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Antibacterial, antifungal, antioxidant activity and polyphenol content of aerial parts and bulbs of Allium schugnanicum.

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ANTIBACTERIAL, ANTIFUNGAL, ANTIOXIDANT ACTIVITY AND POLYPHENOL CONTENT OF AERIAL PARTS AND BULBS OF ALLIUM SCHUGNANICUM

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Objective: To study of antibacterial, antifungal and antioxidant activity, and content of polyphenols in the bulb and aerial parts of an endemic species of onion *A. schugnanicum*.

Methods: An ethanol-based extract was prepared from fresh plants. Total polyphenolic content (Folin) and ABTS antioxidant capacity assays were used to characterized extracts. Extracts obtained from bulbs, peduncle, and seeds demonstrated antibacterial activity against the reference *Staphylococcus aureus* (ATCC 4929), *Pseudomonas aeruginosa* (ATCC 4930) and *Klebsiella pneumoniae* (ATCC 4927) strains, as well as against the hospital strains of the same types of bacteria, i.e. strains isolated from inpatient surgical patients.

Results: The study found that ethanol extracts obtained from all organs of *A. schugnanicum* exhibit high inhibitory activity against the hospital and reference strains. In relation to reference then to hospital strains ethanol extracts were more active. The greatest bactericidal effect on both strains was exhibited by the bulb extracts. The extracts did not inhibit the growth of *Escherichia coli.* Fungicidal action characterized by growth inhibition zone of bulb and seeds, respectively. The extract obtained from the peduncle of this plant exhibited smaller inhibitory activity. The antioxidant capacity of *A. schugnanicum* different parts are shown in all the parts had a large antioxidant activity with dominating antioxidant capacity in seeds. The highest polyphenol level was found in the bulb.

Conclusions: The study showed that *A. schugnanicum* may be a prospective species for developing botanical therapeutics.

Keywords: *Allium schugnanicum, medicinal plants, growth inhibition, extracts, antioxidant activity, hospital strains of bacteria, reference strains of bacteria.*

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АНТИБАКТЕРИАЛЬНАЯ, ПРОТИВОГРИБКОВАЯ, АНТИОКСИДАНТНАЯ АКТИВНОСТЬ И СОДЕРЖАНИЕ ПОЛИФЕНОЛОВ В ЛУКОВИЦЕ И НАДЗЕМНЫХ ЧАСТЯХ ALLIUM SCHUGNANICUM

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Цель: изучение антибактериальной, противогрибковой, антиоксидантной активности и содержания полифенолов в луковице и надземных частях эндемичного вида лука *Allium schugnanicum*.

Материал и методы: Исходные этанольные экстракты были получены из свежих растений. Были изучены общий состав полифенолов (Folin) и ABTS антиоксидантная активность растения. Экстракты, полученные из луковицы, стебля и семян демонстрировали высокую антибактериальную активность относительно трёх видов стандартных музейных микроорганизмов (тест штаммов): *Staphylococcus aureus* (ATCC 4929), *Pseudomonas aeruginosa* (ATCC 4930) и *Klebsiella pneumoniae* (ATCC 4927), а также против госпитальных штаммов этих же видов бактерий, т.е. штаммов, изолированных от стационарных хирургических пациентов.

Результаты: изучение показало, что этанольные экстракты, полученные из различных частей лука *Allium schugnanicum* проявляли высокую ингибирующую активность в отношение как госпитальных, так и референсных штаммов бактерий. *Allium schugnanicum* проявлял более высокую антибактериальную активность к референсным штаммам, чем к штаммам госпитального происхождения. Наибольшей антибактериальной активностью, как к госпитальным, так и к референсным штаммам характеризовался экстракт, полученный из луковицы. Экстракты не подавляли роста *Escherichia coli.* Противогрибковая активность также проявлялась у луковицы и семян, а меньшую фунгицидную активность показал экстракт из стебля. Антиоксидантная активность *Allium schugnanicum* проявлялась в различных частях с доминирующей антиоксидантной активностью в семенах. Наиболее высокое содержание полифенолов обнаружилось в луковице.

