

COMPARATIVE CHARACTERISTICS OF ANTIBACTERIAL ACTIVITY OF PLANTS GROWING IN THE CENTRAL PART OF THE REPUBLIC OF TAJIKISTAN

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Objective: To evaluate the spectrum of antibacterial activity of plants growing in the central part of the Republic of Tajikistan.

Methods: The antibacterial properties of 18 plant species from the 14 families were studied. The extract samples on dry filter discs were prepared according to the method developed at Rutgers University. Screens-to-Nature (STN) and disc diffusion (DD) methods were used to assess antimicrobial properties of the extracts. The antimicrobial activity of plant extracts was determined against four types of pathogenic standard museum strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, as well as by using the same types of microorganisms isolated from inpatients (the hospital strains).

Results: Ethanol extract from leaves of *Artemisia absinthium* demonstrated a broad-spectrum high degree of antimicrobial activity against both reference and hospital strains of *S. aureus*, *P. aeruginosa* and *E. coli*. The *Morus nigra* extract also showed broad-spectrum activity, however overall antibacterial activity was lower than in *A. absinthium*. In general, tested extracts were less effective against *E. coli*. None of the 18 tested extracts showed activity against *K. pneumoniae*.

Conclusions: *Artemisia absinthium* and *Morus nigra* have a broad-spectrum antimicrobial effect and differ only in the degree of activity. The extracts of *Amaranthus tricolor* and *Arctium tomentosum*, *Indigofera tinctoria*, *Punica granatum* were characterized by a somewhat narrower spectrum of action. *Mentha arvensis* and *Allium suworowii* had approximately the same degree of inhibitory ability.

Keywords: Plants, growth inhibition, extracts, hospital strains of bacteria, reference strains of bacteria.

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СРАВНИТЕЛЬНАЯ ХАРАКТЕРИСТИКА АНТИБАКТЕРИАЛЬНОЙ АКТИВНОСТИ НЕКОТОРЫХ РАСТЕНИЙ, ПРОИЗРАСТАЮЩИХ В ЦЕНТРАЛЬНОЙ ЧАСТИ РЕСПУБЛИКИ ТАДЖИКИСТАН

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Цель: Оценить спектр антибактериальной активности некоторых растений, произрастающих в центральной части Республики Таджикистан.

Материал и методы: изучены антибактериальные свойства 18 видов растений из 14 семейств. Из растительных препаратов были выделены экстракты, которыми пропитывались стекловатные диски, затем высушенные диски накладывались на поверхность питательной среды, предварительно засеянной культурой микроба. После инкубации по зоне подавления роста микроорганизмов изучалась активность экстрактов растений относительно микроорганизмов. Антимикробная активность растительных экстрактов определялась диско-диффузионным методом с использованием четырех видов патогенных стандартных музейных штаммов – *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, – а также с применением этих же видов микроорганизмов, изолированных от стационарных больных.

Результаты: экстракт из листьев *A. absinthium* обладает широким противомикробным действием против как музейных, так и изолированных госпитальных штаммов *S. aureus*, *P. aeruginosa* и *E. coli*. Экстракт из *Morus nigra* также продемонстрировал широкую антимикробную активность, однако несколько меньшую, чем *A. absinthium*. Протестированные экстракты были менее активны в отношении *E. coli*. Ни один из используемых 18 экстрактов не проявлял активности по отношению к *K. pneumoniae*.

Заключение: широким противомикробным действием характеризовались экстракты *Artemisia absinthium* и *Morus nigra* и отличились между собой лишь по степени активности. Несколько узким спектром действия характеризовались экстракты *Amaranthus tricolor*, *Arctium tomentosum*, *Indigofera tinctoria*, *Punica granatum*. Экстракты *Mentha arvensis* и *Allium suworowii* имели примерно одинаковую степень ингибирующей способности.

Ключевые слова: растения, ингибирование роста, экстракты, госпитальные штаммы бактерий, референсные штаммы бактерий.

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INTRODUCTION

The discovery of the first antibiotic penicillin in 1928 can rightly be called a revolution in biology, pharmacy and medicine. The synthesis and preparation of this pure antibiotic in the forties of the last century by the "Oxford Group" led by Chain E and Florey HW [1] saved the lives of tens of millions of people who could have died from so-called weak pathogenic bacteria, especially during the World War II [2]. However, the «triumph» did not last long: already during the preclinical trial of penicillin, the problem of antibiotic resistance of microorganisms to this antibiotic arose. A. Fleming in his speech at the Nobel Prize ceremony in medicine warned of the possibility of the formation of resistant microorganisms if antibiotics were used incorrectly, irresponsibly and unnecessarily [3].

The misuse and overuse of antimicrobials are accelerating the development of resistance to antibiotics. The consequences of antimicrobial resistance, besides the failure to treat infections, result in prolonged illness, death, and production losses, including higher costs for treatment and healthcare and drain national and global economies [4, 5]. Along with a decrease in the efficacy of antibiotics, another major problem with their use is possible adverse reactions, including their toxicity, especially in children [6]. As it is known, many synthetic antibacterial and antifungal drugs are characterized by ototoxicity, nephrotoxicity, hepatotoxicity, neurotoxicity and the occurrence of allergic reactions. These properties often cause serious complications and disability of patients, even death [7].

Therefore, there is a need to intensify the search for new sources of antibacterial and antifungal compounds, which should have a broad spectrum of action, be characterized by the least toxicity and not have allergic effects. According to most researchers, the most suitable sources are various plants and herbs [8, 9].

Given the fact that about 500 000 plant species exist on Earth, one can assume that a rich potential supply of medicinal sources, including plants with antibacterial and antifungal properties, is at our disposal [10].

Primitive people began to use plants in the treatment of diseases. Long before our era, written instructions on the types and methods of using herbs in medicine already appeared in ancient Egypt, India, China, and later in Greece and Rome. The number of medicinal plants described in these countries reached 12 thousand species. Of great interest is the so-called Tibetan medicine, which arose about 3 000 years BC based on even more ancient Indian medicine and supplemented by information from Chinese and Mongolian sources [11].

