EXPERIMENTAL RESEARCH

ANTITUBERCULOSIS, ANTIMICROBIC AND ANTIOXIDANT PROPERTIES OF *PERSICA VULGARIS* L. EXTRACTS

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Peach is a medicinal plant which has many traditional applications uses against various diseases. In this study we have evaluated differences in tannins and flavonoids in the composition of flowers and peach leaves and their antioxidant properties. Antibacterial activity of the peach flower and leaf extract was investigated using *Mycobacterium tuberculosis* and *E. coli* by the disk diffusion method. Total fractions of flavonoids and tannins were obtained using ethanol and aqueous extraction, respectively. The antioxidant activity was evaluated using the adrenaline autooxidation test. The results have shown that the peach flower extract contains many flavonoids, tannins that probably account for better antimicrobial effects as compared with the peach leaf extract. This shows perspectives for the use of peach flowers for the treatment of many diseases, especially for tuberculosis, and other diseases associated with overproduction of free radicals.

Key words: peach; flavonoids; tannins; *Mycobacterium tuberculosis; E. coli*

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INTRODUCTION

In the context of medicinal plants, peach is an effective, curative, and natural product. It is used in traditional medicine for the treatment of joints, colds, improving kidney function, pancreatitis, liver cirrhosis, cancer, as well as diuretic and antimicrobial action. External use of the water extract of peach leaves helps to cure eczema, psoriasis, boils, dermatitis, allergic rashes, and other diseases. In addition to vitamins and minerals, peaches are rich in other biologically active composition, such as flavonoids, carotenoids and phenolic acids.

Medicinal compositions based on plant polyphenols demonstrate many pharmacological effects, including antioxidant, anti-inflammatory, anti-carcinogenic, antiviral, or anti-allergic activities [1-5].

Many scientific experiments demonstrated have shown that peaches contain tannic acid, sulfated compounds exhibiting the antibacterial effect and help to heal gum inflammation. *C*-glycosyl compounds play an important role in living organisms. In medicines glycosides are used as anti-oxidants, anti-inflammatory, laxatives, analgesics, and for treatment of heart diseases [6-8].

Flavonoids have antioxidant activity by inhibiting generation of reactive oxygen species (ROS) and by affecting the activity of many detoxifying enzymes, such as cyclooxygenases, lipoxygenases, and inducible nitric oxide synthase [9-11].

The purpose of this study was to investigate the antioxidant and antimicrobial activity of the ethanol extract **of** peach flowers and leaves.

MATERIALS AND METHODS

The flowers and leaves were collected in early spring from the Andijan city of Uzbekistan, dried and milled to a size of 1 mm. All the chemicals used in this study were purchased from «Sigma» (USA)

Total Flavonoids Determination

10000 g of product (leaves or flowers) was placed in a 150 ml flask than was added 30 ml of 90% ethanol containing 1% concentrated hydrochloric acid. The flask is attached to the reflux condenser and heated in a water bath for 30 min at 80°C; the solution was then cooled and filtered through a paper filter into a 100 ml flask. The extraction procedure was repeated in the same plate one more time. The resulting extracts were filtered through the same paper filter into the same flask. The filter was washed with 90% ethanol. The volume of the resultant filtrate was adjusted with 90% ethanol to 150 ml (Solution A).

A 2 ml-aliquot of Solution A (in a 25 ml-flack), was mixed with 1 ml of a solution containing 1% aluminum chloride in 95% ethanol, and the volume was adjusted with 95% ethanol to 25 ml. After 20 min, we measured the optical density of the resultant solution spectrophotometrically at λ=430 nm using a 10 mm light path cuvette. To make a comparison solution, 2 ml of solution A was adjusted with 95% ethanol to the mark in a 25 ml flask. The content of flavonoids in terms of quercetin in percentage (X) was calculated by the formula [12]:

$$x = \frac{D \cdot 25 \cdot 100 \cdot 100 \cdot 100}{764.6 \cdot 2 \cdot (100 - W)}$$

Determination of Tannins

1500 g of the milled sample was placed in a 500 ml flask and after addition of 250 ml of hot water the mixture was boiled for 30 min in the water bath using a reflux condenser. After that the mixture was cooled, filtered and adjusted to 250 ml with water. An aliquot of 25 ml of the filtrate was placed in a 750 ml conical flask, added 500 ml of water and 25 ml of indigosulfonic acid solution. The filtrate was then titrated with 0.02 M potassium

Table 1. The content of tannins, flavonoids in dry extracts of flowers and leafs of *Persica vulgaris* L.