Заключение: исследование показало, что *Allium schugnanicum* может быть перспективным лекарственным растением в фитотерапии.

Ключевые слова: *Allium schugnanicum, лекарственные растения, ингибирование роста, экстракты, антиоксидантная активность, госпитальные штаммы бактерий, референсные штаммы бактерий.*

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Introduction

Plants as sources of antibiotics. The development of bacterial resistance to the currently used antibiotics necessitates the search for new antibacterial drugs [1]. The emergence and rapid spread of hospital and socially significant microorganisms, which quickly acquire the drug polyresistance and cause severe clinical forms of infectious pathology is of particular concern [2, 3]. Gram-positive bacteria such as *Staphylococcus aureus* are mainly responsible for postoperative wound infections, toxic shock syndrome, endocarditis, osteomyelitis, food poisoning as well as many pathologies of women in labor and newborns [4-7]. Gram-negative bacteria, in particular *Escherichia coli*, a representative of the normal intestinal microbial flora, may enter an uncharacteristic habitat and impact various organs and tissues, causing infections of the lower urinary tract, otitis, and septicemia. Other types of gram-negative microorganisms like *Pseudomonas aeruginosa* and various types of *Klebsiella*, are also frequent etiological factors of nosocomial infections and are characterized by wide polyresistance [8, 9].

The current situation, along with the high cost of synthetic drug production and their possible adverse effects on the human body compared with herbal preparations, forces scientists to intensify the search for new antimicrobial compounds from various sources [7, 10-13].

People around the world used plants to combat infections for thousands of years [14-16], even without knowing about the existence of microorganisms. It is difficult to know when exactly this practice has begun due to the lack of written or other material evidence. Some plants were used because people learned by trial and error about their healing properties. Other plants were used in cooking, and along with their taste, they also demonstrated antimicrobial effects [17-19]. These include *Allioideae*, a subfamily of plants in the family *Amaryllidaceae*, order *Asparagales*. Various species of this group have been used since ancient times in cooking and medicine [20]. People in China, Egypt, Persia, India, and other countries knew about the antimicrobial properties of garlic, one of the representatives of this family [21-23]. As per historical overview by Lawson [24] the medicinal use of garlic, which began about 5,000 years ago, was first recorded by both the Sumerians of Mesopotamia as well as by the people of ancient India. Garlic was also of great importance in Egypt, where it has been found preserved in Pharaohs' tombs and the Egyptian Ebers Codex of 1,550 BC mentions the use of garlic. The Greeks used garlic to strengthen Olympic athletes and to treat battle wounds. In World War II, Russia, where garlic use has long been popular, used it again for soldiers' wounds when they ran out of penicillin, which resulted in the nickname of "Russian penicillin".

***Allium*. Botanical description.** Significance and use. Genus *Allium* (Onion) comprises a large group of onion- or garlic-scented bulbous herbs of the *Amaryllidaceae* family. *Allium* species have very characteristic flowers with six petals pungent linear leaves. Black seeds maturate in dry capsule fruits. Onion species are found in a diverse spectrum of habitat almost everywhere from highlands to the coast of the seas. The steppe and semi-desert of lowlands and mountains are known for a large variety of *Allium* representatives including many endemic species [25]. Among the species of the *Allium* genus, there are many valuable plants: food, medicinal, and ornamental. Even Dioscorides and Avicenna describe the beneficial properties of onions.