The content of biologically active substances determines the antibacterial and antifungal properties of plants. Among biologically active substances of plant origin with activity against microorganisms, phenolic compounds occupy a special place. Polyphenols, which make up the active substance, are found in many medicinal plants [12].

When searching for plants with antimicrobial activity, it is necessary to bear in mind the fact that the concentration of physiologically active compounds in various plant organs differs, and the chemical composition of each plant organ also varies significantly in different phases of its development [13, 14]. The Republic of Tajikistan has unique climatic features. The nature of the region is characterized by diversity, sharp contrasts and the presence of magnificent landscapes. The mountainous nature of the terrain and the location of Tajikistan on the border between the temperate and subtropical zones, creates an extreme variety of local climatic conditions [13, 15]. The exceptional richness of the plant world of the republic, covering both the hot lowlands and the cold peaks of the mountains,

depends on this variety of conditions. The flora of Tajikistan is characterized by a tremendous genetic, ecological diversity and exceptional species richness and is represented by 9771 species and 20 types of vegetation [16]. The portion of flowering plants alone includes about 5 000 species. From the mentioned number, about 1 700 species are included in medicinal plants, which are actively used in phytotherapy [17]. Meanwhile, there is no data about their antibacterial and antifungal effects and their possible application with this purpose.

In this context, it should be noted that in the Republic of Tajikistan the production of antibacterial, antifungal and antiviral drugs has not been established. The need for the population is ensured by the import of antibiotics from countries near and far. As a result, it affects the cost and quality of drugs, because the rules of storage and transportation are not always observed during drug delivery. At the same time, the prevalence of imported innovations significantly limits the development of the infrastructure for the production of new drugs in Tajikistan.

Therefore, one of the urgent tasks of the healthcare authorities and specialists of the Republic of Tajikistan in the field of botany, pharmacy, and medicine is the search for plants with effective antibacterial and antifungal activity, the rational use of medicinal plants and herbal raw materials, as well as the creation and implementation of import-substituting drugs in accordance with principles of evidence-based medicine.

PURPOSE OF RESEARCH

To evaluate the spectrum of antibacterial activity of selected plants growing in the central part of the Republic of Tajikistan.

MATERIALS AND METHODS

Plant material. Samples from 18 plant species of the 14 families were collected in an area of the central part of the Republic of Tajikistan in the village of Sufiyon of the Vahdat district and Romit gorge at an altitude of more than 1000 meters above sea level (Table 1).

Preparation of plant extract, filter discs and nutrient agar to determine antibacterial activity. Working concentration and paper discs were prepared according to the method developed at Rutgers University [18]. Briefly, two grams of the plant material was weighed and cut into small pieces with a knife. The sample was placed in a marked by a permanent marker 20 ml scintillation vial. With a clean syringe, four ml of 95% ethanol was measured and added to the vial. Grinding and simultaneous extraction of the plant tissue was performed with the specially adapted cordless, variable speed, Dremel rotary tool, Model 8220, 12V for 10 minutes. The contents of the vial were settled for at least 5 minutes and then filtered 90 µl of the plant extract were instilled onto each 10 mm in diameter Whatman1glass microfiber filters, Grade GF/D (Whatman #1823±010, purchased from Millipore, Sigma) carefully monitoring the distribution of the extract so that it was not particularly concentrated in one section of the disc. Discs were dried at room temperature. When air-dried, discs were placed inside the plastic bags pre-labeled with an identification number.

The antimicrobial activity of plant extracts was determined against four types of pathogenic standard reference microorganisms (reference strains): *Staphylococcus aureus* (ATCC 4929), *Pseudomonas aeruginosa* (ATCC 4930), *Escherichia coli* (ATCC 4928), and *Klebsiella pneumoniae* (ATCC 4927), as well as hospital strains of the same types of bacteria, i.e. strains isolated from inpatient surgical patients.

Table 1 Plant samples used for antibacterial evaluation

Sam- ple ID	Altitude (m)	Longitude	Latitude	Plant		Family	Part used
				Common name	Scientific name		
Collected on September 3, 2018 at upper Sufiyon							
01	1088	380 34' 7.4"	690 83' 4.5"	Armenian Mallow	<i>Althaea armeniaca</i> Ten.	Malvaceae	Flowers
02	1092	380 34' 9"	690 83' 41"	Dog rose	<i>Rosa canina</i> L.	Rosaceae	Fruit
03	1086	380 34' 16.7"	690 83' 5"	Wormwood	<i>Artemisia absinthium</i> L.	Asteraceae	Leaves
04	1090	380 34' 8.3"	690 83' 4.8"	Redroot pigweed	<i>Amaranthus retroflexus</i> L.	Amaranthaceae	Stem
05	1090	380 34' 7.2"	690 83' 5.2"	Joseph's coat	<i>Amaranthus tricolor</i> L.	Amaranthaceae	Stem
06	1089	380 34' 7"	690 83' 4"	Chickweed	<i>Stellaria media</i> (L.) Vill.	Caryophyllaceae	Leaves
07	1085	380 34' 6.7"	690 83' 3.4"	Woolly burdock	<i>Arctium tomentosum</i> Mill.	Asteraceae	Seeds
08	1085	380 34' 7.3"	690 83' 4.7"	Henna tree	<i>Lawsonia inermis</i> L.	Lythraceae	Leaves
Collected on September 10, 2018 at lower Sufiyon							
09	1091	380 34' 7.3"	690 83' 4.1"	White mulberry	<i>Morus alba</i> L.	Moraceae	Leaves
10	1111	380 34' 17.6"	690 83' 3.3"	Blackberry	<i>Morus nigra</i> L.	Moraceae	Leaves
11	1091	380 34' 7.1"	690 83' 3.3"	True indigo	<i>Indigofera tinctoria</i> L.	Leguminosae	Leaves
12	1092	380 34' 7"	690 83' 3.2"	Corn	<i>Zea mays</i> L.	Poaceae	Stigmas
13	1103	380 34' 7.1"	690 83' 3.9"	Mint	<i>Mentha arvensis</i> L.	Lamiaceae	Leaves
14	1107	380 34' 17.9"	690 83' 2.4"	Buckthorn	<i>Hippophae rhamnoides</i> L.	Elaeagnaceae	Leaves
15	1085	380 34' 6.9"	690 83' 3.4"	Pomegranate	<i>Punica granatum</i> L.	Lythraceae	Leaves
16	1090	380 34' 6.9"	690 83' 3.2"	Peppers	<i>Capsicum annuum</i> L.	Solanaceae	Leaves
17	1088	380 34' 6.9"	690 83' 3.7"	Poplar	<i>Populus cataracti</i> Kom.	Salicaceae	Leaves
18	1370	380 44' 4.4"	690 20' 0.9"	Allium	<i>Allium suworowii</i> Regel	Alliaceae	Bulb

