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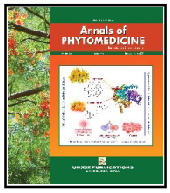

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Isolation of flavonoid extracts from the medicinal plant *Physalis alkekengi* L. and study of antibacterial and immunostimulating properties

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Abstract

This study investigated the optimal conditions for extracting biologically active substances, particularly flavonoids, from the medicinal plant *Physalis alkekengi* L. The research examined how various factors, such as the concentration of the extracting agent, the particle size of the raw material, incubation time, and the ratio of raw material to extracting agent, influenced flavonoid yield. Using ethanol, a dry extract of dark green color was obtained, and both quantitative and qualitative analyses were performed to determine the flavonoid content in the plant leaves, expressed as luteolin. Additionally, the study explored the antibacterial activity of the flavonoid extract from *P. alkekengi* against various bacteria, including *Escherichia coli* 002673/477, *Pseudomonas aeruginosa* 003841/114, *Proteus mirabilis* 9, *Staphylococcus aureus*, *Bacillus subtilis* VKM, *Listeria monocytogenes*, and *Candida albicans*. The immunostimulating effect of the flavonoid extract on the lymphoid organs of immunized animals was also investigated *in vivo*.

1. Introduction

Currently, the majority of drugs used in medical practice for treating various diseases contain synthetic components, which often cause side effects. Therefore, much attention is paid to herbal preparations that affect the body without negative consequences (Niazian and Sabbatini, 2021; Wen *et al*., 2017). The use of various herbal preparations is due, first of all, to their high biological activity and less harmful effects on a living organism than their chemical analogues (Halkuzieva *et al*., 2023). This is due to the fact that biologically active substances of plant origin are much less likely to cause adverse reactions (allergies, dysbacteriosis, blood diseases, gastric and intestinal ulcers and other) and, as a rule, do not accumulate in the body, human and animal tissues (Abdoulahi *et al*., 2023; Sudhakaran *et al*., 1999; Khan *et al*., 2018; Kadirova *et al*., 2024).

In this context, medicinal plants, particularly those containing flavonoids and their extracts, are receiving special attention and are considered more promising alternatives. Having higher biological activity, flavonoids are powerful antioxidants, estrogen regulators and antimicrobial agents. These types of medicinal plants include *P. alkekengi* from the nightshade family (Abdoulahi *et al*., 2023; Sudhakaran *et al*., 1999). The plant is widespread in Europe, America, and Asia has powerful antioxidant properties, as well as anti-inflammatory, antiseptic, analgesic, diuretic, choleretic and hemostatic effects (Ji *et al*., 2012; Kang *et al*., 2011; Wu *et al*., 2005).

Physalis grows in light forests, on the edges, in ravines, and in vineyards. It is found as a weed in bushes, fields, and is also grown in gardens as an ornamental and medicinal plant. *Physalis* has many other names: Peruvian gooseberry, ground cherry, strawberry tomato, field cherry, Chinese lantern, *etc.* Species of *Physalis alkekengi*: *Physalis alkekengi, Physalis glabripes, Physalis praetermissa, Physalis pubescens, Physalis ixocarpa, Physalis peruviana, Physalis floridana*. Genera: *Solanum* (Nightshade), *Lycopersicum* (Tomato), *Capsicum* (Pepper), *Physalis* (Physalis), *Lycium* (Lycium), *Hyoscyamus* (Henbane), *Physochlaina*, *Nikotiana* (Tobacco), Petunia, Datura, Nikandra Adans, Atropa, *Physaliastrum Makina*, Scopolia (Qiu *et al*., 2008; Feng *et al*., 2018; Nanagulyan *et al*., 2020; Yavich *et al*., 2021; Sudhakaran *et al*., 1999; Khan *et al*., 2018). The rich composition of this plant includes many biologically active substances (BAS): Alkaloids (Yamaguchi *et al*., 1965; Zare Zadeh *et al*., 1997), steroids (Qiu *et al*., 2008), flavonoids (phenolic compounds and their glycosides) (Qiu *et al*., 2008; Ghazy *et al*., 2022; Qiu *et al*., 2008), carotenoids (Wen *et al*., 2017; Alirezalu *et al*., 2020), polysaccharides (Guo *et al*., 2017), lectins, pectins, vitamins, fatty oils, tannins and others. These substances are synthesized and accumulated by the plant (Parks *et al*., 2018).

Several diseases, such as urolithiasis, cystitis, hepatitis, bronchitis, edema, ascites, rheumatism, gout, and bruises, can be treated using a decoction and/or water infusion made from the fruit (Wen *et al*., 2017; Qiu *et al*., 2008; Shu *et al*., 2016). The plant extract is rich in various biologically active compounds, including alkaloids, flavonoids,

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carotenoids, and vitamins. It also possesses antibacterial and immunostimulating properties, making it widely used in medicine, pharmaceuticals, the food industry, and agriculture (Gharib Naseri *et al*., 2007; Helvaci *et al*., 2010; Moniruzzaman *et al*., 2016; Kar *et al*., 2006; Feng *et al*., 2018; Li *et al*., 2014). Preparations prepared based on a plant extract have contraceptive properties (Hong *et al*., 2015).

