. Statistical analysis of the results of the chemical experiment" (2015). GraphPad Prism software, version 8, was used to evaluate significant differences between experimental groups using one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. A $p < 0.05$ difference was considered statistically significant.

3. Results and Discussion

3.1. Technological Parameters and Quality Indicators of Raw Materials. Table 1 shows the technological parameters and quality indicators of corn silk (3,00 mm): specific density, volumetric density, bulk density, porosity, spatial layer, free volume of the layer, the absorption coefficient, weight loss on drying, and extractives. These indicators were assessed based on the development of high-quality raw materials, the effectiveness, safety, reproducibility of the imposed collection, and the maximum yield of extractive and active substances [37].

Furthermore, as shown in Table 1, 40% ethanol is the most effective extractant, with a yield of 24,70 ± 0,64% when compared to ethanol in various degrees and water. On the other hand, it is inexpensive and environmentally safe.

3.2. Optimizing Extractability of the CSLE. The best extraction time for obtaining of CSLE was determined experimentally based on the amount of extractives obtained.

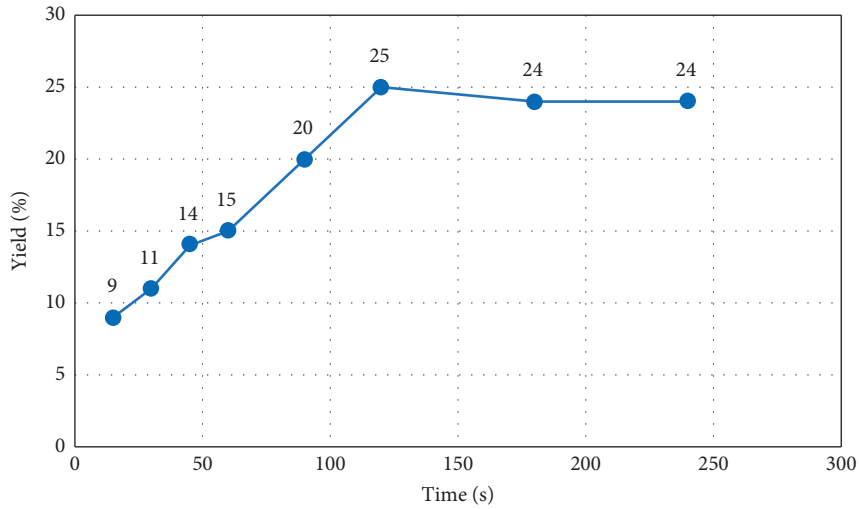

Figure 1: Optimization of the extractability of the CSLE, (%).

Table 2: Preliminary phytochemical screening of corn silk liquid extract.

Test	Free of reduced sugars	Glycosides (bound reduced sugars)	The sum of phenols	Tannins	Flavonoids	Saponins	Hydroxycinnamic acids
Qualitative determination	+	+	+	+	+	+	+
Quantitative content, (%)	–	–	8.25 ± 0.33 (In terms of *p* pyrogallol)	1.4 ± 0.03 (In terms of *p* pyrogallol)	2.20 ± 0.06 (In terms of luteolin 7-glucoside)	–	3.30 ± 0.13 (In terms of chlorogenic acid)

+ = present. – = the indicator is not defined. the quantitative content of saponins and sugars was not determined. data are presented as mean ± standard deviation ($n = 3$).

Figure 1 shows the percentage of extractive substances yield that was achieved depending on time extraction (min).

According to Figure 1, the maximum amount of extractive substances could be extracted in 120 minutes using the selected extractant. Furthermore, after 120 minutes of extraction, the yield was 25,30 ± 0,64%. Meanwhile, there were no significant changes in yield as the extraction time was increased. As a result, the determined extraction time is optimal for this type of raw material.

3.3. Phytochemical Investigation of CSLE. Table 2 depicts the qualitative and quantitative content of BAS of CSLE. Several chemical tests were carried out to detect the presence of BAS. The findings of this research confirmed the presence of free reduced sugars, glycosides (bound reduced sugars), phenols, tannins, flavonoids, saponins, and hydroxycinnamic acids in the CSLE. Based on the availability of the BAS, total phenols, tannins, flavonoids and hydroxycinnamic acids were 8.25 ± 0.33%, 1.4 ± 0.03%, 2.20 ± 0.06%, 3.30 ± 0.13% respectively.

The presence of numerous active substances in the CSLE demonstrated potential health benefits such as antioxidant properties against a variety of chronic and age-related diseases such as diabetes, hypertension, cancer, inflammation, hyperlipidemia, depression, hepatic and cardiovascular diseases [8, 38–40].