Preparation of inoculum [19]. The bacterial strains were scattered over the surface of the corresponding nutrient media in Petri dishes: *S. aureus* – on staphyloagar, *E. coli* – on Endo medium, *P. aeruginosa* and *K. pneumoniae* – on simple agar. Subsequently, in order to obtain a pure culture, one isolated colony of a certain type was reseeded onto the corresponding slant agar medium. Suspensions (inoculums) were prepared from diurnal cultures of the studied strains using McFarland turbidity 10 IU, bringing the final concentration of microorganisms to 2×10^6 CFU/ml.

Evaluation of the antimicrobial activity of plant extract by Screens-to-Nature Method. We used modified Screens-to-Nature (STN) bioactivity assays developed by Global Institute for BioExploration [11, 20, 21]. Specifically, antibacterial assays were performed in Multiwell Polystyrene Greiner Bio-One 24-well plates, which were provided from the plant laboratory of Rutgers University, USA, i.e. 4 plates for reference and 4 plates for hospital strains of microorganisms (1 plate for 1 studied strain).

To prepare the semi-solid nutrient medium, 1.2 g of LB powder and 0.3 g of dry agar were added to 30 ml of distilled water. The mixture was thoroughly mixed and autoclaved at $1,200^\circ\text{C}$ for 30 minutes. The prepared agar was stored in a water bath (so the agar did not harden) and 300 μl were poured into each well. The plates were covered with a plate cover and left for 5-10 minutes at room temperature (so that agar hardened). The wells of each plate were numbered by marking the plate cover with the plant ID (Fig. 1).

Then, extracts of a certain plant species were added to the marked wells. The plates were shaken slightly to distribute the extract evenly over the surface of nutrient agar in the wells. After that, 10 μl of suspensions of the reference and hospital strains of bacteria used during the procedure were added to each well. To confirm bacterial growth, plant extracts were not added to the wells marked with «-». As a positive control, to the marked «+» wells, were added 10 μl of Twaxipim (Cefepime hydrochloride), an antibiotic that has a wide spectrum of action against gram-positive and gram-negative

bacteria. The plates were incubated at 37°C for 24-48 hours. The results of the study were evaluated as follows: the well is covered with a lawn of bacterial growth, no antibacterial activity (0); $>50\%$ – of well-covered colonies – low antibacterial activity (1); $<50\%$ – of well-covered colonies – moderate antibacterial activity (2); no colony growth, as in positive control, – high antibacterial activity (3).

Studying the antimicrobial activity of plant extract by disc diffusion method [22]. For this assay, 10 μl of suspensions of the studied strains of microorganisms were introduced into the center of the Petri dish with the appropriate nutrient medium and with a sterile spatula distributed over its surface. Discs impregnated with an extract of a certain type of plant were placed on the surface of

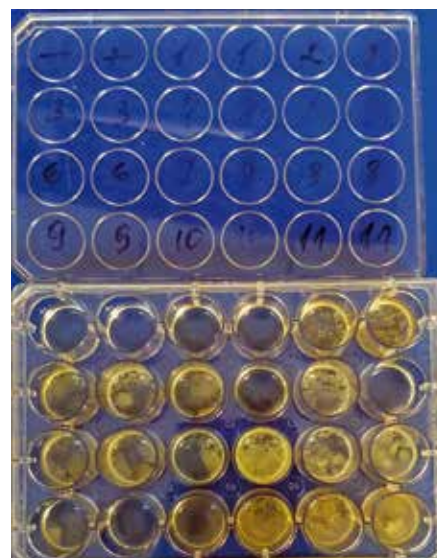


Fig. 1 Determination of antibacterial activity of plant extracts by STN method

the inoculation at a distance of 2.5 cm from the center of the dish in a circle. Inoculations were incubated 24-48 hours in an incubator at 36°C. After incubation in the background of a uniform bacterial lawn around discs with extracts having antibacterial activity, a zone of growth inhibition of colony was observed. The results were taken into account by the presence or absence of growth around a glass wool disc containing an extract of a certain type of plant (Fig. 2).

In the absence of antibacterial activity around the paper disc, a continuous growth of the studied bacterial strain was observed. As a positive control, a paper disc impregnated with Twaxipim (Cefepime hydrochloride) antibiotic solution was used. It was conditionally accepted that the diameter of a growth inhibition of a microorganism over 15 mm constitute high antibacterial activity (3), 10-15 mm – moderate antibacterial activity (2), 6-10 mm – low antibacterial activity (1), no inhibition – no antibacterial activity (0).

RESEARCH RESULTS

Antibacterial activity of plant extracts against the reference and hospital strains of *Staphylococcus aureus*. A gram-positive *S. aureus* is a common part of the body microbiota and an opportunistic pathogen. Given the special clinical significance of *S. aureus* [23], and methicillin-resistant strains in particular, initially, the activity of all the extracts was tested against the reference and hospital strains of this bacteria.