In present day medical care, people with chronic cholecystitis, hypertension, duodenal ulcers, and hypoacid gastritis are treated with physalis fruits as a dietary supplement and multivitamins. Antiviral, antibacterial and anti-inflammatory properties of fruits will help reduce the risk of infection during epidemics and alleviate the course of the disease (Kadirova *et al*., 2024; Halkuzieva *et al*., 2023).

In case of abscesses, ulcers and other skin injuries, a compress of juice or a decoction of *Physalis* can be placed on the sore spot. The fruits of this plant have the ability to remove toxins and free radicals from the body. *Physalis*, this will not only cleanse the body, but will also serve as a prevention of cancer (Guo *et al*., 2017; He *et al*., 2013; Ji *et al*., 2012).

Highly effective, immunostimulating drugs based on medicinal plants today are an urgent task in modern immunobiotechnology and immunology. In this regard, the search and creation of new immunomodulatory drugs with immunological activity remains an urgent problem. According to the literature, more promising in this regard is the study of biologically active substances, in particular flavonoids isolated from medicinal plants *P. alkekengi* (Qiu *et al*., 2008; Tong *et al*., 2008). This is because flavonoids possess a higher activity on protein synthesis and exhibit pronounced anabolic properties. They reduce body fat, help reduce blood glucose levels without affecting insulin levels, and also have an immunostimulating effect (Shu *et al*., 2016). Therefore, in various fields of science, as in medicine, pharmaceuticals and other fields, flavonoid-containing preparations are widely used, in particular for disorders of the cardiovascular and nervous systems and decreased immunity of the body (Yang *et al*., 2016; Li *et al*., 2014). Considering the medical and biological significance of this class of compounds, studies of flavonoids are currently being conducted to identify the most important properties and their functions (De Souza *et al*., 2022).

Plant materials rich in polyphenolic compounds have been used to treat bacterial diseases for centuries. Some of them have the necessary abilities to reduce the virulent properties of pathogenic strains or increase the body's defenses. Especially, plant extracts that inhibit bacterial growth at concentrations less than 10 µg/ml are of great interest for pharmacology (Brown *et al*., 2014).

2. Materials and Methods

The *Physalis alkekengi* L. was authenticated by Professor Zukhra Kadirova, National Herbarium of Uzbekistan (TASH), Tashkent region, Akhangaran District, Lashkarak forests, Uzbekistan. A Voucher Specimen No. 40906903 was placed in the Herbarium unit. Flavonoid extracts from the leaves, stem, and whole plant of *P. alkekengi* were tested against indicator strains, including *Escherichia coli* 002673/477, *Pseudomonas aeruginosa* 003841/114, *Proteus mirabilis* 9, *Staphylococcus aureus*, *Bacillus subtilis* VKM, *Listeria monocytogenes*, and *Candida albicans*.

2.1 Methodology

To obtain a dry flavonoid-rich extract, green leaves and flowers of the medicinal plant *P. alkekengi* were used. For the extraction procedure, 30 g of plant leaves were finely ground to a particle size of 0.5 mm and placed in a 1.0 L flask, followed by the addition of an appropriate extractant. The mixture was incubated at room temperature for 6-8 h. Sequential extraction was then performed using hydroethanolic solutions containing 40-90% ethanol. Absolute ethanol and elevated temperatures are generally unsuitable for the extraction of flavonoids, as they can lead to the degradation or inactivation of these thermolabile compounds. Thus, the use of hydroethanolic solvents is preferred to enhance extraction efficiency while maintaining the structural integrity of flavonoids (Azwanida, 2015). To ensure complete extraction, the mixture was gently heated to 80-90°C, and the ethanol extract was concentrated in portions using a Rotavapor R-210 rotary evaporator at 50-60°C until a thick mass was obtained. The concentrated extract was then subjected to freeze-drying (lyophilization) to yield a dry plant extract. The resulting product was a non-hygroscopic dark green powder with a characteristic odor. The flavonoid content in the dry extract, expressed in terms of luteolin equivalents, was quantified using high-performance liquid chromatography (HPLC) as described by Brown *et al.* (2014) and Li *et al.* (2014). A standard solution of luteolin (1 mg/ml) was used as the reference. Prior to injection, the sample was filtered through a membrane filter. The HPLC conditions were as follows: column Agilent Technologies C18, 5 µm, 150 × 4.6 mm; mobile phase -acetonitrile and trifluoroacetic acid solution (40:60); flow rate –1.0 ml/min; column temperature – 30°C; detection -UV at 365 nm, 254 nm, and 280 nm; injection volume -10 µl.

The antimicrobial activity of plant flavonoids against opportunistic microorganisms was assessed using the agar diffusion method. Melted nutrient medium MPA (Meat-Peptone Agar) (Hi-media, India) was poured into Petri dishes. To formulate the culture, we employed the technique of direct suspension in a sterile isotonic solution of colonies from a pure 18-24 h culture of a test microorganism grown on a dense non-selective nutrient medium. The bacterial suspension was adjusted to a density of 0.5 according to the McFarland turbidity standard, which approximately corresponded to a concentration of 1-2 × 10^8 CFU/ml (*E. coli*), either by diluting the suspension with a sanitized isotonic mixture or by adding microbial significance to it.