3.4. Pharmacological Studies of CSLE

3.4.1. Acute Toxicity Study. The acute toxicity of corn silk extract was investigated. Table 3 shows that a single intragastric administration of the liquid extract in a dose up to 5.0 ml/kg did not result in the death of rats, both females, and males. Physiological excess weight was observed in all groups of animals. White rats actively consumed food and water. They responded adequately to external stimuli an hour after administration. Their body temperature, social relationships, and behavioral responses were all normal [34]. Our findings showed that corn silk extract at a dose up to 5.0 ml/kg is safe.

An increase in the drug's experimental doses exacerbated the symptoms of poisoning, characterized by excitation of animals within 10 minutes of administration of corn silk extract, and a subsequent change in inhibition of motor activity, gradual loss of appetite, and decreased response to external stimuli. The deaths of rats (more animals) were mostly observed in the first two days following a single injection of the liquid extract [34].

The extract's average Lethal dose per os was almost the same for males (5.15 ml/kg) and females (5.64 ml/kg). The findings confirm the absence of sexual sensitivity to corn silk extract.

The animals were euthanized by an overdose of diethyl ether 14 days after receiving corn silk extract, and their cross-section was taken. Binocular dandruff was used for

Table 3: Study of acute toxicity of CSLE.

Route of administration	Sex	Dose, (ml/kg)	Weight of rats, (g) ($M \pm m$)		Result*	LD_{50} ml/kg
			Initial	At the end of the experiment		
Per os	Male	2.0	164.3 ± 5.4	174.0 ± 5.0	0/2	5.15
		2.5			0/2	
		3.1			0/2	
		3.9			0/2	
		5.0			1/2	
		6.3			2/2	
		7.9			2/2	
		10.0			0/2	
	Female	2.0	160.0 ± 4.2	170.4 ± 2.8	0/2	5.64
		2.5			0/2	
		3.1			0/2	
		3.9			0/2	
		5.0			0/2	
		6.3			2/2	
		7.9			2/2	
		10.0			2/2	

Note. * - the number of animals that died about the total number of rats in the group.

Table 4: Rat internal organ mass (g) following a single injection.

Treatment	Sex	Liver	Kidneys	Heart	Adrenal glands	Lungs	Testicles
Control (intact animals)	Male	7.80 ± 0.15	0.80 ± 0.06	0.86 ± 0.07	0.019 ± 0.002	0.77 ± 0.07	1.20 ± 0.12
	Female	7.95 ± 0.60	0.75 ± 0.08	0.80 ± 0.04	0.020 ± 0.002	0.85 ± 0.08	—
CSLE	Male	8.10 ± 0.07	0.77 ± 0.05	0.88 ± 0.07	0.021 ± 0.001	0,82 ± 0,06	9,50 ± 0,67
		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
		$p1 > 0.05$	$p1 > 0.05$	$p1 > 0.05$	$p1 > 0.05$	$p1 > 0.05$	$p1 > 0.05$
	Female	7.90 ± 0.07	0.80 ± 0.06	0.79 ± 0.05	0.021 ± 0.002	0.92 ± 0.07	
		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	—
		$p1 > 0.05$	$p1 > 0.05$	$p1 > 0.05$	$p1 > 0.055$	$p1 > 0.05$	

Note. data are expressed as mean ± SEM. CSLE - corn silk liquid extract; p - the level of significance of the difference when compared to intact animals; $p1$ - the level of consistency of the difference when compared to animals of the opposite sex.

Table 5: Effect of CSLE on blood clotting time in white rats.

Treatment	Start of blood coagulation, $M \pm m$, s	End of coagulation (blood clot formation), $M \pm m$, s
Control (intact animals), $n = 8$	62.1 ± 3.58	132.5 ± 5.58
CSLE, $n = 8$	33.1 ± 2.48*	81.3 ± 2.48*
WPLE, $n = 5$	33.0 ± 3.85*	92.0 ± 12.05*

Note. CSLE-corn silk liquid extract; WPLE-water paper liquid extract; Data are expressed as mean ± SEM; * = $p < 0.05$ significant difference in comparison to intact animals values.

macroscopic examination of the internal organs, and the mass of the internal organs was recorded. Table 4 displays the results.

According to the findings, a single injection of a liquid extract does not cause visible changes in the internal organs; they remain in their normal location and color, the lungs retain their specific structure, the intestines are not filled with air, and the size and mass of internal organs do not change significantly ($p > 0.05$). Thus, the obtained results confirm the absence of organotropism for corn silk extract [34].

3.4.2. Investigation of the Liquid Extract's Specific Hemostatic Activity in terms of Blood Coagulation. The presence of an effect on blood clotting time in white rats was demonstrated by a study of the specific hemostatic activity of corn silk liquid extract in terms of blood coagulation determining the beginning of blood clotting by the appearance of fibrin filaments and its ending by the formation of a blood clot (Table 5).