As shown in Table 2, from 18 tested plants 14 (*A. suworowii*, *A. armeniaca*, *A. tricolor*, *A. tomentosum*, *A. absinthium*, *C. annuum*, *H. rhamnoides*, *I. tinctoria*, *L. inermis*, *M. arvensis*, *M. alba*, *M. nigra*, *P. granatum*, *S. media*) showed different degrees of antibacterial activity against *S. aureus* strains.

The extracts of *A. absinthium* and *A. tomentosum* exhibited the highest antibacterial activity, both against reference and hospital strains of *S. aureus*. However, for the hospital strain, the antibacterial effect of both extracts was somewhat lower and the growth inhibition zone around the discs impregnated with these extracts was just over 10 mm. The methods used in our assays showed a high

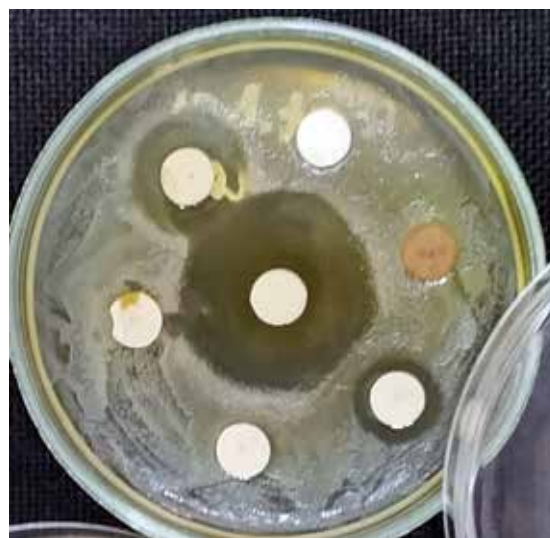


Fig. 2 Determination of antimicrobial activity of plant extracts by disc-diffusion method

consistency of the results. The results obtained by different methods were identical for 83% tests in the hospital strain and 55% in the reference strain. When the results were different, with the exception of the reference strain for *A. suworowii* and *M. arvensis*, the higher level of antibacterial activity was observed in the STN assay. For six species (*C. annuum*, *L. inermis*, *M. alba*, *M. nigra*, *P. granatum*, *S. media*) a higher level of antibacterial activity against the reference strain of *S. aureus* was observed using the STN method compared to the disc diffusion method. Against the hospital strain of *S. aureus*, a higher level of antibacterial activity was observed for three species (*A. suworowii*, *A. tomentosum*, *A. absinthium*) using the STN method compared to the disc diffusion method. Overall, tested extracts were more active against the reference *S. aureus* strain compared to the hospital strain. In the STN assay six species (*C. annuum*, *L. inermis*, *M. alba*, *M. nigra*, *P. granatum*, *S. media*) and in the disc diffusion

Table 2 Antimicrobial activity of extracts against the reference and hospital strains of *Staphylococcus aureus*

Species	Reference strain		Hospital strain	
	STN	Disc diffusion	STN	Disc diffusion
<i>Allium suworowii</i>	1	2	2	1
<i>Althaea armeniaca</i>	1	1	1	1
<i>Amaranthus retroflexus</i>	0	0	0	0
<i>Amaranthus tricolor</i>	1	1	1	1
<i>Arctium tomentosum</i>	3	3	3	2
<i>Artemisia absinthium</i>	3	3	3	2
<i>Capsicum annuum</i>	1	0	0	0
<i>Hippophae rhamnoides</i>	1	1	1	1
<i>Indigofera tinctoria</i>	1	1	1	1
<i>Lawsonia inermis</i>	1	0	0	0
<i>Mentha arvensis</i>	1	2	1	1
<i>Morus alba</i>	1	0	0	0
<i>Morus nigra</i>	2	1	1	1
<i>Populus cataracti</i>	0	0	0	0
<i>Punica granatum</i>	1	0	0	0
<i>Rosa canina</i>	0	0	0	0
<i>Stellaria media</i>	1	0	0	0
<i>Zea mays</i>	0	0	0	0

0 – no activity, 1 – low activity, 2 – moderate activity, 3 – high activity

Table 3 Antibacterial activity of extracts against the reference and hospital strains of *Pseudomonas aeruginosa*

Species	Reference strain		Hospital strain	
	STN	Discdiffusion	STN	Discdiffusion
<i>Allium suworowii</i>	1	1	0	0
<i>Althaea armeniaca</i>	1	1	1	1
<i>Amaranthus retroflexus</i>	0	0	0	0
<i>Amaranthus tricolor</i>	1	1	1	1
<i>Arctium tomentosum</i>	3	3	3	2
<i>Artemisia absinthium</i>	3	3	3	2
<i>Capsicum annuum</i>	1	0	0	0
<i>Hippophae rhamnoides</i>	1	1	1	1
<i>Indigofera tinctoria</i>	1	1	1	1
<i>Lawsonia inermis</i>	1	0	0	0
<i>Mentha arvensis</i>	1	2	1	2
<i>Morus alba</i>	1	0	0	0
<i>Morus nigra</i>	2	1	1	1
<i>Populus cataracti</i>	0	0	0	0
<i>Punica granatum</i>	1	0	0	0
<i>Rosa canina</i>	0	0	0	0
<i>Stellaria media</i>	1	0	0	0
<i>Zea mays</i>	0	0	0	0

0 – no activity, 1 – low activity, 2 – moderate activity, 3 – high activity

test, four species (*A. suworowii*, *A. tomentosum*, *A. absinthium*, *M. arvensis*) exhibited a high level of antimicrobial activity against the reference strain compared to the hospital strain. It is noticeable that plants of the same family and even genus were quite different in antimicrobial properties. For example, extract from *A. tricolor* exhibited at least some antibacterial activity in all tests and *A. retroflexus* did not inhibit bacterial growth. Of the 18 tested extracts, extracts from four plants (*P. cataracti*, *R. canina*, *A. retroflexus*, and *Z. mays*) did not show any antibacterial activity against the reference or hospital staphylococcus strains. In addition, extracts from five species (*C. annuum*, *L. inermis*, *M. alba*, *P. granatum*, *S. media*) did not suppress the growth of the *S. aureus* hospital strain.

