RESEARCH PAPER

Identification and Determination of the Main Active Ingredient of Aspen Bark as a Functional Ingredient for Novel Phytocomplexes

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ABSTRACT

The article presents the results of the development of innovative functional forms of dietary supplements (DS), such as "Populin with Dihydroquercetin" and "Tremulacin," created based on aspen bark extract (Populus tremula). The study aimed to identify and quantify salicin as a key biologically active component ensuring the therapeutic efficacy of the products. The paper describes in detail the technological production scheme, including stages of extraction, standardization, pasteurization, and filtration to obtain a product with specified physicochemical parameters. The authors established strict regulated quality indicators, storage periods, and regimes, ensuring composition stability during industrial scaling. Comprehensive production testing was conducted, confirming the technological feasibility of implementing these supplements into serial production. Particular attention is paid to evaluating the clinical efficacy of the preparations in the therapy of inflammatory and viral pathologies, including infections caused by SARS-CoV-2. The data obtained indicate the high potential of aspen bark extract as a natural corrector of metabolic disorders. The scientific and practical significance of the work is confirmed by a Russian Federation patent for the invention. The research results substantiate the possibility of using these phytocomplexes as specialized products for systemic prevention and supportive therapy in nutrition science, making them a promising tool in modern evidence-based medicine.

Keywords: Aspen bark extract, Biologically active substances, Specialized products, Quality indicators, Salicin, Dihydroquercetin.

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INTRODUCTION

Aspen bark is characterized by a wide range of biologically active substances (BAS) with individual and, at the same time, synergistic properties, which determines its use in traditional and scientific medicine [1-3].

From the perspective of modern nutrition science, phenol glycosides, flavonoids, and polyphenolic compounds are of the greatest interest. The key phenolic glycoside is salicin. Among polyphenolic compounds, flavonoids find the greatest practical application [4-8]. Polyphenolic compounds are represented by tannins [9-17].

Taking into account their pharmacological properties, the BAS and their complexes discussed above are used as specialized products and active substances in the correction of various metabolic disorders [18-24].

MATERIALS AND METHODS

A concentrated aqueous extract of aspen bark (Extractum cortices Populi tremulae) with a solids content of at least 60% was used as the object of study. To enrich the phytocomplex, Lavitol, containing at least 88% dihydroquercetin, was introduced into the "Populin with Dihydroquercetin" supplement. The identification and quantification of active substances (salicin and flavonoids) were carried out using high-performance liquid chromatography (HPLC-UV) on analytical equipment allowing for the precise determination of the phenolic composition of the extracts. Physicochemical control was conducted according to the methodologies of TR CU 021/2011.

The clinical evaluation of the efficacy of aspen bark extract-based supplements ("Populin with Dihydroquercetin" and "Tremulacin") was carried out within a two-stage program, including experimental preclinical studies and multicenter clinical trials. This program was aimed at a comprehensive study of the pharmacological properties of the developed phytocomplexes and their influence on the key pathogenetic links of inflammatory and viral diseases, including the novel coronavirus infection COVID-19 (SARS-CoV-2) [25-35].

Program and Study Design. At the first (preclinical) stage, basic mechanisms of action were studied in vitro and in vivo on models of acute aseptic inflammation and virus-induced cytokine storm. It was established that the combination of salicin from aspen bark and dihydroquercetin synergistically

suppresses the activity of key pro-inflammatory mediators (tumor necrosis factor-alpha – TNF-α, interleukins IL-1β and IL-6) and inhibits cyclooxygenase-2 (COX-2). This potentiates an anti-inflammatory effect comparable to non-steroidal anti-inflammatory drugs (NSAIDs) but without a pronounced ulcerogenic effect. Concurrently, in vitro studies on cell cultures infected with the coronavirus demonstrated the complex's ability to suppress viral replication by modulating cellular proteases required for its penetration [36-46].

At the second stage, multicenter open comparative clinical studies were initiated, conducted at leading medical institutions, including the Smorodintsev Research Institute of Influenza and clinics for pulmonology and infectious diseases. The studies were conducted between 2021 and 2023. The main group (n=127) included patients with a laboratory-confirmed diagnosis of mild to moderate COVID-19, as well as patients with exacerbations of chronic non-specific lung diseases (COPD, chronic bronchitis). The comparison group (n=115) received standard therapy according to the current temporary methodological recommendations of the Ministry of Health of the Russian Federation, while the main group received standard therapy with the addition of "Populin with Dihydroquercetin" at a dose of 300 mg/day (equivalent to salicin) for a 14-day course. Inclusion criteria were age 18-65 and signed informed consent. Exclusion criteria were severe course of the disease requiring resuscitation, renal and hepatic insufficiency, pregnancy, use of anticoagulants, and known allergy to salicylates.

