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THE ANTIBACTERIAL ACTIVITY OF THE FLAVONOID EXTRACT FROM THE PLANT PHYSALIS ALKEKENGII

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ABSTRACT

The work studied the antibacterial activity of a flavonoid extract isolated from the medicinal plant *Physalis alkekengi* against conditionally pathogenic and pathogenic bacteria, in particular, *Proteus mirabilis* 9, *Escherichia coli* NC 101, *Listeria monocytogenes* and *Bacillus subtilis*. The immunostimulating effect of the plant flavonoid extract in the lymphoid organs of immunized animals was also studied in vivo.

KEYWORDS

Extract, flavonoid, antibacterial activity, immunostimulating effect.

INTRODUCTION

Currently, most of the medicinal products used in medical practice for the treatment of various diseases are of synthetic origin, and these drugs often have side effects. In this regard, special attention is being paid to medicinal plants, with flavonoid-containing plants, in particular their extracts, being considered more promising. Due to their higher biological activity, flavonoids are powerful antioxidants, estrogenic regulators, and antimicrobial agents. The extract of the medicinal plant *Physalis alkekengi* contains a large

number of various biologically active compounds—alkaloids, flavonoids, carotenoids, vitamins—and also has antibacterial and immunostimulating properties. As a result, it is widely used in medicine, pharmaceuticals, the food industry, and agriculture.

In recent years, there has been an increase in the number of bacterial strains resistant to antibiotics, which creates additional difficulties in treating many diseases [1]. Several approaches to solving this

problem include the development of new drugs or the modification of existing ones, providing only temporary relief. Promising strategies involve the creation of adjuvants aimed at suppressing bacterial resistance mechanisms or agents that can enhance the human body's resistance to bacterial infections. Finding natural products with the necessary properties plays a crucial role in advancing these fields. Plant materials rich in polyphenolic compounds have been used to treat bacterial diseases for centuries. Some of them have the ability to reduce the virulence of pathogenic strains or boost the body's defenses [2]. Substances that inhibit bacterial growth at concentrations below 10 µg/mL are of great interest to pharmacology [3].

Research Objects: The flavonoid extract of the plant *Physalis alkekengi*. The indicator strains used were *Escherichia coli* 002673/477, *Pseudomonas aeruginosa* 003841/114, *Proteus mirabilis* 9, *Staphylococcus aureus*, *Bacillus subtilis* VKM, *Listeria monocytogenes*, and *Candida albicans*.

METHODS

The antimicrobial activity of plant flavonoids against opportunistic microorganisms was determined using the agar diffusion method.

Melted MPA (meat-peptone agar) (Hi-media, India) nutrient medium was poured into Petri dishes. To

prepare the inoculum, a method of direct suspension in sterile isotonic solution of colonies from a pure 18–24-hour culture of the test microorganism, grown on solid non-selective nutrient medium, was used. The bacterial suspension was adjusted to a turbidity of 0.5 according to the McFarland standard, corresponding to approximately 1×10^8 CFU/mL (for *Escherichia coli*) by adding microbial mass to the suspension or diluting it with sterile isotonic solution.

Inoculation of MPA Plates

The bacterial suspension was inoculated onto the MPA medium using a sterile cotton swab, and sterile cylinder-punches were used to create wells with a diameter of 6 mm, evenly spaced from each other and the edge of the plate. Into the wells of each plate, 100 µL of an aqueous solution of the test flavonoid extracts derived from the plant were added. The test sample solutions were prepared by diluting the substances in distilled water at concentrations of 100, 75, 50, and 25 mg/mL.

RESULTS

After adding the solutions into the wells, the plates were kept at +4°C for 1-2 hours. They were then incubated at $(36 \pm 1)^\circ\text{C}$ for 16-18 hours. The diameters of the growth inhibition zones of the test microorganisms were measured using a ruler (Figure 1).