As a result, as shown in Table 5, the time of onset of blood clotting in white rats after the use of corn extract and the reference drug is reduced significantly ($p < 0.05$) by nearly 47% when compared to intact animals. Furthermore, the time of onset of blood clotting in white rats treated with CSLE is comparable to that of white rats treated with WPLE. Moreover, CSLE reduces the end of blood coagulation by 38.6%, while the reference preparation reduces it by 30.5%.

The coagulometric determination of blood clotting time and prothrombin time are shown in Table 6. Blood clotting

Table 6: The effect of CSLE on hemocoagulation parameters in rats.

Treatment	Blood clotting time, $M \pm m$, s	Prothrombin time, $M \pm m$, s	Retraction index
Control (intact animals), $n = 8$	138.9 ± 6.78	23.23 ± 1.58	0.40 ± 0.03
CSLE, $n = 8$	$89.1 \pm 2.48^*$	$13.9 \pm 1.48^*$	0.52 ± 0.04
WPLE, $n = 5$	$94.8 \pm 8.85^*$	$15.6 \pm 2.05^*$	0.49 ± 0.06

Note. CSLE-corn silk liquid extract; WPLE-water paper liquid extract; data are expressed as mean ± SEM; $^* = p < 0.05$ significant difference in comparison to intact animals values.

times for CSLE, WPLE and the control were 89.1 ± 2.48, 94.8 ± 8.85 and 138.9 ± 6.78 seconds, respectively. The prothrombin times for CSLE, WPLE and control were 13.9 ± 1.48, 15.6 ± 2.05 and 23.23 ± 1.58 seconds, respectively.

In addition Table 6 also shows that when white rats are given CSLE, their blood clotting rate is shorter by 35.9% and their prothrombin time is reduced by 40% when compared to the control group. Similarly, both CSLE and WPLE showed the same effect (Table 6).

The retraction index increased by 30% and 22.5% with the use of corn silk extract and liquid pepper water extract, respectively.

The results of this experiment demonstrated that CSLE contributes to hemostasis by shortening the time required for plasma-coagulation (PT) and blood clotting. BAS, particularly polyphenols (tannins, flavonoids) may play an important role in hemostasis by preventing bleeding from injured vessels through protein coagulation to form a vascular plug. Hemostasis is defined by spontaneous blood stoppage, which includes vascular spasms of ruptured vessels, platelet aggregation, and blood coagulation [35]. These findings imply that CSLE may cause vasoconstriction and reduce bleeding from injuries or wounds.

Blood clotting is a complex phenomenon that involves a cascade of reactions in addition to platelet aggregation and vasoconstriction. This process begins with prothrombin activation, followed by thrombin conversion, which converts fibrinogen to insoluble fibrin. CSLE reduces coagulation time and raises the possibility that the extract affects with the blood coagulation process [35]. These findings suggested that CSLE may compel the hemostasis effect via the coagulation pathway, resulting in a decrease in clotting time and vasoconstriction, both of which are required to reduce blood loss from injuries.

The current study's findings suggest that CSLE may have clinical implications as a coagulant in the treatment of various pathological states.

Thus, a five-fold preliminary administration of CSLE into the stomach of white rats results in a reduction in blood clotting time, a decrease in prothrombin time, and an increase in the blood clot retraction index. Changes in the studied parameters with the use of CSLE were comparable in the degree of manifestation to those observed with the introduction of the reference drug. The reduction in blood clotting time in white rats when using CSLE, as well as the comparison drug, could be due to an increase in prothrombinase formation.

4. Conclusions

Technological parameters and quality indicators of corn silk were studied: specific density, volumetric density, bulk density, porosity, spatial layer, free volume of the layer, the absorption coefficient, weight loss on drying, and extractives. These results could be used during the industrial production of corn silk medicines.

The technology of obtaining a corn silk liquid extract was developed. It was found that the most effective extractant, according to the extractives yield, is ethanol 40% and the time of extraction is 120 min.

Phytochemical investigation of liquid extract confirmed the presence of free reduced sugars, glycosides (bound reduced sugars), phenols, tannins, flavonoids, saponins, and hydroxycinnamic acids. The research of quantitative content allows us to draw a conclusion that phenolic compounds are a main BAS group of CSLE: total phenols −8.25 ± 0.33%, tannins −1.4 ± 0.03%, flavonoids −2.20 ± 0.06% and hydroxycinnamic acids −3.30 ± 0.13%.

An acute toxicity study showed that corn silk extract at a dose up to 5.0 ml/kg is safe. A single injection of a liquid extract does not cause visible changes in the internal organs. The obtained results confirm the absence of organotropism for CSLE.

The preliminary administration of CSLE to white rats results in a reduction in blood clotting time, a decrease in prothrombin time, and an increase in the blood clot retraction index.

According to the findings, corn silk liquid extract is high in phytochemicals and has therapeutic potential in bleeding disorders. Furthermore, it could be used in pharmaceutical sciences and industry to develop medicines for testing in the treatment of a variety of diseases.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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