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DETERMINATION OF THE ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACTS OF SOME *Passiflora* L. SPECIES, FIRST INTRODUCED IN AZERBAIJAN

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ОПРЕДЕЛЕНИЕ АНТИМИКРОБНОЙ АКТИВНОСТИ ЭТАНОЛЬНЫХ ЭКСТРАКТОВ НЕКОТОРЫХ ВИДОВ *Passiflora* L., ВПЕРВЫЕ ИНТРОДУЦИРОВАННЫХ В АЗЕРБАЙДЖАНЕ

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Abstract. The article presents the research results on the microbiological activity of some *Passiflora* genus species, first introduced in Azerbaijan. Phytochemical compounds were analyzed *in vitro* in ethanolic extracts of *Passiflora incarnata* L., *P. edulis* Sims, *P. caerulea* L. leaves and fruits to determine the antimicrobial activity against six pathogenic bacteria. The activity indicators of ethanolic extracts from leaves and fruits of all three species were compared. Leaf extracts were found to have higher antibacterial activity compared to fruit extracts. Ethanolic extracts of *Passiflora edulis* Sims were more active than extracts from *P. incarnata* L. and *P. caerulea* L. The results of the study may contribute to the development of new medications against pathogenic microbes and the progress of the pharmaceutical industry in the future.

Аннотация. В статье представлены результаты исследования микробиологической активности некоторых видов рода *Passiflora*, впервые интродуцированных в Азербайджане. Фитохимические соединения анализировали *in vitro* в этанольных экстрактах листьев и плодов *Passiflora incarnata* L., *P. edulis* Sims, *P. caerulea* L. для определения антимикробной активности в отношении шести патогенных бактерий. Сравнивали показатели активности этанольных экстрактов листьев и плодов всех трех видов. Было обнаружено, что экстракты листьев обладают более высокой антибактериальной активностью по сравнению с экстрактами плодов. Этанольные экстракты *P. edulis* Sims оказались более активными, чем экстракты *P. incarnata* L. и *P. caerulea* L. Результаты исследования могут способствовать разработке новых препаратов против патогенных микробов и прогрессу фармацевтической промышленности в будущем.

Keywords: plant extract, lyophilizer, disc diffusion method.

Ключевые слова: растительный экстракт, лиофилизатор, диско-диффузионный метод.

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Introduction

Because the world is facing a growing number of pathogenic microorganisms that are resistant to various medications, extensive studies have been conducted to select compounds of new natural sources that are extremely important [19, 23]. The plants are, admittedly, a valuable reservoir of bioactive compounds of substantial medical importance [12].

Innovative technologies and new therapies have brought new drugs with them. However, the dangerous side effects of these medications are inevitable, so interest in plant research has increased and research on their bioactive properties has deepened [16]. Although there are many antimicrobial agents, research on new ones is of great importance. Treatment of bacterial infections is a common problem due to the emergence of many antibiotic-resistant bacterial strains [15, 25]. The lethal effects of plants on pathogens and their important properties for human health have been studied in laboratories since 1926 [27]. It has been already proven that plants with antimicrobial activity, playing an important role in the fight against infectious diseases, have a greater potential in the treatment compared to modern drugs [8, 20].

Plants are not evenly distributed on Earth and the tropics are the richest in terms of plant diversity. Towards the poles, the number of species begins to decline. The richest places in terms of species are the Indonesian islands and the northern parts of South America (http://www.oran.org.tr/). *Passiflora* L. is the largest genus of about 520 species in the Passifloraceae family. This genus is composed of five subgenera: *Astrophea, Decaloba, Passiflora, Tetrapathea,* and *Deidamioides.*

In general, some species of the *Passiflora* genus, especially those having sedative and anxiolytic effects, including Homoeopathic Pharmacopoeia [1, 11] have been officially registered for use in the treatment of central nervous system diseases such as insomnia and anxiety in many countries [24, 28] and various institutions [3, 9, 26]. Some species of the *Passiflora* genus have been used as a source of therapeutic drugs for many years. It has been used as a medicinal plant in traditional medicine since ancient times [24]. According to the literature data, most species of *Passiflora* L. are grown in South America and used in folk medicine in the form of tea by Native Americans. Today, this plant is an integral part of phytopharmaceutical products worldwide. Biological active substances of the *Passiflora* species were explored and isolated. These studies were also performed on phenols and flavonoids [22]. Passicol, one of the chemical compounds in *Passiflora* L. flowers, was reported to have antimicrobial activity [7, 18].