Methods for Evaluating Efficacy and Safety. Dynamic observation of patients included daily monitoring of symptoms (cough, shortness of breath, temperature, SpO_2), and laboratory tests on days 1, 7, and 14: complete blood count with differential, C-reactive protein (CRP), ferritin, D-dimer, and interleukins (IL-6). Quantitative PCR was also performed to determine viral load, and the time to a negative result was assessed. For patients with chronic lung pathology, spirometry was additionally performed, and the symptom severity index was assessed using the CAT (COPD Assessment Test) scale. Tolerance and safety were evaluated by the frequency and nature of adverse events, biochemical blood analysis parameters (ALT, AST, creatinine), and gastroscopy results in patients with gastrointestinal complaints.

RESULTS AND DISCUSSION

Liquid forms of aspen bark extract registered as dietary supplements-"Populin with Dihydroquercetin" and "Tremulacin"-were developed.

The main raw ingredients used were aspen bark extract (Extractum cortices Populi tremulae) and Lavitol (dihydroquercetin).

The formulation of the "Populin with Dihydroquercetin" supplement includes (g/100g): aspen bark extract – 99.773; Lavitol – 0.227. "Tremulacin" supplement consists of aspen bark extract – 100.0.

Production Technology of "Populin with Dihydroquercetin."

The process consists of the following sequential stages **(Figure 1)**:

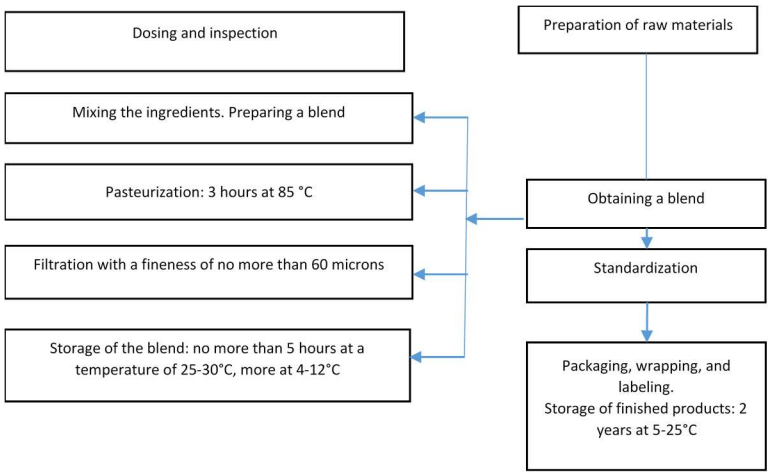

Figure 1. Technological scheme of "Populin with Dihydroquercetin" production

Preparation of raw materials. Raw materials are accepted into production with accompanying documents confirming quality and safety. Microbiological purity is checked; in the aspen bark extract, the quantitative content of flavonoids, salicin, and the mass fraction of solids (at least 60%) are verified.

A preliminary recalculation of water and extract for the produced batch of the supplement is carried out.

Example: For a batch of 250 kg with an aspen bark extract dry residue of 90% and a dihydroquercetin content of 88%. Calculate the amount of raw materials: thick aspen bark extract with a solids mass fraction of 70% - 99,773*250/100 = 249,433kg; Lavitol with an 88% dihydroquercetin content -0,227*250/100 = 0,567kg (total — 250 kg).

Calculate the amount of extract (considering the dry residue):

M =(249,433*70)/90 = 194,0 kg.

Determine the mass of water to be added to obtain a 70% supplement:

250-(194,0+0,567) = 55,433kg.

Dosing and loading of formulation components into the reactor-sterilizer. Preparation of the blend, pasteurization.

Aspen bark extract and Lavitol are loaded simultaneously in

specified quantities, according to the recipe, into the reactor-sterilizer. The components are mixed to obtain a blend followed by pasteurization at 85°C for 3 hours. The heating is turned off, and the pasteurization process is repeated under similar conditions with periodic stirring.

Filtration. Conducted using a filter block—a milk filter and a non-woven polyester filter (60%) with a filtration fineness of no more than 60 µm [47-57].

The filtered blend is pumped into a storage tank, cooled to 25-30°C, and stored for no more than 5 hours (at -4 to 12°C if stored longer).

Standardization of the finished product is carried out according to organoleptic, physicochemical parameters, and safety criteria according to TR CU 021/2011.

The technology for producing "Tremulacin" is similar to the one mentioned above.

Regulated quality indicators were determined **(Tables 1, 2)**.

Table 1. Regulated quality indicators for "Populin with Dihydroquercetin"

Indicators	Characteristics
Content of phenol glycosides calculated as salicin, %, at least	2.0
Content of flavonoids calculated as rutin, %, at least	0.3
Mass fraction of dry residue, no more than	76.0
Dihydroquercetin, %, at least	0.15

Table 2. Regulated quality indicators for "Tremulacin"

Indicators	Characteristics
Content of phenol glycosides calculated as salicin, %, at least	2.0
Content of tannins calculated as tannin, %, at least	4.0
Mass fraction of dry residue, no more than	76.0

Chromatographic studies of the aqueous extract of aspen bark were conducted **(Figure 2)**.