Inoculation of plates with MPA. The bacterial suspension was inoculated with a sterile cotton swab onto the MPA, and holes with a diameter of 6 mm were made using a sterile punch cylinder at an equal distance from each other and from the edge of the dish. 100 il of an aqueous solution of the tested flavonoid extracts obtained from the plant was added to the wells of each dish. Solutions of the test sample were made by diluting the substances in purified water with a concentration of 100, 75, 50 and 25 mg/ml.

3. Results

To determine the optimal conditions for extracting flavonoids from the *P. alkekengi* plant, research was conducted to quantify flavonoid extraction. The influence of several factors on flavonoid yield was examined, including the concentration of the extractant, the particle size of the raw material, the incubation time, and the ratio of raw material to extractant. Additionally, to ensure the completeness of flavonoid extraction from *P. alkekengi*, the effect of extractant concentration was investigated. The obtained data are shown in Figure 1.

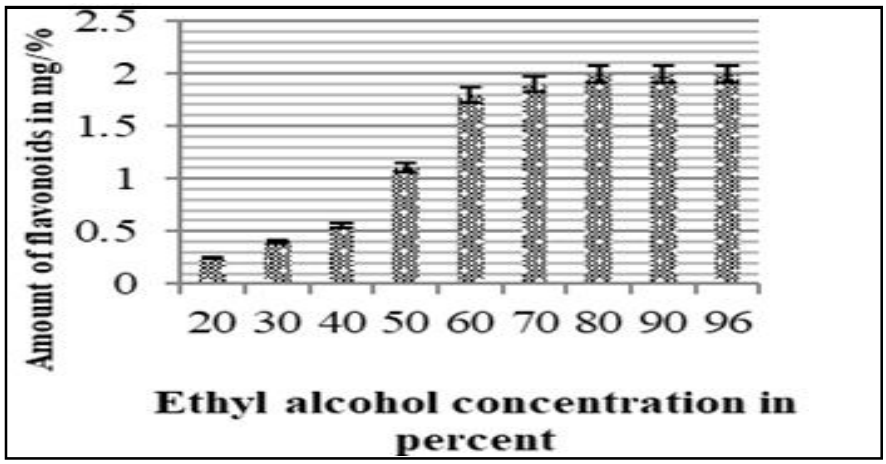


Figure 1: Effect of ethanol concentration on flavonoid extraction efficiency.

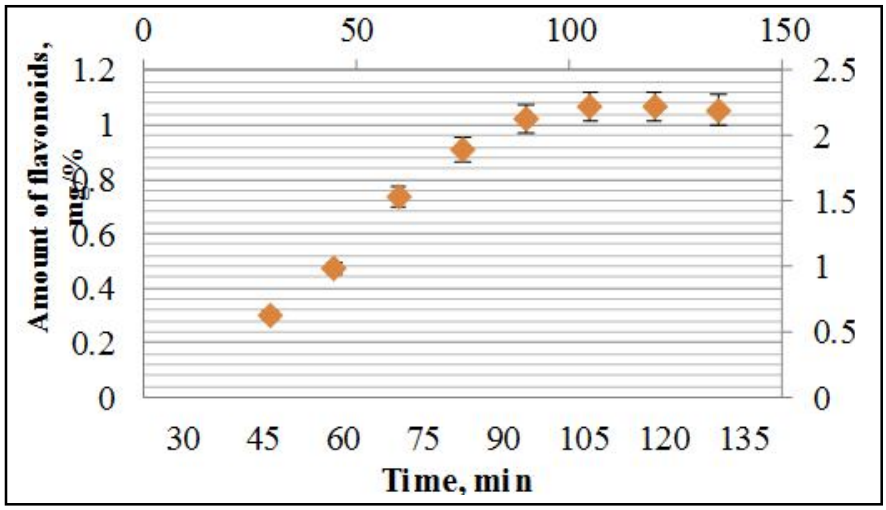


Figure 2: Effect of incubation time on flavonoid extraction efficiency.

Table 1: Impact of raw material particle size and raw material-extractant ratio on flavonoid yield

S.No.	Ratio of raw material: extractant, g/ml	Yield of flavonoids, mg/%	Degree of raw material grinding, mm	Yield of flavonoids, mg/%
1	1:30	1.51 ± 0.87	0.5	1.79 ± 0.91
2	1:50	1.62 ± 1.03	1.0	1.81 ± 1.12
3	1:60	1.79 ± 1.12	2.0	1.93 ± 1.01
4	1:80	1.91 ± 0.92	3.0	1.90 ± 1.71
5	1:100	1.95 ± 0.81	4.0	1.65 ± 0.98
6	1:150	1.95 ± 0.99	5.0	1.48 ± 0.91
7	1:200	1.94 ± 1.12	6.0	1.48 ± 091

Figure 1 demonstrates that at lower ethanol concentrations (20%, 30%, and 40%), the flavonoid yield ranged between 0.25 and 0.55 mg. The highest extraction efficiency was achieved using 80% ethanol, resulting in a maximum flavonoid yield of 1.92 mg. Further increases in ethanol concentration beyond 80% did not lead to any significant enhancement in extraction efficiency, indicating that 80% ethanol is the optimal concentration for flavonoid extraction from *P. alkekengi*. Additionally, the influence of incubation time on flavonoid extraction was examined over a range of 30 to 135 min (Figure 2), providing insight into the time-dependent kinetics of flavonoid release. As illustrated in Figure 2, flavonoid extraction from *P. alkekengi* plant material reached its maximum within 105 min of incubation. Extending the incubation period beyond this duration did not result in a further increase in flavonoid yield, indicating that 105 min is the optimal extraction time under the given conditions.