A substance with a polyacetylene structure called Passicol was derived from aqueous extracts of *P. mollissima* (Kunth) Bailey fruit peels and shown to have antifungal and antibacterial properties [13]. Besides, the seeds of the fruit contain a protein (Passiflin) that prevents fungal infections [2]. Antimicrobial compounds in plants are defined as chemicals or biological substances that are able to stop or limit reproduction and most importantly kill disease-causing microorganisms [8]. Unlike synthetic antimicrobials, due to the action mechanisms of plant antimicrobial compounds, they inhibit bacterial growth by a number of metabolic reactions [5]. Freshly plucked leaves of *Passiflora edulis* L. can be used green because they contain vitamin A and niacin. In addition, the leaves contain polyphenols, triterpenes, carotenoids, polysaccharides, especially flavonoids and amino acids that have a positive effect in the prevention of degenerative diseases. In addition to polyphenols, triterpenes, carotenoids, polysaccharides, the leaves contain also flavonoids and amino acids that especially contribute to the prevention of degenerative diseases [17]. Most pharmacological studies on *Passiflora* L. revealed its anxiolytic, antiepileptic, and sedative effects [29]. The cytotoxic, anti-inflammatory and antihypertensive effects of some species were also studied [14].

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Currently, the demand for tropical plants is growing rapidly. The relevance of the cultivation of the tropic *Passiflora* species introduced in Azerbaijan and research on the effects of their substances are substantiated by the richness of biologically active substances in them, the need for identification of new types of raw materials, and the production of these substances. Many studies have focused on the discovery of natural sources and clinically useful antimicrobial drugs and functional foods for pharmaceutical and nutraceutical use [10, 21].

Today, the use of compounds derived from plant extracts is growing rapidly, especially in the pharmaceutical industry. The purpose of this research was to evaluate the antimicrobial and antifungal effects of extracts from some promising species of the *Passiflora* genus: *P. edulis* L., *P. incarnata* L., *P. caerulea* L. It should be noted that no pharmacochemical, biochemical, microbiological analysis of this unique plant has been conducted in our country so far. In addition to antioxidant and anti-inflammatory activities, antibacterial and antifungal properties of its tropical fruit have been studied by scientists around the world.

The purpose of our research was to analyze the secondary metabolic products and antimicrobial activity of some promising species of *Passiflora*, introduced in our country for the first time.

Materials and Methods

Research objects: P. edulis L., *P. incarnata* L., *P. caerulea* L. that are some of the promising species of the *Passiflora* genus, introduced in the experimental field of the Institute of Dendrology of the Azerbaijan National Academy of Sciences in 2018, were chosen as the research objects. The identification and botanical naming of the specimens were based on the scheme of Ulmer and MacDougal [17]. The introduction of all three species has been successfully completed. The above-ground parts of the plants (leaves and ripe fruits) were collected and dried separately under a tent equipped with special racks to prevent their exposure to sunlight and moisture.

Preparation of extracts: To study the antibacterial activity, solutions of 1 mg/ml of ethanolic extracts from leaves and fruits of *P. edulis* L., *P. incarnata* L., *P. caerulea* L. were prepared and analyzed. The extracts were prepared under laboratory conditions using the Maceration method. After the drying process, raw materials were cleaned of the defective parts to provide a condition meeting the requirements of normative and technical documents [4].

The dried plant samples were separated into small pieces using a blender and 20 grams of samples were collected in Erlenmeyer flasks, 100 ml of a solvent (methanol, water, or acetone) was added, shaken, and kept in a water bath at 48–50°C for 6 hours. Then, it was poured through a filter paper [29], into a volumetric flask, which was round and swollen at the bottom and cylindrical at the top, and the solvent was added again and kept for 6 hours. After filtration, the samples were placed in a Rotary Evaporator to completely remove the solvent. After the solvent had evaporated, the remaining extracts in the flask were poured into Petri dishes with water. The extract samples were kept in a lyophilizer at -54°C, for 8–10 hours to completely remove the water inside.

Samples from the lyophilization process were scraped from the Petri dishes with a spatula and placed in glass jars with lids. The samples were stored at -20°C until used [26].