Antibacterial activity of plant extracts relative to the reference and hospital strains of *Pseudomonas aeruginosa*. A gram-negative *P. aeruginosa*, found in soil, water, is associated with the infection of plants and animals. It is an opportunistic human pathogen linked to a wide range of infections. Human colonization usually begins within the gastrointestinal tract, and subsequently spreads to skin, ear, eye, urinary tract, heart, airway and lung tissues. Nosocomial infections associated with *P. aeruginosa* include gastrointestinal infection, urinary tract infections and bloodstream infection and are difficult to treat due to *P. aeruginosa* initiate resistance to many antibiotics and its ability to acquire further resistance mechanisms to multiple class of antibiotics [24]. The results for assays with *P. aeruginosa* were similar to the plant extracts antibacterial properties demonstrated with *S. aureus* strains. As exhibited in (Table 3), out of 18 tested plant extracts, 14 (*A. suworowii*, *A. armeniaca*, *A. tricolor*, *A. tomentosum*, *A. absinthium*, *C. annuum*, *H. rhamnoides*, *I. tinctoria*, *L. inermis*, *M. arvensis*, *M. alba*, *M. nigra*, *P. granatum*, *S. media*) showed different degrees of antibacterial activity against *P. aeruginosa* strains.

The extracts of *A. absinthium* and *A. tomentosum* exhibited the highest antibacterial activity, both against reference and hospital strains of *P. aeruginosa*. However, for the hospital strain, the antimicrobial effect of both extracts was somewhat lower and the growth inhibition zone around the discs impregnated with these extracts was just over 10 mm. The methods used in our assays showed a high

consistency of the results. The results obtained by different methods were identical for 83% tests in the hospital strain and 61% in the reference strain. When the results were different, with the exception of *M. arvensis*, the higher level of antibacterial activity was observed in the STN assay. For seven species (*A. suworowii*, *C. annuum*, *L. inermis*, *M. alba*, *M. nigra*, *P. granatum*, *S. media*), a higher level of antibacterial activity against the reference strain of *P. aeruginosa* was observed using the STN method compared to the disc diffusion method. Against the hospital strain of *P. aeruginosa*, a higher level of antibacterial activity was observed for two species (*A. tomentosum*, *A. absinthium*) using the STN method compared to the disc diffusion method. Overall, tested extracts were more active against the reference *P. aeruginosa* strain compared to the hospital strain. In the STN assay seven species (*A. suworowii*, *C. annuum*, *L. inermis*, *M. alba*, *M. nigra*, *P. granatum*, *S. media*) and in the disc diffusion assay, three species (*A. suworowii*, *A. tomentosum*, *A. absinthium*) exhibited a high level of antimicrobial activity against the reference strain compared to the hospital strain. It is noticeable that plants of the same family and even same genus were quite different in antimicrobial properties. For example, extract from *A. tricolor* exhibited at least some antibacterial activity in all tests and *A. retroflexus* did not inhibit *P. aeruginosa* growth. The biggest difference between affecting the growth of *P. aeruginosa* compared to *S. aureus* was observed with activity of *A. suworowii*. This species demonstrated high antibacterial properties against both *S. aureus* strains and was not active against the hospital strain of *P. aeruginosa*. Of the 18 tested extracts, extracts of four plants (*P. cataracti*, *R. canina*, *A. retroflexus*, and *Z. mays*) did not show any antibacterial activity against the reference or hospital *P. aeruginosa* strains. In addition, extracts from six species (*A. suworowii*, *C. annuum*, *L. inermis*, *M. alba*, *P. granatum*, *S. media*) did not suppress the growth of *P. aeruginosa* hospital strain.

Antibacterial activity of plant extracts against the reference and hospital strains of *Escherichia coli*. Most strains of a gram-negative bacterium *E. coli* are harmless and are part of the normal microflora of the lower intestine. However, some serotypes of *E. coli* can cause serious infections of the gastrointestinal tract and purulent in-

flammatory processes in various organs. The antibiotic-resistant hospital serotypes of *E. coli*, which cause severe clinical forms of nosocomial infectious pathologies, are of particular interest. Overall the activity of tested extracts against *E. coli* was much lower compared to activity against *S. aureus* and *P. aeruginosa*. Only seven (*A. armeniaca*, *A. retroflexus*, *A. absinthium*, *H. rhamnoides*, *M. nigra*, *S. media*, *Z. mays*) out of 18 extracts showed at least some antimicrobial activity (Table 4). Just the extract of *A. absinthium* exhibited the high antibacterial activity against both reference and hospital strains of *E. coli*. The methods used to evaluate the antimicrobial potential of plant extracts against *E. coli* showed high consistency of the results.

For the hospital strain – 89% and for the reference strain – 78% of the results obtained by different methods were identical. The extract from *A. retroflexus* was moderately active against the reference strain of *E. coli* in both STN and disc diffusion assays. However, only low antibacterial activity was found for this extract against the hospital strain. The extracts of three species (*A. armeniaca*, *H. rhamnoides*, and *M. nigra*) were found to be moderately active against the reference strain of *E. coli* in the STN assay. In the remaining assays, these species had low activity or, in the case of *H. rhamnoides*, did not affect the hospital strain of *E. coli*. It is notable that the *Z. mays* extracts showed some low activity against the reference but was ineffective against the hospital *E. coli* strain. It is also noteworthy that the majority of tested extracts (*A. suworowii*, *A. tricolor*, *A. tomentosum*, *C. annuum*, *I. tinctoria*, *L. inermis*, *M. arvensis*, *M. alba*, *P. cataracti*, *P. granatum*, *R. canina*) did not show any activity against *E. coli* strains.