Subsequently, the study examined the effects of raw material particle size and the ratio of plant material to extractant on flavonoid yield. Table 1 presents the findings, demonstrating that the degree of grinding significantly impacts the extraction efficiency of active constituents, particularly flavonoids. The plant material was ground to various particle sizes, corresponding to sieve openings of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mm. The results revealed that the highest flavonoid yield (1.93 mg) was obtained when the plant material was ground to a particle size of 2.0 mm, emphasizing the importance of optimizing particle size for efficient extraction. Additionally, the study examined the effect of the ratio of raw material to extractant on flavonoid yield, as depicted in Table 1.

As shown in Table 1, the optimal raw material-to-extractant ratio for flavonoid extraction was 1:100, yielding a maximum flavonoid content of 1.95 mg. Increasing the extractant volume beyond this ratio did not result in any further improvement in flavonoid yield, suggesting that 1:100 is the most efficient ratio under the experimental conditions. Following this, a quantitative analysis of flavonoids in *P. alkekengi* raw material was performed using high-performance liquid chromatography (HPLC) (Figure 3). The concentration of luteolin in the extract was determined to be 5.6 µg/ml.

Chromatographic separation was performed using an Agilent 1200 System Technologies (USA) liquid chromatograph equipped with a diode-array detector and an Agilent Technologies column (150 cm × 4.6 mm, 5 µm). The column thermostat temperature was set at 30°C. Sample injection was carried out using an autosampler, with an injection volume of 10 µl. For the preparation of the mobile phase, acetonitrile of Chromasolv gradient grade, for HPLC > 99.9% (Sigma-Aldrich), was used. Gradient elution was employed for the separation of phenolic compounds, utilizing a mixture of bidistilled water acidified with trifluoroacetic acid and acetonitrile (40:60).

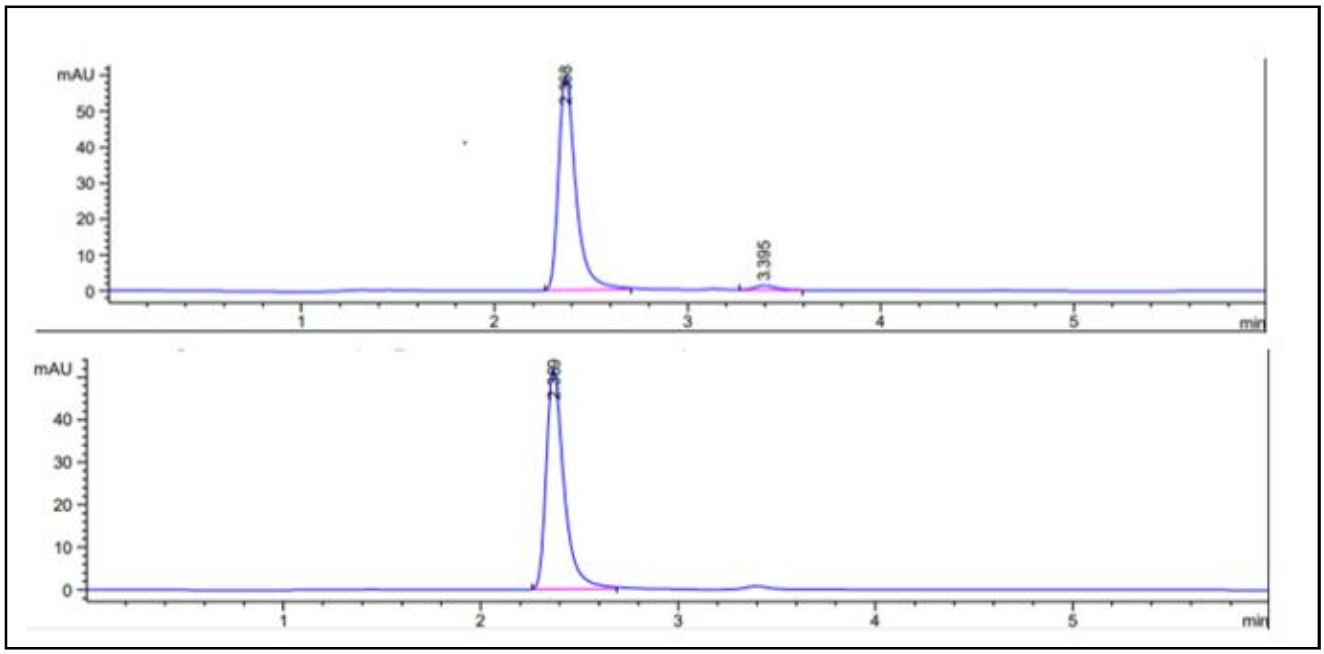
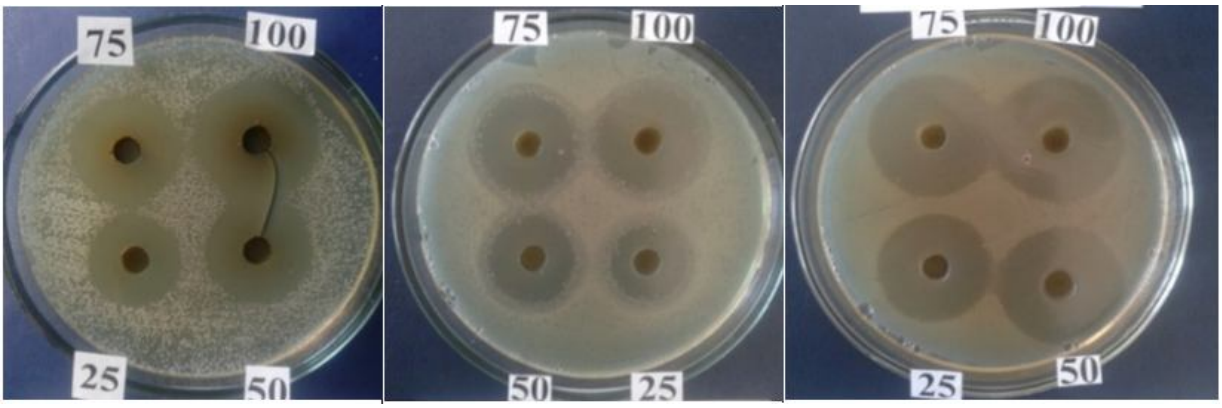