The most commonly used method in the study of antimicrobial effects under laboratory conditions is a disc diffusion test. This test is based on the principle of the diffusion of the antimicrobial substance impregnated on paper disks into the nutrient medium containing microorganisms whose susceptibility is under study. Therefore, paper discs impregnated with antimicrobial substances in certain proportions are placed in a concentrated nutrient medium in which the bacteria to be tested are penetrated. As the discs dissolve and diffuse into agar, the

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penetrated bacteria begin to multiply. Bacteria grow fast in an agar medium. Bacterial colonies appear as small creamy spots on the agar surface. After some time, no multiplication occurs around the disc, where the antimicrobial substance is completely diffused. The more susceptible microorganisms are to the antimicrobial agent, the larger is the size of the inhibitory area around the disc [6].

Gram-negative and gram-positive bacteria, which are the main indicators of purulent-inflammatory processes, were used as a test culture to study the antimicrobial activity of the obtained Passiflora extracts. 0.5 McFarland standard was used in the preparation of microorganism strains. Gram-positive bacteria, which are disease-causing microbes found in the soil, and in the gastrointestinal tract of humans and animals, such as *Bacillus subtilis (*ATCC 6633*), Listeria monocytogenes* (ATCC 7644), *Staphylococcus aureus* (MTCC 554231), and gram-negative bacteria, such as *Escherichia coli* (MTCC 423155), *Proteus vulgaris* (ATCC 49132) of the Enterobacteriaceae genus, a spore-free and capsule-free bacterium found in soil, water, and feces, and blue-green pus bacterium *Pseudomonas aeruginosa* (ATCC 27853) were used as a test culture (cultivation). The method of disc diffusion was used to study antimicrobial activity [9]. The research was conducted in the Natural Products and Biotechnology Laboratory of the Mughla Sitki Kochman University of the Republic of Turkey.

Bacterial strains were provided by the Microbiology Laboratory of the Medical Faculty of the Afyon University, Republic of Turkey. The 6 mm discs made of Whatman paper were sterilized in an autoclave and dried. 0.5 McFarland standard was used during the preparation of microorganism strains [17]. In the method of disc diffusion, a suspension was prepared from a daily culture of a microorganism with a pH of 7.2–7.4 (for bacteria) as a nutrient medium (containing 1 mg of microbial cells per 1 ml). The suspension was then added to a Petri dish containing meat peptone agar (Nutrient Broth) and spread evenly over the surface. The excess liquid was sucked up with a pipette and the Petri dishes were dried in a thermostat for 10–15 minutes. Sterile filter paper discs were soaked in the preparations and placed in a thermostat. The results were recorded after incubation for 24 hours at 37°C in an anaerobic medium. Then, inhibition zones were measured with High Contrast Vernier Caliper. Inhibited areas were measured with three repetitive samples. The antibacterial activities of plant extracts were assessed by measuring the inhibitory zones, formed around the discs after the incubation period, and comparing them with control.

Statistical Analysis

A one-dimensional ANOVA test was used for the variation analysis of the obtained results. The Minitab notation was used to calculate the significant difference between the mean values of the variations and was rated at $p < 0.05$ according to the Tukey Test for Pairwise Mean Comparisons. The results obtained are shown as the mean value ± standard deviation (mean ± SD).

Results and Discussion

Passiflora species are new tropical plant species introduced in Azerbaijan. It is economically and scientifically important to conduct research on the production and adaptation of these species to appropriate environmental conditions.

Passiflora edulis, *P. incarnata*, *P. caerulea* leaf and fruit ethanol extracts obtained by Maceration method were examined for antimicrobial activity. The extracts were tested against 6 pathogenic microbes and showed antibacterial activity against all of them.

The comparison of ethanolic and water extract activities of leaves and fruits of each species revealed a higher antibacterial effect in leaf extracts compared to fruit extracts. The results

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confirmed the ethnobotanical properties of *Passiflora* species used in traditional medicine to treat various diseases caused by microbes. The comparison of leaf extracts showed that ethanolic extracts of the *P. edulis* species had the highest susceptibility to a gram-positive bacterium *Listeria monocytogenes* (ATCC 7644) (15.2±0.6 mm) and a gram-negative bacterium *Pseudomonas aeruginosa* (ATCC27853) (16.7±0.1 mm). While the leaf extracts showed the lowest effect on *Escherichia coli* (MTCC 423155), a gram-negative bacterium (10.4±0.3 mm).