The importance of onions as vitamin-bearing plants has also been long recognized by humans. *Alliums* are characterized by the presence in the tissues of volatile sulfur-containing oils that determine their characteristic onion or garlic odor, which have a strong bactericidal effect. Beneficial properties of *Allium* are often linked to the organosulfur compounds [26]. Bulbs and leaves at the same time have several vitamins including Vitamin C (ascorbic acid), Vitamin B_1 (thiamin), Vitamin B_2 (riboflavin), Vitamin B_3, Vitamin B_6 (piridoxine), Folate, Vitamin E (α-tocopherol), Vitamin K (phylloquinone), minerals (Ca, Fe, Mg, P, K, Na, Zn, Se), and essential oils [27]. All this determines the widespread use of onions in medicine.

Their bactericidal properties of *Allium*, which have long been used in traditional medicine, are best known. Modern medicine uses about ten drugs from various types of onions. The most common are Allylchep and Allylglycer. Their main purpose is the treatment of infectious diseases. In addition, they enhance motor and secretory activity. Mannitol, extracted from onions, is a product intended for nutrition for diabetics [28]. The Natural Medicines Comprehensive Database, one of the most comprehensive and reliable natural medicine resources, lists 746 products containing onion [29] and 3,791 products containing garlic [30].

Over 30 different wild onions grow in Tajikistan, of which more than 14 species found in Badakhshan. Among those are *A. afghanicum Wendelbo, A. carolinianum DC., A. Ramosum L., A. oshaninii O. Fedtsch., A. schugnanicum* Vved. Many of the species are endemic and their biological activity, including antimicrobial activity, was not studied [31, 32].

Among these species, *Allium schugnanicum* Vved., which grows at an altitude of 2100-2600 m, is of great economic interest, including medicinal interest [33]. This species, a narrow endemic from shady montane slopes of western Pamir mountain massifs, belongs to *Allium* L. subg. *Melanocrommyum*. The classification and phylogeny of this group were recently extensively reviewed [34-36].

Purpose of research

The study of antimicrobial, antifungal and antioxidant activity of ethanol extract from different parts of *A. schugnanicum*.

Materials and methods

Plant material. The object of the study was the bulb, peduncle and seeds of *A. schugnanicum*, collected at the Pamir Botanical Garden of the Gorno-Badakhshan Autonomous Region (GBAR), the Republic of Tajikistan (altitude –2300 m). Ethanol extracts obtained from various parts of *A. schugnanicum*, plant with well known antimicrobial activity, were used for comparison in the study of antimicrobial and antifungal properties (Fig. 1).

Fig. 1 *Allium schugnanicum Vved.*

Sample preparation. Working concentration and paper discs were prepared according to the method developed at Rutgers University (Skubel, Dushenkov et al, 2018). 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 5 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.

Culture preparation. The antimicrobial activity of plant extracts was determined against four types of pathogenic standard reference microorganisms (reference strains): *Klebsiella pneumoniae* (ATCC 4927), *Escherichia coli* (ATCC 4928), *Staphylococcus aureus* (ATCC 4929), and *Pseudomonas aeruginosa* (ATCC 4930) as well as hospital strains of the same types of bacteria, i.e. strains isolated from inpatient surgical patients. Antifungal activity was studied against fungi of the genus Candida, the most common cause of fungal infection. 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, to obtain a pure culture, one isolated colony of a certain type was reseeded onto the corresponding slant agar medium. McFarland turbidity 10 IU was used to prepare suspension (inoculum) of 24-hour bacterial cultures of the studied strains with the final concentration of microorganisms 2×10^6 CFU/ml.

The study of antimicrobial and antifungal properties

Chemicals. The chemicals used in the analysis were obtained respectively from Sigma-Aldrich St. Louis, MO: Folin&Ciocalteu's phenol reagent and ABTS [2, 2 azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid)], Fisher Scientific Co., Fair Lawn, NJ: Sodium Carbonate, JTBaker Chemical Co., Phillipsburg, NJ: Potassium Persultfate, and Acros Organics, Morris Plains, NJ: Gallic Acid Monohydrate.