Samples	Tannins, mg/g, dry weight	Flavonoids, QUE, mg/g, dry weight
Flowers of *Persica vulgaris* L.	123.6±3.7	121.5±13.3
Leaves of *Persica vulgaris* L.	68.3±27.3	101.5±15.4

Table 2. Antioxidant activity (AA) the flower and leaf extracts of *Persica vulgaris* L.

Samples	AA,%		
	3 min	5 min	10 min
Flowers of *Persica vulgaris* L.	75.5±4.17	60.9±1.41	46.9±0.37
Leaves of *Persica vulgaris* L.	51.5±5.46	34.7±1.89	21.1±0.32

permanganate solution to yellow staining. In parallel, we conducted a control experiment with distilled water.

The relative content of tannins (X) has been calculated using the following formula:

$$X = \frac{(V - V1) \cdot 0.004157 \cdot 250 \cdot 100 \cdot 100}{a \cdot 25 \cdot (100 - W)}$$

where V is the volume of potassium permanganate solution (0.02 mol / L) consumed for titration extraction, in ml; V1 is the volume of potassium permanganate solution (0.02 mol / l) consumed in titration in a control experiment, in ml; A is a sample of raw materials, g; 0.004157 – amount of tannins corresponding to 1 ml of potassium permanganate solution (0.02 mol / l) (in terms of tannin), in g; W - loss in mass during drying of raw materials, in%.

Antioxidant Properties of Extracts

The antioxidant effect of the extract was studied by the ability to inhibit adrenaline autooxidation of *in vitro* and thereby to prevent ROS formation [13]. For this purpose, 0.2 ml of 0.1% epinephrine solution was added to 4 ml of 0.2 M sodium carbonate buffer, pH = 0.65, thoroughly mixed and the optical density was measured spectrophotometrically at λ=347 nm after 30 seconds, and from 3 min to 10 min using a 10 mm light path cuvette (D_1).

Then, 0.06 ml of the leaves or flowers extract and 0.2 ml of 0.1% epinephrine solution were added to 4 ml of 0.2 M sodium-carbonate buffer, pH=0.65, and the optical density (D_2) was measured as above. The antioxidant activity was calculated using the formula [13] :

$$AA = \frac{D_1 - D_2}{D_2} \cdot 100,\%$$

Determination of Antibacterial Activity

The antibacterial activity was tested using cultures of three gram-negative strains *Escherichia coli* Dh5a, *Mycobacterium Smegmatis* and *Mycobacterium tuberculosis* Rv2349c gene. The cultures were gifted by the Department of Immunology and Microbiology of Life Science College of Southwest University (China). Phospholipase c is considered virulent factor in many bacteria. From the experiment we found that Rv2349c increases the permeability of the cell wall of *M. tuberculosis* for hydrophilic components, but reduces the permeability of hydrophobic components [14]. Based on this study we used this gene for learning the effect of the extracts on *M. tuberculosis*. The Rv2349c gene was successfully cloned (the DNA sequence is derived from https://mycobrowser.epfl.ch/) using *M. tuberculosis H37Rv* genomic DNA and special primers [14].

To test the antibacterial activity about 20 g of leaves and flowers were extracted in 130 ml of ethanol at room temperature, respectively [15]. The filtrates were kept at 40 °C for 2 days for evaporation of the solvent and the dry ready extracts were stored for further use.

The antimicrobial activity was tested using the method of disk diffusion in agar [16].These experiment were performed using liquid medium Middlebrook (MB) 7H9 with the addition of 0.05% Tween-80, 0.5% glycerol, and 0.2% glucose or agar Middlebrook (MB) 7H10, supplemented with antibiotic hygromycin (25 μg/ml for mycobacteria and 50 μg/ml for *E. coli*) and ampicillin (50 μg/ml). All cultures were incubated at 37 ° C for 1-3 days.