Figure 3: Quantitative determination of luteolin in the plant extract by HPLC.

Detection was carried out at three UV wavelengths: γ = 365 nm, 254 nm, and 280 nm. The mobile phase flow rate was maintained at 1.0 ml/min. The results of the chromatographic analysis are presented in Figure 3, which confirms the presence of a single identified flavonoid aglycone luteolin, in the hydroethanolic extract of *P. alkekengi*. As illustrated in Figure 3, luteolin was detected at a concentration of 5.6 µg/ml, indicating its significant presence in the plant extract. These findings validate the effectiveness of the optimized extraction and HPLC conditions for the separation and quantification of flavonoids in *P. alkekengi*. The established method provides a reliable approach for the identification, quantification, and potential isolation of flavonoids from plant-derived raw materials.This study successfully established optimal conditions for the extraction of biologically active compounds, specifically flavonoids, from the medicinal plant *P. alkekengi*. Key parameters influencing flavonoid yield, including extractant concentration, particle size of the plant material, incubation time, and the raw material-to-extractant ratio, were systematically evaluated. The optimal conditions identified were 80% ethanol as the extractant, a particle size of 2.0 mm, a raw material-to-extractant ratio of 1:100, and an incubation period of 105 min. Additionally, a dark green dry extract was obtained using ethanol, and both quantitative and qualitative analyses were conducted to quantify flavonoid content in the plant leaves, specifically luteolin.

Furthermore, the antimicrobial activity of the flavonoid extract from *P. alkekengi* against opportunistic microorganisms, including *E. coli* 002673/477, *P. aeruginosa* 003841/114, *P. mirabilis* 9, *S. aureus*, *B. subtilis* VKM, L. *monocytogenes*, and *C. albicans*, was investigated. Following the addition of the dry flavonoid extract to wells, dishes were incubated at (36 ± 1)°C for 16-18 h after initial chilling at +4°C for 1-2 h. The diameters of growth inhibition zones around the test microorganisms were measured using a ruler (Figure 4). During the measurement of the inhibition zone, it is important to ensure that the nutrient medium on which the microorganisms are growing is not contaminated with pathogenic or conditionally pathogenic microorganisms. Contaminant agents may interfere with the formation of the inhibition zone in the nutrient medium.



Bacillus subtilis BKM *Escherichia coli NC101* *Proteus mirabilis isolate*

Figure 4: Antimicrobial activity of flavonoid extract from *P. alkekengi* against opportunistic microorganisms.

Out of the six test strains examined, the flavonoid extract showed sensitivity against two strains. *Candida albicans* was not affected by the flavonoid extract. The antibacterial activity of the flavonoid extract from *Physalis alkekengi* was investigated. The extract exhibited activity against conditionally pathogenic and pathogenic microorganisms, including *E. coli* NC101, *P. mirabilis, and B. subtilis* VKM, at flavonoid concentrations of 100, 75, 50, and 25 mg/ml (Figure 4).

The flavonoid extract exhibited inhibitory effects on other test microorganisms: *B. subtilis* VKM (inhibition zone diameter of 32 mm), *L. monocytogenes* (18 mm), *S. aureus* (13 mm), and *P. aeruginosa* 003841/114 (12 mm). *P. mirabilis* 9 and *E. coli* NC 101 were particularly sensitive to the flavonoid extract, showing inhibition zone diameters of 32 mm and 27 mm, respectively (Table 2).

