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DETERMINATION OF PHENOLIC COMPOUND CONTENT AND ANTI-INFLAMMATORY DOSE OF THE DIETARY SUPPLEMENT "PULMACLIN"

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Annotation:

The content of polyphenols in the dietary supplement "Pulmaclin," which is derived from certain local plants and used for the treatment and prevention of upper respiratory tract diseases, was determined using High-Performance Liquid Chromatography (HPLC).

It was established that the content of apigenin is 2,485 ± 0,02 mg/L, quercetin is 1,153 ± 0,045 mg/L, rutin is 0,714 ± 0,014 mg/L, salicylic acid is 0,159 ± 0,004 mg/L, and gallic acid is 0,154 ± 0,004 mg/L.

The study utilized the carrageenan-induced paw edema model, a classic experimental model of inflammation. The efficacy of the "Pulmaclin" dietary supplement was evaluated by administering it at various doses (low, medium, and high) and observing its effect on the degree of edema. The main goal of this research was to determine the effective anti-inflammatory dose and activity level of the "Pulmaclin" dietary supplement.

How to Cite

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Introduction: The use of medicinal plants in traditional medicine is a practice that has continued since ancient times. Plants have important therapeutic significance for the human body, not only as a food source but also due to the presence of a wide range of biologically active substances. Naturally occurring biologically active compounds increase the body's resistance to negative environmental influences and strengthen the immune system. Furthermore, they play a crucial role in activating the antioxidant defense system and preventing various chronic diseases [1, 2].

Since ancient times, people have obtained curative substances from certain foods, roots, fruits, bark, leaves, stems of plants, body parts and organs of various animals, as well as from minerals. These products were considered a source of compounds necessary to eliminate the deficiency of biologically active

substances in the human body. Nutrition is one of the most important factors that ensures the connection between a person and the external environment and determines their health [3].

Medicinal plants contain a number of biologically active chemical compounds that serve a protective function for the plant and also exert a therapeutic effect on the human body. These biologically active compounds include phenolic compounds, which are widely distributed in the plant world. Phenolic compounds are secondary metabolites widely present in plants, and to date, over 8000 types have been identified [4]. It is known that phenolic compounds are mainly divided into four classes: phenolic acids, flavonoids, stilbenes, and lignans [5].

The primary pharmacological significance of medicinal plants is manifested in their properties, such as antioxidant and anti-inflammatory effects. Most phenolic compounds possess antioxidant activity and can be used for the prevention and treatment of inflammatory processes [6].

Under the influence of various factors, a condition called "oxidative stress" arises in the body. Oxidative stress results from an imbalance between free radicals and antioxidants in the body, which leads to cell damage and the development of chronic diseases. Phenolic compounds, as powerful antioxidants, neutralize free radicals and protect cells from oxidative damage. Polyphenols in plants reduce oxidative stress by increasing the activity of antioxidant enzymes in the body, such as glutathione peroxidase [7].

The initiation and resolution of inflammation require dynamic equilibrium. Certain plant substances, such as polyphenols, contribute to the inflammation resolution process by stimulating the formation of Specialized Pro-resolving Mediators (SPMs). The activation of AMPK (5'-adenosine monophosphate-activated protein kinase) is essential for the repair of tissues damaged as a result of inflammation [8].

Inflammation is a common and widespread pathological process underlying most diseases. The main mechanism of inflammation is often associated with the body's reaction to a local injury (the focus of inflammation). Migrating cells, such as leukocytes and macrophages, which form part of the inflammatory infiltrate, play a key role at the focus of inflammation. The inflammatory reaction includes a series of interconnected processes: the neuroendocrine system's stress response (the hypothalamus-pituitary-adrenal complex), the immune response, and acute-phase reactions. The primary mechanism for triggering the inflammatory process is linked to the release of cytokines and other mediators from the focus of inflammation into the bloodstream. Experimental models used to study anti-inflammatory activity are based on such mediators as carrageenan, serotonin, histamine, and formalin [9].

Research Results. Since the biological activity of phenolic compounds is also related to their antioxidant activity, this study investigated the content and quantity of phenolic compounds in the dietary supplement "Pulmaclin" using the High-Performance Liquid Chromatography (HPLC) method. The amounts of gallic acid, quercetin, apigenin, rutin, and salicylic acid were determined (Table 1).