Screens-To-Nature assays. Both total polyphenolic content (Folin) and ABTS antioxidant capacity assays are commonly used for medicinal plants methanolic extracts characterization (Gonçalves, Moreira et al, 2017).

Total polyphenolic content (Folin) and ABTS antioxidant assays are part of the Screens-To-Nature (STN) technology assays panel adopted by the Global Institute for BioExploration (GIBEX) (Dushenkov and Raskin, 2008; Kellogg, Joseph et al, 2010). The STN technology is aimed at overcoming the controversies associated with the conventional approach of bringing plant material (nature) from the developing regions to laboratories in the developed world for testing. It makes possible the initial evaluation of locally grown plant material in just a few days without transporting extracts across international borders. The STN kit consists of a number of field-deployable assays requiring only two grams of fresh plant tissue (root, stem, leaves, etc.). All STN bioassays have been lab-validated and proved to be a viable tool for early-stage discovery of bioactive substances in plants, eliminating the destructive harvesting of plants required for the offsite screening.

Total polyphenolic content (Folin) assay. The protocol based on Folin-Ciocalteu reagent was used to estimate total phenolic content (TPC) in plant tissues with reliable results (Blainski, Lopes et al, 2013). The total polyphenolic content (Folin) assay described elsewhere (Ainsworth and Gillespie, 2007; Gonçalves, Moreira et al, 2017) was performed with some modifications. Briefly, an aliquot of the plant extract (200 µL) was mixed with 200 µL 1N Folin-Ciocalteu reagent extract in Eppendorf tubes. After 10 minutes at room temperature, 300 µl of the 20% Na_2CO_3 solution was added into each tube. All tubes were well shaken and kept at 40°C for 20 minutes, thereafter the tubes were quickly cooled to the room temperature and the absorbance was measured at 760 nm by a portable USB-650-VIS-NIR Red Tide Spectrometer linked to the SpectraSuite software. The concentration of TPC was calculated using gallic acid (0-20 µg mL^{-1}) as a standard. When needed the extracts were diluted to obtain results within the linear range of the gallic acid standard curve. The final results were expressed as µg gallic acid equivalents (GAE) per mL of the extract (µg GAE mL^{-1})

ABTS antioxidant assay. The antioxidant assay was performed based on a technique broadly used in plant sample analyses (Gonçalves, Moreira et al, 2017) as described by Walker and Everette (Walker and Everette, 2009) with some modifications. Briefly, the ABTS [2, 2 azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid)] radical scavenging activity of plant extracts was determined through reaction with potassium persulfate ($K_2S_2O_8$). Potassium persulfate converts ABTS to its radical cation and the initially colorless solution turns dark green. The ABTS radical cation is reactive towards antioxidants, which, when added, convert the dark green ABTS radical cation back to its colorless neutral form. Trolox (6-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) solution was used as a reference standard. Absorbance was read at 734 nm using a portable USB-650-VIS-NIR Red Tide Spectrometer linked to the Spectra Suite software. When needed the extracts were diluted to obtain results within the linear range of the Trolox standard curve. The antioxidant capacity results were expressed as "µg mL^{-1} Trolox equivalents (TE)".

Research results

Antibacterial and fungicidal activity of the extracts. The study found that ethanol extracts obtained from all organs of *A. schugnanicum* exhibit high inhibitory activity against the hospital and reference strains of *P. aeruginosa.* The greatest bactericidal effect on both strains was exhibited by the bulb extract. As can be seen from Table 1, the zone of growth inhibition of the reference strain around the paper disk, impregnated with the extract from the bulb, was 15 mm. For the hospital strain, this indication did not exceed 13 mm. Regarding the reference strain, the same inhibitory activity was characterized by ethanol extracts from the peduncle and seeds – 12 mm each, which is slightly larger than the zone of growth inhibition of the hospital strain – 10 mm and 9 mm, respectively. Similar bactericidal activity was observed in relation to strains of Klebsiella. Thus, the inhibitory growth zone of the reference strain was equal to the growth inhibition zone of a similar strain of *P. aeruginosa*, i.e. 15 mm. Bactericidal activity relative to this strain also did not differ in extracts from the peduncle and seeds – 10 mm inhibition growth zone of the reference; 9 mm and 8 mm, respectively, of the hospital strain.