In subsequent experiments, the minimum inhibitory concentration of the flavonoid extract against these test microorganisms was determined (Table 3). The zone of inhibition diameter for *L. monocytogenes* decreased from 18 mm at 100 mg/ml extract concentration to 12 mm at 25 mg/ml. Similarly, for *B. subtilis* VKM, the inhibition zone diameter decreased from 32 mm to 24 mm as the flavonoid extract concentration decreased. The antimicrobial activity against *P. mirabilis* 9 and *E. coli* NC 101 was concentration-dependent, with inhibition zone diameters decreasing from 32 mm to 26 mm and from 26 mm to 19 mm, respectively, across the tested concentrations.

Table 2: Antimicrobial effectiveness of flavonoid extract from *P. alkekengi* against opportunistic microorganisms, measured in millimeters (mm) of inhibition zone diameter

S. No.	Test microorganisms	Flavonoid extract			
		100 mg/ml	75 mg/ml	50 mg/ml	25 mg/ml
1	*L. monocytogenes*	18	16	14	12
2	*B. subtilis* ÂÊÌ	32	29	27	24
3	*E. coli* NC 101	26	24	22	19
4	*P. mirabilis* 9	32	30	28	26

Table 3: Minimum inhibitory concentration of flavonoid extract from the plant *P. alkekengi* against opportunistic micro-organisms, mm in diameter

Flavonoid substance	Flavonoid extract
E. coli NC 101	27
P. aeruginosa 00384/114	12
P. mirabilis 9	32
S. aureus	13
B. subtilis ÂÊÌ	32
C. albicans	0
L. monocytogenes	18

Based on the findings, the flavonoid extract derived from *P. alkekengi* demonstrated broad-spectrum antimicrobial activity against the tested opportunistic microorganisms. The extract effectively inhibited the growth of *P. mirabilis* 9, *E. coli* NC 101, *L. monocytogenes*, and *B. subtilis* VKM in a dose-dependent manner. Therefore, the flavonoid extract from *P. alkekengi* holds potential as a basis for developing antimicrobial drugs.Additionally, this study investigated the impact of *Physalis alkekengi* flavonoid extract on enhancing the population of immunocompetent cells in the lymphoid organs of animals, as presented in Table 4. The results indicate a huge growth in total cell count in the thymus of immunized mice compared to the control group, with a 1.77-fold increase ($65.2 \pm 2.1 \times 10^6$ cells versus $36.8 \pm 2.0 \times 10^6$ cells). Similar results were observed in the bone marrow, where the studied herbal extract from *Physalis alkekengi* increased total cell count by 1.89 times (Table 4).

Table 4: The effect of the herbal preparation on the number of cells in the central and peripheral immune organs in mice (M ± m, n=6)

S.No.	Group	Dose of substance	Thymus cells in × 10^6	IP	Bone marrow cells in × 10^6	IR	Lymph node cells in × 10^6	IR
1.	Control	-	36.8 ± 2.0	-	11.0 ± 0.5	-	20.5 ± 0.8	-
2.	P. alkekengi	1.0 mg/g	65.2 ± 2.1*	+1.77	20.8 ± 0.5*	+1.89	29.2 1.1*	+1.42

Note: IR - index of ratio to control, * - reliably to control

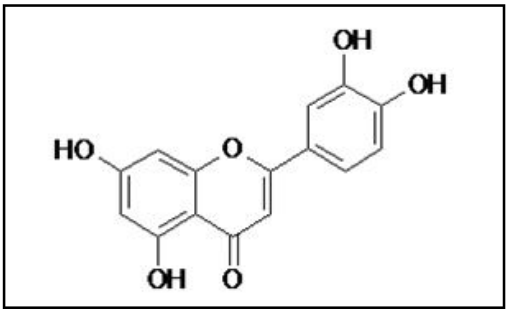


Figure 5: Structure of luteolin.

The flavonoid extract derived from *P. alkekengi* exhibited a broad spectrum of antimicrobial activity against the tested opportunistic microorganisms. It effectively inhibited the growth of *P. mirabilis* 9, *E. coli* NC 101, *L. monocytogenes*, and *B. subtilis* VKM in a dose-dependent manner. This suggests that the flavonoid extract could serve as a basis for developing antimicrobial drugs. Furthermore, the extract demonstrated potent immunostimulating effects. It significantly increased the population of immunocompetent cells in the lymphoid organs of animals: by 1.77 times in the thymus, 1.89 times in the bone marrow, and 1.42 times in the lymph nodes. These findings highlight the potential therapeutic benefits of the flavonoid extract from *P. alkekengi* beyond its antimicrobial properties.