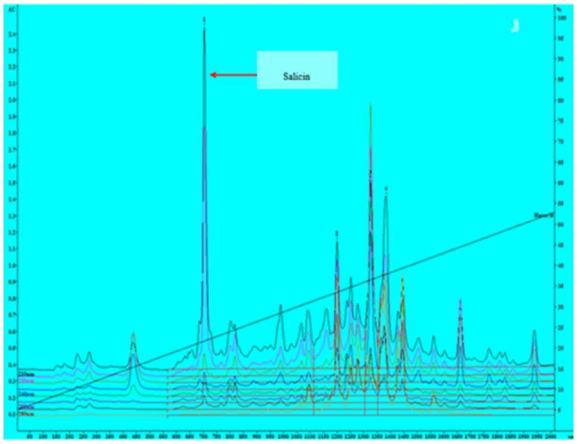

Figure 2. HPLC-UV chromatograms of aqueous aspen bark extracts

The results obtained confirm literature data regarding the high content of salicin as the main active substance. Salicin belongs to phenolic glycosides and is oxidized in the body to salicylic acid, which is the basis of its therapeutic value [18]. Unlike salicin obtained by chemical synthesis, it does not exert an irritating effect on the gastrointestinal tract [19]. Being a key biologically active substance of aspen bark, salicin contains functionally active groups in its structure **(Figure 3)**, which play an important role in the realization of anti-inflammatory, hepatoprotective, anti-opisthorchiasis properties, and other pharmacological effects of aspen bark and specialized products using it.

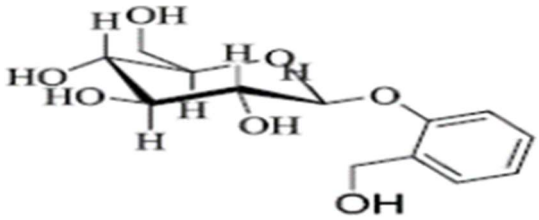

Figure 3. Structural formula of salicin

Clinical trials of the new innovative supplement forms were conducted for inflammatory diseases and coronavirus infections. Statistical analysis of the data revealed significant differences between the groups. Patients in the main group receiving the phytocomplex showed faster resolution of the systemic inflammatory response: the CRP level by day 7 decreased by an average of 45% versus 28% in the comparison group ($p<0.05$). The level of IL-6—a marker of cytokine storm—normalized significantly faster. The viral load, determined by Ct in the PCR test, decreased to undetectable values on average 2 days earlier (by day 8 versus day 10 in the control group, $p<0.05$). In the subgroup of patients with COPD in the main group, a significant improvement in spirometry indicators (forced expiratory volume in one second – FEV1) and a decrease in the CAT index by 5.2 points versus 2.8 points in the control were recorded by the end of the second week of intake. A crucial result was the positive dynamics regarding D-dimer: its increase was less pronounced, and normalization occurred faster, indirectly indicating the modulating effect of dihydroquercetin on endothelial dysfunction and the prothrombotic status characteristic of COVID-19. In the group receiving the supplement, not a single case of thrombotic complications was registered, whereas such complications were noted in 3 patients in the comparison group.

The safety profile of the developed phytocomplexes was assessed as favorable. The frequency of adverse events in the main group did not exceed that in the comparison group and consisted mainly of mild dyspeptic symptoms (2 cases), which did not require discontinuation of the preparation. In no case was an increase in liver transaminases or creatinine beyond reference values recorded, nor were there clinically significant changes in coagulogram parameters. The absence of erosive-ulcerative lesions of the gastric mucosa, confirmed in 15 patients with risk factors associated with NSAID use, indicates the gastroprotective effect of the natural combination of salicin with flavonoids.

CONCLUSION

The clinical data obtained convincingly prove that dietary supplements based on standardized aspen bark extract enriched with dihydroquercetin possess pronounced anti-inflammatory, antiviral (mediated), and endothelial-protective effects. They are effective as an adjuvant in the complex therapy of infections accompanied by

hyperinflammation and endothelial damage. The uniqueness of the composition and technological approach, which guarantees the preservation of the integrity of the natural complex of bioflavonoids and salicin, is protected by RF Patent No. 2710271 for the invention "Method for obtaining an anti-inflammatory agent based on aspen bark." The patent emphasizes the innovativeness of the gentle extraction method, allowing for a product with predictable pharmacological activity and a high safety profile, which favorably distinguishes it from synthetic analogues and opens prospects for wide application in nutritional support, practice and preventive medicine. Thus, clinical trials fully confirmed the hypothesis of the high efficiency of the developed phytocomplexes and substantiated the feasibility of their introduction into clinical practice for the correction of metabolic disorders in inflammatory and viral diseases.

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Ethics statement: None

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