Noteworthy is the relatively low bactericidal activity of ethanol extracts obtained from all organs against *S. aureus* strains. In particular, the diameter of the zone of growth inhibition of the reference strain around the disk with the bulb extract was just 11 mm, which is 4 mm less than that of the *P. aeruginosa* and *Klebsiella* strains. Though, the antibacterial activity of extracts from the peduncle and seeds was close to that of the two tested microorganisms listed above and ranged from 7 mm to 10 mm. Notably, we did not record inhibition to *E. coli* growth by any of the tested extracts.

***Table 1** Antibacterial activity of extracts of A. schugnanicum*

Part of plant	*P. aeruginosa*		*K. pneumoniae*		*S. aureus*		*E. coli*	
	Zone of inhibition around of disk (in mm)							
	Reference	Hospital	Reference	Hospital	Reference	Hospital	Reference	Hospital
Bulb	15	13	15	12	11	9	0	0
Peduncle	12	10	10	9	10	8	0	0
Seeds	12	9	10	8	8	7	0	0

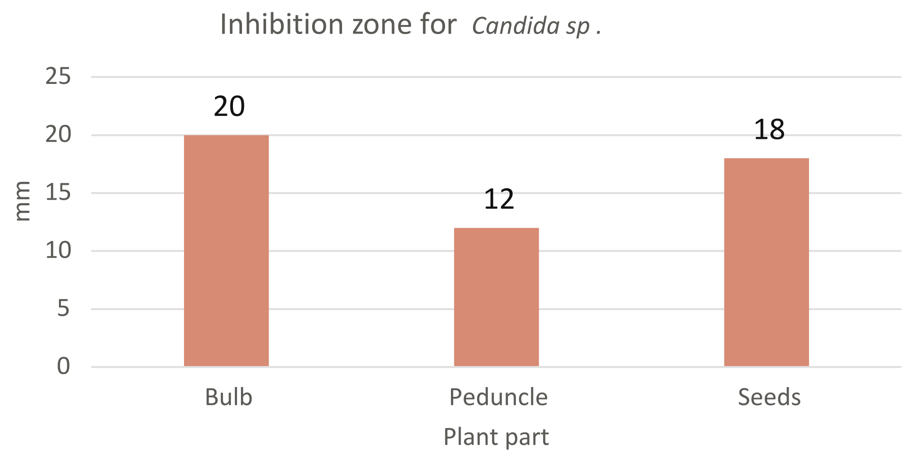

***Fig. 2** Antifungal activity of A. schugnanicum extracts*

Research results indicate that ethanol extracts of *A. schugnanicum* are characterized by a diverse spectrum of fungicidal action.

As shown in Fig. 2, the extracts from the bulb and seeds had the greatest fungicidal activity: the growth inhibition zone around paper disks was 20 mm and 18 mm, respectively. The extract obtained from the peduncle of this plant exhibited smaller inhibitory activity (12 mm).

The antioxidant capacity of *A. schugnanicum* different parts is shown in Fig. 3. All the parts had a large antioxidant activity with dominating antioxidant capacity in seeds (95.4 μg mL^{-1} TE) and bulb (94.6 μg mL^{-1} TE). Peduncle antioxidant capacity was lower at 81.2 μg mL^{-1} TE.

The total polyphenolic content is dramatically different in tested parts of *A. schugnanicum* (Fig. 4). The absolute majority of polyphenols are concentrated in bulb with total polyphenolic content measured at 39.49 μg GAE mL^{-1}. As shown in Fig. 3 peduncle and seeds had the total polyphenolic content measured respectively only at 5.44 μg GAE mL^{-1} and 2.38 μg GAE mL^{-1}.