Table

ANTIBACTERIAL ACTIVITY OF CRUDE ETHANOLIC EXTRACT AND ISOLATED COMPOUNDS OF THE LEAVES AND FRUITS OF *Passiflora* SPECIES

Microorganisms	*Antibiotic and negative control*		*Diameter of inhibition Zone (mm) Ethanolic extract 10 mg/ml extract*					
			Passiflora edulis		*Passiflora incarnata*		*Passiflora caerulea*	
	Antibiotic (A)	*Fruit ethanolic extract*	*leaf*	*fruit*	*leaf*	*fruit*	*leaf*	*fruit*
Gram-positive bacteria *Bacillus subtilis* (ATCC 6633)	23.00 (VA)[a]	14.4±0.8	14.4±0.8	7.2±0.2	12.4±0.3	7.4±0.2	9.2±0.8	13.8±0.3
Listeria monocytogenes (ATCC 7644)		13,1±0.4	15.2±0.6	10.5±0.4	11.2±0.1	11.1±0.2	8.4±0.7	12.6±0.7
Staphylococcus aureus (MTCC 554231)	28.00 (A)[b]	15.2±0.6	11.6±0.2	9.4±0.3	13.5±0.3	10.3±0.3	9.2±0.4	10.5±0.1
Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC 27853)	30.00 (MEM)[c]	16.7±0.1	16.7±0.1	6.2±0.6	14.1±0.3	7.5±0.5	8.2±0.7	15.4±0.3
Proteus vulgaris (ATCC 49132)		11.4±0.5	12.1±0.3	10.8±0.4	13.2±0.4	10.6±0.4	9.5±0.2	11.2±0.5
Escherichia coli (MTCC 423155)	24.00 (AMC)[d]	10.2±0.6	10.2±0.6	10.7±0.7	11.6±0.7	9.3±0.3	13.2±0.5	10.4±0.3

p — value < 0.05 is considered significant

A. Antibiotic, a. Penicillin (10 units), b. Vancomycin (30 mg), c. Meropenem (10 mg), d. Amoxycillin / Clavulanic acid 2:1 (30 mg)

The difference in the final results of laboratory tests during the study is due to the difference in the total amount of biologically active substances, antioxidants, flavonoids, phenols in different parts of the species *Passiflora* and in the composition of individual species.

When evaluating the antimicrobial activity of fruit extracts, the strongest effect was manifested by *Passiflora caerulea* ethanolic extracts on *Escherichia coli* (MTCC 423155) (13.2±0.5 mm), and the lowest effect was on a gram-positive bacterium *Bacillus subtilis* (ATCC6633) by ethanolic extracts of *Passiflora edulis* (7.2±0.2) (Table 1). As seen in the table, ethanolic extracts of *Passiflora edulis* have stronger activity than the extracts of *Passiflora*

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incarnata and *Passiflora caerulea*. At the same time, ethanol gave a better result among the solvents used in the production of extracts.

In our study, the antimicrobial activity of *P. edulis* leaf ethanol extracts against *S. aureus*, (10±1.03 mm) and (12.0 mm) corresponds to the indicated level [28, 29].

In microbiological studies, *Passiflora* species showed various antimicrobial activities. According to the results of our microbiological analysis, the antimicrobial activity of the tested plant extracts depends on the part of the plant used (raw material) and the type of extract. Thus, plant extracts are very valuable as natural antimicrobials and can be safely used both in medicine and as preservatives in the food industry.

Our research is designed to investigate and develop clinically important antimicrobial drugs and functional foods from natural sources for pharmaceutical and nutraseptic use, and these experiments have yielded positive results.

In general, *P. incarnata* L. is used in medicine, *P. edulis* L. is a very important tropical species in terms of agriculture and is widely used commercially in the fruit industry.

As a result of our in vitro microbiological analysis, it has been found that *Passiflora* species have antibacterial, antioxidant, antiviral-immune-boosting effects. The spectra of antibacterial actions of some species were found to be wider.

This research contributed to the creation of conditions for gaining new values in addition to the use of leaves and fruits of *Passiflora* species for the treatment of various diseases, as well as fruit juices and other products. Thus, plant extracts are very valuable as natural antimicrobials and can be safely used both in medicine and as preservatives in the food industry.

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