Each experiment was performed three times. Data represent mean ± SD.

RESULTS AND DISCUSSIONS

The quantitative determination of flavonoids and tannins is given in Table 1, which shows that peach flowers contain 121.5 mg/g of flavonoids and 123.6 mg/g of tannins. This indicates the highest amount of flavonoids and tannins as compared to peach leaves, which contain tannins 68.3 mg/g and 101.5 mg/g of flavonoids.

In this study, the method of antioxidant activity of antioxidants at the initial stage of free radical oxidation was used to inhibit superoxide radical production in the reaction of adrenaline autooxidation [13, 17]. The study has shown that the flower and leaf extracts exhibit the antioxidant activity (Table 2), which was more pronounced in the case of peach flower extract.

Two parts of peach were studied for antimicrobial activity against *E. coli*, *M. smegmatis* and *M.tuberculosis* Rv2349c. The results of the experiment shown in Table 3 indicate, all bacterial strains were sensitive to peach extracts. Flower extracts have shown somewhat higher activity against all types of bacteria than the leaf extract. The results of the experiment confirm the antimicrobial activity and effectiveness of this plant species in relation to various diseases, including tuberculosis. It also shows the promising potential of this plant for therapeutic use and supporting their traditional use in folk medicine.

Table 3. Different bacterial strains and inhibition zone induced by the flower and leaf extracts of *Persica vulgaris* L.

Bacteria used for test	**Diameter of inhibition zone(mm)**	
	flowers	**leaves**
Escherichia coli	18.21±0.34	16. 70±3.57
Mycobacterium smegmatis	16.93±0.95	14.32±4.96
Mycobacterium tuberculosis Rv2349c	15.80±2.69	14.00±0.74

CONCLUSIONS

In recent years, more than 10 million tuberculosis diseases have been recorded in the world. Despite the availability of anti-TB drugs, cases of multidrug-resistant tuberculosis (MDR) have spread [18]. One of the important problems today is the identification of effective drugs from nature is the study of various experiments of traditional medicine. Discover a new drug from biologically active compounds or supplements are considered very relevant. Chemical identification biological additives can lead to identification of many effective medicines and manufacturing broad-spectrum antibiotics.

The study confirmed that the antioxidant power of flowers was somewhat higher than that of leaf extracts.

The presented results encourage the use of peach flowers as food additives in diet during various diseases, including tuberculosis, as well as to prevent human health from depression, stress and diseases caused by free radicals and the pathogenesis of certain diseases.

The data of the study indicate the need for further study of peach flowers to identify specific components responsible for the spread of anti-tuberculosis, antimicrobial and antioxidant properties. It will be interesting to determine, which particular constituents of the extracts studied are responsible for observed biological/pharmacological activities.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

Mamatova Irodakhon designed, performed all experiments, analyzed the data and wrote the article; *Ibragim Askarov* and *Mukhamatdin Mamarakhmonov* analyzed the data and wrote manuscript. All authors read and approved the manuscript.

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ПРОТИВОТУБЕРКУЛЁЗНОЕ, АНТИМИКРОБНОЕ, АНТИОКСИДАНТНОЕ СВОЙСТВО ЭКСТРАКТОВ *PERSICA VULGARIS* L.

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Персик - лекарственное растение, которое имеет много традиционных применений при лечении различных заболеваний. В данном исследовании мы оценили количественные и антиоксидантные свойства танинов и флавоноидов, содержащихся в цветах и листьях *Persica vulgaris* L. Антибактериальную активность экстракта цветов и листьев персика исследовали на культурах *Mycobacterium tuberculosis* и *E. coli* методом дисковой диффузии. Суммарные фракции флавоноидов и танинов были получены с использованием спиртовой и водной экстракций. Антиоксидантную активность оценивали с помощью теста аутоокисления адреналина. Результаты показали, что экстракт цветов персика содержит больше флавоноидов и танинов по сравнению с экстрактом листьев, что обеспечивает лучший антимикробный эффект . Это показывает перспективы использования цветов персика для лечения многих заболеваний, связанных с перепроизводством свободных радикалов, в том числе туберкулеза.

Ключевые слова: персик; флавоноиды; танины; *Mycobacterium tuberculosis; E. coli*

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