Discussion

The antibacterial and antifungal effects of the most common species of the *Allium* genus – *Allium cepa* (onions), *Allium sativum* (garlic), *Allium suworowii* (anzur) and some other widely used onion varieties have been known since historical times [37, 38]. There is sufficient scientific information on their biological properties, including bactericidal and antioxidant activities, as well as the content of polyphenols [39-41]. Recently, more attention was devoted to a variety of wild *Allum* species [42]. However, despite the widespread onion plants in various climatic areas of our country, their bactericidal and antifungal activity was not studied before the start of this study.

Based on the foregoing, we were set a goal to study the antibacterial, antifungal, antioxidant activity, as well as the content of common polyphenols of one of the endemic species of onion plants – *A. schugnanicum*. It mainly grows on the territory of the Shugnan ridge of the Gorno-Badakhshan Autonomous Region of the Republic

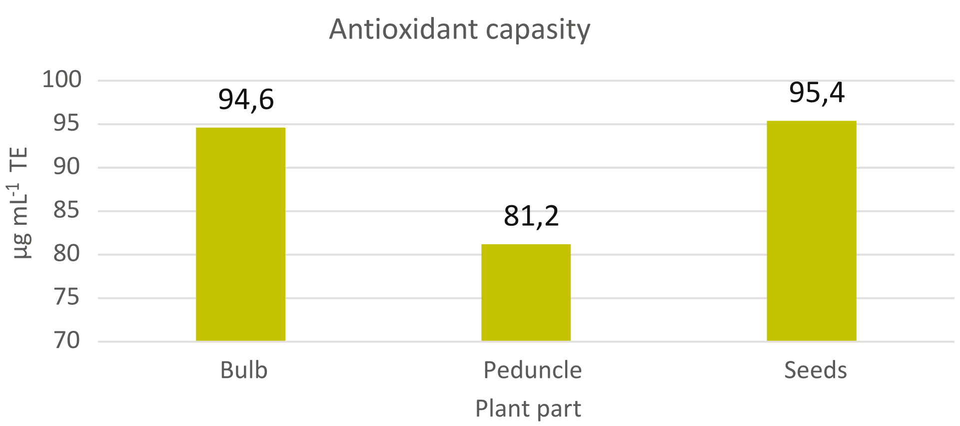

***Fig. 3** Antioxidant capacity of A. schugnanicum extracts*

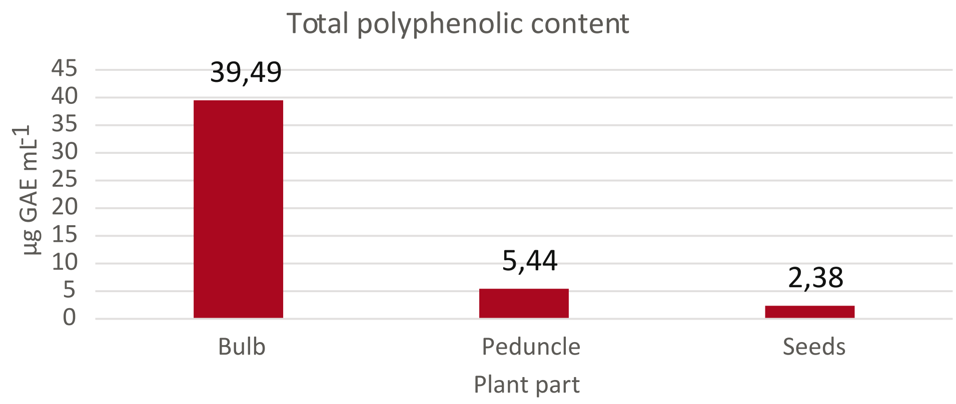

Fig. 4 *Total polyphenolic content of A. schugnanicum extracts*

of Tajikistan and the northern regions of Afghanistan at an altitude of 2100 m above sea level.