4. Discussion

P. alkekengi is renowned in Asian and European traditional medicine for its therapeutic properties across all parts of the plant. Historical records, including those by Avicenna, document its use in treating conditions such as gastric and duodenal ulcers, hepatitis, and anemia. Iranian researchers have explored the anti-inflammatory effects of alcoholic extracts and highlighted the fruit extract's efficacy in anemia treatment. The plant has also been traditionally used in Iran for its anti-infective properties, with studies confirming antibacterial and antifungal activities of aqueous-alcoholic extracts (Wen *et al*., 2017; Shu *et al*., 2016; Zare Zadeh *et al*., 1997; Parks *et al*., 2018; Huang *et al*., 2010). In addition to its medicinal uses in Iran, *P. alkekengi* is extensively utilized in traditional Chinese medicine. Various parts of the plant, including fruits, calyxes, roots, leaves, and stems, have been employed to treat ailments ranging from sore throats and coughs to hepatitis, urinary issues, and tumors. Japanese and Chinese research has underscored the effectiveness of *P. alkekengi* extracts in treating conditions like pharyngitis, leveraging components like physalins and flavones known for their antibacterial and anti-inflammatory properties (Ji *et al*., 2012; Kang *et al*., 2011; Laczkó-Zöld *et al*., 2009).

Western studies, notably in England, France, and the USA, have isolated alkaloids and studied their antibacterial properties from various *Physalis* species. Additionally, researchers have investigated polysaccharides from *P. alkekengi* calyxes for their antidiabetic effects, demonstrating reductions in blood glucose levels and protection against pancreatic cell necrosis in diabetic mice (Shu *et al*., 2016; Zare Zadeh *et al*., 1997; Nanagulyan *et al*., 2020).

Japanese scientists have extensively characterized over 124 biologically active components from *P. alkekengi*, including steroids, flavonoids (such as luteolin O-β-D-glucopyranoside), and phenolic acids (Barupal *et al*., 2019; Yamaguchi *et al*., 1965; Feng *et al*., 2018). These components display various pharmacological activities, including anti-inflammatory, antitumor, antimicrobial, diuretic, alzheimer's disease effects (Wu *et al*., 2005), antidiabetic, antiasthma, immunomodulatory, and antioxidant effects (Hong *et al*., 2015; Shu *et al*., 2016; Li *et al*., 2018; De Souza *et al*., 2022; Barupal *et al*., 2019; Wang *et al*., 2021; Guo *et al*., 2017).

Russian researchers have explored *P. alkekengi* for its potential benefits in age-related visual impairment, carotenoid content, and biochemical composition. Concurrently, studies at the Institute of Chemistry of Plant Substances in Tajikistan and the Institute of Botany in Uzbekistan have focused on the botanical and chemical properties of *Physalis* species, emphasizing their rich composition of biologically active substances (BAS) such as alkaloids, steroids, flavonoids, carotenoids, polysaccharides, and vitamins.

Overall, *P. alkekengi* stands out for its diverse and rich array of biologically active compounds, contributing to its wide-ranging therapeutic applications in traditional and modern medicine alike (Sudhakaran *et al*., 1999; Khan *et al*., 2018).

Luteolin, a common flavonoid found in various plants including fruits, vegetables, and medicinal herbs, is utilized in traditional Chinese medicine for treating conditions like hypertension, inflammatory diseases, and cancer (Hong *et al*., 2015; Shu *et al*., 2016; Halkuzieva *et al*., 2023). It exhibits diverse biological effects such as anti-inflammatory, antiallergic, and antitumor properties, and can act either as an antioxidant or prooxidant depending on biochemical

conditions (Gharib Naseri *et al*., 2007; Yavich *et al*., 2021; Sudhakaran *et al*., 1999; Moniruzzaman *et al*., 2016). Interest in flavonoids extends beyond their natural benefits in plant-based diets; there is also considerable interest in synthesizing derivatives with enhanced medicinal properties. Flavonoids serve a crucial role in protecting plants from environmental stressors such as UV radiation, temperature fluctuations, heavy metal exposure, and microbial infections. Their potent antioxidant activity is particularly notable for mitigating oxidative stress in plants (Khan *et al*., 2018; Ji *et al*., 2012; Feng *et al*., 2018).

In conclusion, the flavonoid extract from *P. alkekengi* demonstrates a broad spectrum of antimicrobial activity. Moreover, it exhibits significant immunostimulating effects, underscoring its potential therapeutic applications in medicine and health care (Ji *et al*., 2012; Kang *et al*., 2011; Laczkó-Zöld *et al*., 2009; Moniruzzaman *et al*., 2016).

Research on flavonoids is driven by the potential to produce synthetic versions of these compounds with therapeutic effects and the potential benefits of these compounds seen when consumed in plant products. New, potent medications with anti-inflammatory, anticarcinogenic, antiviral, antiparasitic, or bactericidal properties can be made from flavonoids (Yamaguchi *et al*., 1965; Feng *et al*., 2018). Flavonoids play the most noticeable role in protecting plants from various unfavorable environmental factors. These include the effects of ultraviolet radiation, temperature stress, and increased concentrations of heavy metals. Flavonoids play a huge role in protecting plants from bacterial, viral and fungal infections, from parasites and insect damage. One of the most notable functions of flavonoids is their participation in protecting plants from oxidative stress due to their pronounced antioxidant activity (Qiu *et al*., 2008; Shu *et al*., 2016). Research on humans, animals, and cell cultures has demonstrated that flavonoids are beneficial to both human and animal health. Flavonoids act as vitamins, antioxidants, hormone regulators, and antimicrobials because they are abundant in foods including fruits, vegetables, and medicinal herbs.