Antibacterial activity of plant extracts against the reference and hospital strains of *Klebsiella pneumoniae*. A gram-negative *K. pneumoniae* is a part of the normal flora of the mouth, skin, and intestines. It is one of the most common nosocomial opportunistic pathogens. The emergence and spread of *K. pneumoniae* antibiotic resistance is well documented [25]. In our assays, none of the tested extracts had an inhibitory effect on strains of these bacteria.

Comparative evaluation of the antimicrobial activity of the plant extracts against bacteria of various groups. The performed STN

and disc diffusion antimicrobial assays showed the variability of plant extract activity against different bacteria. Since no extracts demonstrated antimicrobial activity against *K. pneumoniae*, we concentrated on comparative analysis of antimicrobial activity against reference and hospital strains of *S. aureus*, *P. aeruginosa* and *E. coli*. The methods used in our assays showed a high consistency of the results. For the three aforementioned bacteria, the results obtained by different methods were identical in 85% for the hospital strain and 65% for the reference strain. Table 5 summarized antimicrobial activity identified for tested extracts.

In general, tested extracts were more active against the reference strains of particular bacteria. The extracts were less effective against *E. coli* compared to *S. aureus* and *P. aeruginosa*. The extract from *A. absinthium* leaves demonstrated a broad-spectrum high degree of antimicrobial activity against both reference and hospital strains of *S. aureus*, *P. aeruginosa* and *E. coli*. The *M. nigra* leaves extract also showed broad-spectrum activity however, the overall antibacterial activity of this species was lower than of *A. absinthium*. Extracts from *A. armeniaca* and *H. rhamnoides* moderate antibacterial properties against all three types of bacteria. *A. tomentosum* and *M. arvensis* extracts suppressed the growth of *S. aureus* and *P. aeruginosa* while being absolutely ineffective against *E. coli*. Ten species (*A. suworowii*, *A. retroflexus*, *A. tricolor*, *C. annuum*, *I. tinctoria*, *L. inermis*, *M. alba*, *P. granatum*, *S. media*, *Z. mays*) showed very low antibacterial activity against at least some of the bacteria strains. No antimicrobial activity was observed for *P. cataracti* and *R. canina* extracts.

DISCUSSION

The increasing multi resistance of human and animal infections to antibacterial drugs is one of the key factors in the search for new antibiotic compounds. In the etiological structure of human infectious pathology, a special place is occupied by antibiotic-resistant clinically significant bacteria, which can be called the leading hospital microorganisms: *Staphylococci*, *P. aeruginosa*, and pathogenic

Table 4 Antibacterial activity of extracts against the reference and hospital strains of *Escherichia coli*

Species	Reference strain		Hospital strain	
	STN	Disc diffusion	STN	Disc diffusion
<i>Allium suworowii</i>	0	0	0	0
<i>Althaea armeniaca</i>	2	1	1	1
<i>Amaranthus retroflexus</i>	2	2	1	1
<i>Amaranthus tricolor</i>	0	0	0	0
<i>Arctium tomentosum</i>	0	0	0	0
<i>Artemisia absinthium</i>	3	3	3	3
<i>Capsicum annuum</i>	0	0	0	0
<i>Hippophae rhamnoides</i>	2	1	1	0
<i>Indigofera tinctoria</i>	0	0	0	0
<i>Lawsonia inermis</i>	0	0	0	0
<i>Mentha arvensis</i>	0	0	0	0
<i>Morus alba</i>	0	0	0	0
<i>Morus nigra</i>	2	1	1	1
<i>Populus cataracti</i>	0	0	0	0
<i>Punica granatum</i>	0	0	0	0
<i>Rosa canina</i>	0	0	0	0
<i>Stellaria media</i>	1	0	0	0
<i>Zea mays</i>	1	1	1	0

0 – no activity, 1 – low activity, 2 – moderate activity, 3 – high activity

Table 5 Comparative analysis of the antibacterial activity of plant extracts against the reference and hospital strains of bacteria. Numbers represent the summary of activity identified by both STN and disc diffusion methods

Species	<i>S. aureus</i>		<i>P. aeruginosa</i>		<i>E. coli</i>		Cumulative	
	Reference	Hospital	Reference	Hospital	Reference	Hospital	Total	Average
<i>Artemisia absinthium</i>	6	5	6	5	6	6	34	2.83
<i>Arctium tomentosum</i>	6	5	6	5	0	0	22	1.83
<i>Morus nigra</i>	3	2	3	2	3	2	15	1.25
<i>Althaea armeniaca</i>	2	2	2	2	3	2	13	1.08
<i>Hippophae rhamnoides</i>	2	2	2	2	3	1	12	1.00
<i>Mentha arvensis</i>	3	2	3	3	0	0	11	0.92
<i>Allium suworowii</i>	3	3	2	0	0	0	8	0.67
<i>Amaranthus tricolor</i>	2	2	2	2	0	0	8	0.67
<i>Indigofera tinctoria</i>	2	2	2	2	0	0	8	0.67
<i>Amaranthus retroflexus</i>	0	0	0	0	4	2	6	0.50
<i>Stellaria media</i>	1	0	1	0	1	0	3	0.25
<i>Zea mays</i>	0	0	0	0	2	1	3	0.25
<i>Capsicum annum</i>	1	0	1	0	0	0	2	0.17
<i>Lawsonia inermis</i>	1	0	1	0	0	0	2	0.17
<i>Morus alba</i>	1	0	1	0	0	0	2	0.17
<i>Punica granatum</i>	1	0	1	0	0	0	2	0.17
<i>Populus cataracti</i>	0	0	0	0	0	0	0	0.00
<i>Rosa canina</i>	0	0	0	0	0	0	0	0.00

0 – no antibacterial activity, 6 – maximum of antibacterial activity with the highest 3 points recorded for each of two methods

strains of *E. coli* and *K. pneumoniae*. Our goal was to study the antimicrobial activity of extracts obtained from 18 plant species growing in the central region of the Republic of Tajikistan against these bacteria.