The bactericidal and antifungal activity of the extracts against gram-positive and gram-negative bacteria was determined by the disk diffusion method by measuring the growth inhibition zone of test microorganisms around paper disks.

Overall antibacterial activity of *A. schugnanicum* was more pronounced against reference strains compared to hospital strains. In our evaluation bulb extract demonstrated the highest antibacterial activity, followed by peduncle and seeds extracts. The highest antifungal activity was also observed for bulb extract (Fig. 2). The seeds extract exhibit slightly lower antifungal activity. The lowest inhibition zone in Candida experiments was observed for peduncle (12 mm). High antibacterial and antifungal activity of bulb extracts may be connected to a high level of total polyphenols (39.49 µg GAE mL^{-1}). The observed antioxidant capacity was similar for all *A. schugnanicum* parts with a peduncle having the lower levels of antioxidant capacity (Fig. 3). In general, the bulb had the most promising health beneficial properties including antibacterial, antifungal capabilities and high antioxidant capacity paired with high total polyphenol level.

Due to the lack of data on the bactericidal activity of *A. schugnanicum* in the scientific literature and taking into account the presence of common biological characters in representatives of the genus *Allium*, we compared our results with data from other authors who studied similar properties of other types of onions. The analysis shows that our data partially agree with the results of a study by the authors [43, 44] who studied the antibacterial activity of six *Allium* species against gram-positive and gram-negative microorganisms, which report the bactericidal effect of extracts of all the studied species against *E. coli*, the main representative of the normal intestinal microbial flora. In our experiments, the extract of the endemic species *A. schugnanicum* did not affect *E. coli* growth.

A comparative analysis of antibacterial and antifungal activity indicates that extracts obtained from all organs of *A. schugnanicum* are characterized by higher fungicidal activity compared to antibacterial activity. Here, our data are consistent with the results of most authors from Asia, Africa and Latin America [45, 46]. Some authors report the lack of antifungal activity of extracts of various onion varieties, in particular, garlic [47]. However, the objects examined by us and foreign colleagues belonged to different plant species of the genus *Allium* and additional and deeper studies are required.

It is known that plants are considered as a promising source of biologically active compounds possessing anti-oxidative activity. However, in various plants, including representatives of the genus *Allium*, there is a large variability of antioxidant activity [48, 49], therefore it becomes necessary to determine the antioxidant activity of each test plant [50, 51]. An analysis of the literature data suggests that the content of substances of a phenolic and polyphenolic nature plays a role in the level of antioxidant activity [52-55]. Based on the available information, we studied the antioxidant activity and the presence of polyphenols in various parts of *A. schugnanicum*. Our studies show that extracts obtained from the underground and aboveground parts of this plant exhibit approximately the same antioxidant activity. Differences in the concentrations of polyphenolic compounds in extracts from various organs were revealed.

Further research allowed us to establish some correlation between the content of total polyphenols and their bactericidal activity, which was more pronounced in relation to a strain of fungi of the genus Candida. Here, the results of our study are consistent with published data [56] which studied the possible relationship between the content of phenolic compounds and the antibacterial activity of the most common species of the genus *Allium*.

Conclusions

A study of antibacterial, antifungal and antioxidant activity, as well as the content of polyphenols in the bulb and aerial parts, was conducted for the first time for *A. schugnanicum*, an endemic species of onion representatives growing on the territory of the GBAR of the Republic of Tajikistan. Bulb ethanol extract of this species is characterized by more pronounced bactericidal and fungicidal activity compared to other parts. Extracts from all parts of *A. schugnanicum* have similar antioxidant activity. The highest concentration of phenolic compounds was found in the bulb extract. A correlation between the content of polyphenols in plant materials and the bactericidal activity of the studied extract was revealed. The study showed that *A. schugnanicum* may be a prospective species for developing botanical therapeutics.

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