The results of the HPLC analysis showed that the "Pulmaclin" dietary supplement contains various polyphenolic compounds, and their concentration and quantitative ratio are of significant importance for determining the potential pharmacological action of the biologically active supplement.

Table 1

Content of Phenolic Compounds in the Dietary Supplement "Pulmaclin" (mg/L)

Samples	Gallic acid	Rutin	Salicylic acid	Quercetin	Apigenin	Kaempferol
Experiment 1	0,159	0,714	0,155	1,102	2,471	0
Experiment 2	0,152	0,7	0,159	1,187	2,508	0
Experiment 3	0,152	0,728	0,163	1,172	2,477	0
Mean	0,154	0,714	0,159	1,153	2,485	0
Relative standard deviation, %	2,543	1,964	2,495	3,921	0,801	0

Maximum	0,159	0,728	0,163	1,187	2,508	0
Minimum	0,152	0,7	0,155	1,102	2,471	0
Std..deviation	0,004	0,014	0,004	0,045	0,02	0
Mean (mg/L)	**0,154±0,004**	**0,714±0,014**	**0,159±0,004**	**1,153±0,045**	**2,485±0,02**	**0±0**

The object of the study, the dietary supplement "Pulmaclin," contains apigenin in the largest quantity, with an average content of 2,485 ± 0,02 mg/L. This result indicates that apigenin is the main active component of this biologically active supplement, as it possesses powerful antioxidant and anti-inflammatory properties. Apigenin's relaxing effect on the smooth muscles of the airways suggests its bronchodilatory property [10].

Quercetin was determined to be the next most abundant compound (1,153 ± 0,045 mg/L). Quercetin is known to possess strong antioxidant and membrane-stabilizing properties, as well as the ability to inhibit (suppress) the production of inflammation mediators (such as leukotrienes and prostaglandins) [11]. This property scientifically validates the effectiveness of the "Pulmaclin" dietary supplement in treating diseases associated with airway inflammation.

At the next stage of our study, an experimental evaluation of the anti-inflammatory efficacy of the "Pulmaclin" dietary supplement was conducted. This parameter is one of the key criteria for determining the pharmacological quality of the product and its potential as a dietary supplement. To determine the anti-inflammatory effect, the "carrageenan-induced paw edema" test was used as a standard experimental model. This model is widely applied in pharmacological research due to its ability to reproduce the main stages of the acute inflammatory process—vasodilation, increased capillary permeability, and the development of edema.

Experimental animals were divided into control and treatment groups. The control group was administered only the inflammation-inducing agent—a 1% carrageenan solution—subplantarly (under the paw sole). The treatment groups were preventively given the "Pulmaclin" dietary supplement at various doses prior to carrageenan injection. The paw volume or thickness was measured at specific time intervals as the main indicator of the inflammatory response, and the percentage reduction in edema was calculated relative to the control group.

Table 2

The Effect of the "Pulmaclin" Dietary Supplement at Various Doses on Carrageenan-Induced Paw Edema, as a Percentage of the Initial Level (M±m; n=5)

Groups	Inhibition of inflammation, %			
	1st hour	2nd hour	3rd hour	4th hour
Control	35,4 ± 3,4	59,0 ± 5,8	83,6 ± 8,2	47,9 ± 4,6
Pulmaclin 50 mg/kg	18,6 ± 1,7	25,8 ± 2,5	38,2 ± 3,6	30,7± 3,0
Pulmaclin 100 mg/kg	41,0 ± 4,0	52,8 ± 5,1	64,6 ± 6,3	52,8 ± 5,1

In the control (diseased, untreated) group, maximum edema was observed after 3 hours, reaching 83,6 ± 8,2% of the initial level, and remained high even after 4 hours at 47,9 ± 4,6%. In the study groups (treated with Pulmaclin), maximum edema was also observed 3 hours after carrageenan injection, but the effect of the preparations had almost normalized after 4 hours. The percentage results for 1, 2, 3, and 4 hours are presented in Table 2.

According to the data presented, the anti-exudative effect of all studied substances (various doses of the "Pulmaclin" dietary supplement, 50 and 100 mg/kg) on carrageenan-induced edema ranges from 22,7\% to 54,3\%.