One of the striking differences revealed during the resulting analysis and comparison of our findings with the data of researchers from other countries, is the lack of antimicrobial activity of all tested extracts against the reference and hospital strains of *K. pneumoniae*. Multiple researchers from different countries [26, 27] reported a high antimicrobial activity against *K. pneumoniae* of extracts from many plants also used in this work.

In the available literature, there are numerous reports of the pronounced antibacterial activity of extracts obtained from various parts and organs of *A. absinthium* against *S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*. Interestingly, the opinions of researchers on this issue differ. In the work of Moslemi et al. [28], it has been experimentally shown that this species of *Artemisia* is characterized by high anti-staphylococcal activity. Ahamad et al. [29] also reported high inhibitory effect for the extract from this plant species against *S. aureus*, *P. aeruginosa*, as well as *K. pneumoniae* and not having ability to inhibit the growth of *E. coli*. These data do not always coincide with the results of our studies. The extract we obtained did not exhibit antimicrobial activity against *K. pneumoniae* and simultaneously effectively suppressed the growth of the reference and hospital strains of *E. coli* as well as *S. aureus*, *P. aeruginosa*.

In many countries, the fruits of *M. nigra*, along with being a food source, are widely used to lower blood pressure and as anti-hyperglycemic, diuretic, antioxidant, anthelmintic, antiparasitic and antibacterial treatments [30, 31]. However, information related to the antimicrobial activity of various organs and parts of *M. nigra* varies significantly. According to Aulifa et al. [31], extracts obtained from the stem bark, fruits, and fresh black mulberry juice had an inhibitory effect on *S. epidermidis*, but leaf extract had only a moderate antimicrobial effect on strains of these bacteria. A substantial

degree of bactericidal action of the extract from the leaves of this type of mulberry against the reference strains of *S. aureus*, *E. coli* and *K. pneumoniae* were reported by Khalid et al. [32]. In our case, the extract from the leaves of *M. nigra* inhibited the growth of the reference and hospital strains of *S. aureus*, *P. aeruginosa*, and *E. coli* and did not have effect *K. pneumoniae* strains.

A. armeniaca reportedly is being used in folk medicine as treatment for pneumonia, kidney ailments, and anti-hemorrhagic agent [33]. Similar to *A. absinthium* and *M. nigra*, extracts from *A. armeniaca* flowers had broad-spectrum antibacterial effect. However, *A. armeniaca* effect on the growth of reference and hospital strains of *S. aureus*, *P. aeruginosa*, and *E. coli* was very low. It was demonstrated that methanol extracts of aerial parts of other *Althaea* species, *A. officinalis* L. and *A. cannabina* L. exhibited significant antibacterial activity against a range of food borne bacteria [34].

Health benefits of sea buckthorn (*H. rhamnoides*) fruits and seeds are well known [35-37]. Much less is known about the potential biological activity of leaves [38]. Rich in bioactive phytochemicals [39] the extract from *H. rhamnoides* leaves had a broad-spectrum moderate antibacterial activity and inhibited the growth of all tested strains of *S. aureus*, *P. aeruginosa* and *E. coli*. This concurs with the results of other authors. Marked antibacterial activity of aqueous and hydroalcoholic extracts of Sea buckthorn leaves was shown against *E. coli*, *Enterococcus durans*, *Bacillus cereus*, *P. aeruginosa*, *S. aureus* and *Enterococcus faecalis* [38-40].

According to the scientific literature, in folk medicine of different countries, various species of the *Arctium* genus have been widely used since the ancient times [41] as food [42] and medicine [43]. The chemical composition and healing properties of *Arctium lappa* L. are well researched [44, 45]. Infusions and extracts from various parts and organs of this species of burdock have bactericidal activity against many human infectious agents and are used to treat pathologies of a bacterial, viral and parasitic nature [46, 47]. Experimental studies have established that extracts of this plant species have

an anti-tuberculosis effect [48]. However, in the scientific literature we found insufficient information about the antimicrobial activity of *A. tomentosum*, the most widespread species of burdock in Central Asia. In the Republic of Belarus, fresh leaves of *A. tomentosum* are commonly used as anti-inflammatory agent to alleviate localized pain [49]. Our studies showed that the extract from the leaves of this species of burdock shows antibacterial activity against strains of *S. aureus* and *P. aeruginosa* but does not inhibit the growth of *E. coli* and *K. pneumoniae* strains. It is necessary to note that scientific information on the antimicrobial properties of representatives of the *Arctium* genus, in particular the activity of the *A. lappa*, is ambiguous. Some authors [50] report that extracts obtained from the roots, trunk, or leaves of *A. lappa* are characterized by high bactericidal activity against *E. coli*. Other researchers [51] reported the absence of antimicrobial activity of this species against *E. coli*, which coincides with the results of our observation, despite the fact that we used an extract from another species of burdock (*A. tomentosum*).

Peppermint (*M. arvensis*) one of the world oldest and popular herbs used in cosmetics, food and medicine [52, 53]. At the present stage, its extracts and oil are used in various branches of medicine – in dentistry, dermatology, therapy, treatment of infectious diseases and as an anti-inflammatory agent [53]. In our studies, the extract from the leaves of this plant showed low inhibitory activity against reference and hospital strains of *S. aureus* and *P. aeruginosa*, did not affect strains of *E. coli* and *Klebsiella*, which does not coincide with the results obtained by other researchers [54–56], who reported a high inhibitory activity against the above mentioned bacteria.