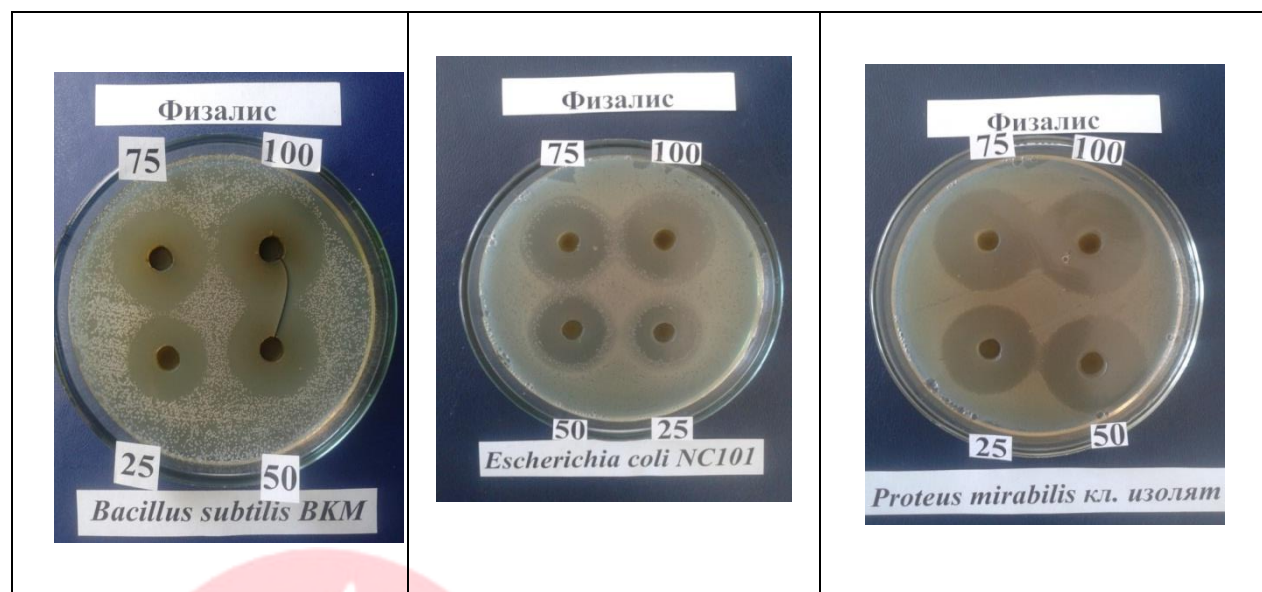


Figure 1. Antimicrobial activity of substances against opportunistic microorganisms

Two of the six tested strains exhibited sensitivity to the action of the flavonoid extract. The flavonoid extract did not show any inhibitory effect against *Candida albicans*.

The flavonoid extract had a detrimental effect on the growth of the following test microorganisms: *Bacillus*

subtilis VKM (with a growth inhibition zone diameter of 32 mm), *Listeria monocytogenes* (18 mm), *Staphylococcus aureus* (13 mm), and *Pseudomonas aeruginosa* 003841/114 (12 mm). *Proteus mirabilis* 9 and *Escherichia coli* NC 101 were the most sensitive to the action of the flavonoid extract, with inhibition zone diameters of 32 mm and 27 mm, respectively (Table 1).

Table 1

Antimicrobial activity of the flavonoid extract from the plant *Physalis alkekengi* against opportunistic microorganisms, in mm diameter

№	Test Microorganisms	Flavonoid Extract			
		100 mg/ml	75 mg/ml	50 mg/ml	25 mg/ml
1	<i>Listeria monocytogenes</i>	18	16	14	12
2	<i>Bacillus subtilis BKM</i>	32	29	27	24

3	<i>Escherichia coli</i> <i>NC 101</i>	26	24	22	19
4	<i>Proteus mirabilis</i> 9	32	30	28	26

In further work, the minimum inhibitory concentration (MIC) of the flavonoid extract against these test microorganisms was determined (Table 2). At concentrations of 100, 75, 50, and 25 mg/mL, the inhibition zone diameters for *Listeria monocytogenes* were 18, 16, 14, and 12 mm, respectively. The inhibition zone diameters for *Bacillus subtilis* VKM decreased with decreasing flavonoid extract concentration,

measuring 32, 29, 27, and 24 mm, respectively. The antimicrobial activity of the flavonoid extract against *Proteus mirabilis* 9 and *Escherichia coli* NC 101 exhibited a dose-dependent pattern, with inhibition zone diameters of 32, 30, 28, and 26 mm for *Proteus mirabilis* 9 and 26, 24, 22, and 19 mm for *Escherichia coli* NC 101, respectively.