Flavonoids have been shown to have potential anti-cancer properties. Through kinase inhibition, transcription factor downregulation, cell cycle regulation, and apoptotic cell death, flavonoids prevent the progression of carcinogenesis at multiple stages, including invasion, metastasis, angiogenesis, and cellular transformation (Wang *et al*., 2021; Niazian *et al*., 2021). Researchers in Italy, Spain, Greece, France have firmly proven the positive effect of consumption of plant polyphenols, especially flavonoids on human health and daily consumption of at least 400 g of fruits and vegetables can prevent certain types of cancer and cardiovascular diseases, as well as obesity and diabetes.

The antitumor properties of luteolin isolated from the plant *P. alkekengi* have been studied by Japanese, Chinese, Iranian and many other researchers, especially in the USA, and the molecular mechanism of luteolin's action against cancer cells has been established. Scientists' observations showed that luteolin isolated from the plant *P. alkekengi* served as an anticancer agent for various types of cancer and prevention. Steroidal components, physalins (Physalin A and Physalin B), with high biological activity, were isolated from different parts of *P. alkekengi* and various pharmacological actions of the isolated compounds, such as antimicrobial and antiparasitic, were studied.

The plant's tannins are amorphous or crystalline substances soluble in water and alcohol. They have anti-inflammatory, wound-healing effects and are able to destroy microorganisms in the intestines and stop the infectious process. The calyxes of the *P. alkekengi* plant are rich in polysaccharides, which help normalise the functional activity of T-lymphocytes, stabilize the level of normal antibodies in the blood and reduce the level of circulating immune complexes during cardiac activity. A characteristic feature of the *P. alkekengi* plant is the rich content of carbohydrate polymers-pectins in the composition of the fruit, which are of great importance for the body as a radioprotective, antitoxic, complexing effect. Pectins remove cholesterol from the body, reduce the toxicity of antibiotics, stimulate wound healing, also prevent obesity, normalize intestinal motility, and prevent the growth of putrefactive bacteria.

Lectins are found in *P. alkekengi* plants complex proteins, metal containing glycoproteins that interact with cell receptors in a natural reaction. They have the property of reversibly and selectively binding carbohydrates without causing their chemical transformation, provide transportation and accumulation of carbohydrates, determine the specificity of intermolecular interactions (processes of recognition of macromolecules and cells), and intercellular interactions. Lectins mimic the action of insulin by reducing adenylate cyclase activity in lymphocytes; stimulate tissue immunity, increasing the phagocytic activity of leukocytes; and differentially affect T and B lymphocytes.

Plant pigments - carotenoids are found in large quantities in *P. alkekengi* plants, especially beta-carotenes, zeaxanthins, and cryptoxanthins, which have antioxidant activity, that is, the ability to bind reactive oxygen species formed during the peroxidation of lipids and other organic compounds. They are also used for the prevention and treatment of malignant neoplasms, for the treatment of precancerous diseases such as hepatoma, for the treatment of diabetes mellitus, hereditary photodermatoses and other diseases. It was recently discovered that preneoplastic growths of the oral mucosa in smokers can be eliminated by local and general exposure to beta carotene.

Currently, immunoactive drugs are obtained from various sources and synthetically. Obtaining new, highly effective, non-toxic, safe immunomodulatory drugs based on local raw materials is today an urgent task in modern immunobiotechnology and immunology. In this regard, the search and creation of new immunomodulatory drugs with immunological activity remains an urgent problem. According to the literature, more promising in this regard is the study of biologically active substances, in particular flavonoids isolated from the medicinal plant *Physalis alkekengi*. This is due to the fact that flavonoids have a higher activity on protein synthesis and exhibit pronounced anabolic properties. They reduce body fat, help lower blood glucose levels without affecting insulin levels, and also have an immunostimulating effect. Therefore, in various fields of science, as in medicine, pharmaceuticals and other fields, flavonoid-containing preparations are widely used, in particular for disorders of the cardiovascular and nervous systems and decreased immunity of the body.

5. Conclusion

In this regard, in this work, the optimal conditions for obtaining *P. alkekengi* flavonoid extract were studied. The resulting extract had broad antimicrobial and immunostimulating activity. When studying

the antibacterial activity of flavonoid extract against opportunistic and pathogenic bacteria, *E. coli* 002673/477, *P. aeruginosa* 003841/114, *P. mirabilis* 9, *S. aureus*, *B. subtilis* VKM, *L. monocytogenes*, and *C. albicans* served as indicator strains. The flavonoid extract effectively inhibited the growth of microorganisms and was dose-dependent. As the concentration of flavonoid extract increased, the zones where microbial growth was inhibited increased. The immunostimulatory activity of the flavonoid extract was evaluated using cells isolated from central lymphoid organs (thymus and bone marrow) and peripheral lymphoid organs (lymph nodes and spleen) of animals. The flavonoid extract obtained from the *P. alkekengi* plant had a high immunostimulating effect. The plant extract increased the number of immunocompetent cells in the lymphoid organs of animals: in the thymus, bone marrow and lymph nodes.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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