We found that the extract from *A. tricolor* has a pronounced antibacterial effect against the reference and hospital strains of staphylococcus. Here, our results do not coincide with the observations of other authors. For example, Musa et al. [57], who studied the antimicrobial activity of extracts from various organs of this type of *Amaranthus* did not find any antibacterial effect, not only against staphylococcus strains, but also against other test bacteria. The results of our observation do not coincide with the data of other authors and, according to the spectrum of action of the extract from *A. tricolor* on the reference and hospital strains of microorganisms used in the work. In our study, this extract showed a distinct effect on strains of *S. aureus* and *P. aeruginosa*, not having activity against strains of *E. coli*. At the same time, several authors reported a pronounced degree of bactericidal activity of *A. tricolor* against these bacteria [58–60].

Usma or black henna (*I. tinctoria*) is used not only in cosmetics but is also widely used in traditional medicine. Based on this fact, we studied the antibacterial properties of the extract obtained from the leaves of this plant. Our observations show that the extract from *I. tinctoria* has negligible antibacterial activity relative to the reference and hospital strains of *S. aureus*, *P. aeruginosa* and did not affect *E. coli* and *K. pneumoniae* strains, which corroborates the results of a study by some authors [61, 62] but is not consistent with the data of other researchers who demonstrated antimicrobial activity of *I. tinctoria* extracts [63] against *S. aureus*, *E. faecalis*, *K. pneumoniae*, *Enterobacter aerogenes*, and *Salmonella paratyphi* [64].

In folk medicine in different countries, different parts of the pomegranate plant (*P. granatum*) has been used since biblical times [65, 66]. It is reported that extracts extracted from the leaves, peel, fruits and root of this plant have antimicrobial activity and are effective against many diseases of an infectious nature [67–71]. It was recently once more confirmed that ethanol extracts from aril, albedo and peel have suppressed growth of *E. coli*, *S. aureus*, *P. aeruginosa* and *Bacillus subtilis* [72]. The leaves hydro-methanolic extract has

antimicrobial activity against *Listeria monocytogenes*, *Clostridium perfringens*, *S. aureus*, *B. cereus*, *Enterococcus faecalis*, *Shigella flexneri*, *Vibrio parahaemolyticus*, *Proteus vulgaris* [73]. According to Nozohour et al. [74] seeds and peel showed inhibitory effects on the growth and proliferation of clinical isolates of *P. aeruginosa* and *S. aureus*. This is similar to our results even though in our case, the activity was much lower.

Henna (*L. inermis*) leaves have been extensively used for centuries in India and many other Asian and African countries for cosmetic dye and in treating skin problems, headache, jaundice, amebiasis and enlargement of the spleen [75]. However, the opinions of the authors [63, 76–78] with regard to its antimicrobial activity differ and do not in all cases agree with the results of our study. There are reports of a high inhibitory ability of its extract against the reference and clinical strains of *P. aeruginosa*, *K. pneumoniae*, *E. coli* and many other etiological agents of purulent inflammatory diseases and pathogens of intestinal infections. For example, Kouadri reported that ethanol extract had moderate anti-inflammatory effect on *S. aureus* and *K. pneumoniae*, while the methanol extract of *L. inermis* exhibited a higher degree of antimicrobial activity against *S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *E. faecalis*, *Enterobacter cloacae* [79]. In our study, the extract from the leaves of this plant only partially inhibited the growth of only reference strain of *S. aureus* and *P. aeruginosa*.

The beneficial properties of representatives of the *Allium* genus are well known [80, 81]. In folk medicine in many countries, their use is especially common as antiviral and immunomodulating agents due to the high content of aromatic oils and volatile products. Natural and climatic factors can also influence their composition. Particular attention is paid to the endemic species of *Allium*. Onion of Suvorov (*A. suworowii*), also known as Anzur, has long been used in Central Asia for canning. It is characterized by a specific taste and aroma. In the course of the study, *A. suworowii* antibacterial properties were studied, where they showed an inhibitory effect on *S. aureus* in an average degree against standard and museum strains, while in case of *P. aeruginosa*, it showed activity only against reference strains and did not show an inhibitory effect against hospital strain and also did not demonstrate antibacterial activity against strains of *E. coli* and *K. pneumoniae*.

The results of our study showed that the extract from *S. media* leave extract has minimal antimicrobial activity against *S. aureus*, *P. aeruginosa* and *E. coli* reference strains, which does not coincide with the data of Melnyk et al. [82], who report its high activity against both *S. aureus* and *P. aeruginosa*. Yildirim et al. [83], on the other hand showed that this species extract does not inhibit the growth of strains of *S. aureus*, *P. aeruginosa*, *E. coli* and was active against *K. pneumoniae*.

The results of our research are not in accord with the data of other authors on the antibacterial activity of the extract from *Z. mays*. According to Saleh et al. [84] and Iwalokun et al. [85], extracts from this plant species give a high inhibition effect against gram-positive bacteria, including *S. aureus* and many gram-negative bacteria – *P. aeruginosa*, *K. pneumoniae*, *E. coli* with a suppression zone. In our case, this extract showed minimal activity against hospital and reference strains of *E. coli*.

The study of the spectrum of antibacterial activity of the examined plants showed that the extract from *C. annuum* also has a minimal suppressive activity against reference strains of *S. aureus* and *P. aeruginosa*. Here, there is some agreement between the results of our research and the data obtained by Koffi-Nevry et al. [86] and Nurjanah et al. [87].

CONCLUSIONS

The results of current study showed that among the 18 plant species growing in the central part of the Republic of Tajikistan, extracts of 16 species have antimicrobial effects of varying strengths. The studied plant extracts differ among themselves, both in the spectrum and degree of antibacterial activity against the reference and hospital strains used in the assays. Extracts from the leaves of *A. absinthium* and *M. nigra* have a broad antimicrobial effect and

differ only in degree of activity. The extracts of *A. tricolor* and *A. tomentosum*, *I. tinctoria*, *P. granatum* were characterized by a narrow spectrum of action; *M. arvensis* and *A. suworowii* – with approximately the same degree of inhibitory ability. In our opinion, the difference between the results of our study and published data may be explained by the effects of intra specific variability in the formation of biologically active compounds, seasonality, methods of extraction of biologically active substances and methods for testing biological activity.

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