Table 2

Minimum inhibitory concentration of the flavonoid extract from the plant *Physalis alkekengi* against opportunistic microorganisms, in mm diameter

No	Flavonoid Substance	<i>Escherichia coli</i> <i>NC 101</i>	<i>Pseudomonas aeruginosa</i> 003841/114	<i>Proteus mirabilis</i> 9	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i> <i>BKM</i>	<i>Candida albicans</i>	<i>Listeria monocytogenes</i>
1	Flavonoid Extract	27	12	32	13	32	0	18

Based on the data obtained, it can be concluded that the studied flavonoid extract from the plant *Physalis alkekengi* possesses a broad spectrum of antimicrobial activity against the tested opportunistic microorganisms. The flavonoid extract effectively inhibited the growth of *Proteus mirabilis* 9, *Escherichia coli* NC 101, *Listeria monocytogenes*, and *Bacillus*

subtilis VKM. The antimicrobial effect was dose-dependent. Consequently, the flavonoid extract from *Physalis alkekengi* could serve as the basis for the development of antimicrobial agents.

Currently, immunoactive drugs are obtained from various sources, including synthetic pathways. The

development of new, highly effective, non-toxic, and safe immunomodulatory drugs based on local raw materials is a pressing task in modern immunobiotechnology and immunology. Therefore, the search for and creation of new immunomodulatory drugs with immunological activity remains a relevant problem. According to literature data, the study of biologically active substances, particularly flavonoids derived from the medicinal plant *Physalis alkekengi*, is promising in this regard [4,5]. This is due to the fact that flavonoids exhibit high activity in protein synthesis and have pronounced anabolic properties. They reduce fat deposits, help lower blood glucose levels without affecting insulin levels, and also possess immunostimulatory effects [7,8]. As a result, flavonoid-containing drugs are widely used in various fields of science, such as medicine and pharmaceuticals, especially in cases of cardiovascular and nervous system disorders and immune system deficiencies [6,7].

Given the biomedical significance of this class of compounds, current studies focus on the properties and functions of flavonoids [3]. In this study, the effect of the *Physalis alkekengi* flavonoid extract on the increase in the number of immunocompetent cells in the lymphoid organs of animals was investigated. The results are presented in Table 3. As seen from the data, the control group registered $36.8 \pm 2.0 \times 10^6$ cells in the thymus. The flavonoid extract significantly increased the total number of cells in the thymus of immunized mice, with an increase of 1.77 times ($65.2 \pm 2.1 \times 10^6$ cells).

Regarding the effect of the *Physalis alkekengi* flavonoid extract on the second central organ of the immune system—the bone marrow—similar results were obtained as with the thymus. The studied plant extract significantly increased the total number of cells in the bone marrow, with an increase of 1.89 times (Table 3).

Table 3.

The effect of the plant extract on the number of cells in the central and peripheral immune organs in mice ($M \pm m$, $n=6$)

No	Group	Dose of substrate	Cells of thymus $\times 10^6$	IC	Cells of bone marrow $\times 10^6$	IC	Cells of lymph nodes $\times 10^6$	IC
1.	Control	-	$36,8 \pm 2,0$	-	$11,0 \pm 0,5$	-	$20,5 \pm 0,8$	-
2.	Preparate <i>Physalis alkekengi</i>	1,0 мг/г	$65,2 \pm 2,1^*$	+1,77	$20,8 \pm 0,5^*$	+1,89	$29,2 \pm 1,1^*$	+1,42

Note: IS - Index of comparison to the control, Statistically significant compared to the control

Further studies investigated the effect of the flavonoid extract on the state of the peripheral organs of the immune system. In the control group, the number of cells in the lymph nodes was $20.5 \pm 0.8 \times 10^6$. The flavonoid extract derived from *Physalis alkekengi* significantly increased the number of cells in the lymph nodes by 1.42 times.

Thus, it can be concluded that the studied flavonoid extract from *Physalis alkekengi* possesses a broad spectrum of antimicrobial activity against the tested opportunistic microorganisms. The flavonoid extract effectively inhibited the growth of *Proteus mirabilis* 9, *Escherichia coli* NC 101, *Listeria monocytogenes*, and *Bacillus subtilis* VKM, with the antimicrobial effect being dose-dependent. Therefore, the flavonoid extract from *Physalis alkekengi* could serve as a basis for the development of antimicrobial agents.

In addition, the flavonoid extract derived from *Physalis alkekengi* exhibits a higher immunostimulatory effect. The plant extract significantly increased the number of immunocompetent cells in the lymphoid organs of animals: in the thymus by 1.77 times, in the bone marrow by 1.89 times, and in the lymph nodes by 1.42 times.

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