In the study group, maximum edema was also observed 3 hours after carrageenan injection; however, the anti-exudative activity of the preparations was calculated as a percentage relative to this 3-hour mark. The obtained results are presented in Table 2.

The anti-exudative activity is calculated using the following formula:

$$AEF = \frac{\Delta V_n - \Delta V_{taj.}}{\Delta V_n} \cdot 100\%$$

Where: AEF – Anti-Exudative Activity

ΔV_n – Change in hind paw volume of the Control group, cm3;

ΔV_{taj} – Change in hind paw volume of the Treatment group, cm3.

Table 3

Anti-Exudative Activity of the "Pulmaclin" Dietary Supplement at Different Doses (M ± m; n=5).

Groups	Dose, mg/kg	Foot volume increase, %, 3 hours after induction	Antiexudative effect %
Control	-	83,6 ± 8,2	-
Pulmaklin	50	38,2 ± 3,6	54,3
Pulmaklin	100	64,6 ± 6,3	22,7

The chemical composition of the "Pulmaclin" dietary supplement was investigated using the HPLC method. It was established that flavonoids, such as apigenin and quercetin, which are present in high concentration among the phenolic compounds, demonstrate an acute anti-inflammatory effect by inhibiting the synthesis and activity of key inflammation mediators—prostaglandins, leukotrienes, and various cytokines (TNF-α, IL-6) [12]. Furthermore, the biologically active compounds in the composition of the "Pulmaclin" dietary supplement provide a basis for suppressing the inflammatory process by neutralizing free radicals and reducing immune system infiltration.

Experimental Section. The object of the study was the "Pulmaclin" dietary supplement, which was taken in the form of a syrup produced by "MMF FARM GROUP TRADE" LLC (Andijan region) based on extracts of medicinal plants. For comparison, the following substances were used as reference standards: gallic acid (≥97,5%), rutin (≥94%), salicylic acid (>99%), quercetin (>95%), apigenin (≥95%), and kaempferol (≥97.5%). Acetonitrile (for chromatography) and acetic acid (analytical grade, Sigma-Aldrich, USA) were used in the experiments. The quantity of polyphenols in the composition of the "Pulmaclin" dietary supplement was determined using the HPLC (High-Performance Liquid Chromatography) method according to established procedures [13].

For the preliminary evaluation of the anti-inflammatory activity of the "Pulmaclin" dietary supplement, the classical "carrageenan model" was used. The study was conducted on 15 rats (190±20 gr body weight, single sex). The experimental animals were divided into 3 groups of 5 animals each: a control group and two treatment groups. Inflammation was induced by the subplantar injection of 0.1 mL of a 1% carrageenan solution into the hind paw of the rats. The "Pulmaclin" dietary supplement was administered to the rats orally at doses of 50 and 100 mg/kg. The inflammatory response was evaluated by measuring the change in paw volume at 1, 2, 3, and 4 hours post-injection. Anti-exudative activity was determined based on the results obtained 3 hours after carrageenan injection [14-17].

Conclusion.

As a result of the quantitative analysis of phenolic compounds in the composition of the "Pulmaclin" dietary supplement, the main bioactive substances responsible for its pharmacological efficacy were determined. Among the phenolic compounds contained in the dietary supplement, apigenin is present in the highest concentration (2,485 ± 0,02 mg/L). It is the primary phenolic component of this dietary supplement and allows for the explanation of its effectiveness in treating respiratory tract diseases through its strong antioxidant, anti-inflammatory, and bronchodilatory effects.

The experiment to study the anti-inflammatory activity of the "Pulmaclin" dietary supplement showed that the dose of 50 mg/kg significantly inhibits acute carrageenan-induced inflammation. At this dose, a 54,3% anti-exudative effect was observed, which reduced the development of edema by almost half. The diminished effect at the higher dose (100 mg/kg) may be due to the complex pharmacokinetics of the "Pulmaclin" dietary supplement. In conclusion, the "Pulmaclin" dietary supplement, especially at the 50 mg/kg dose, exhibits significant anti-inflammatory activity, which forms the pharmacological basis for its use in respiratory tract diseases.

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