A comparative assessment of microbicidal properties of some medicinal plants of Azerbaijan

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Abstract: A comparative study of microbicidal activity of seven medicinal plants (Carum carvi, Pastinaca umbrosa, Geranium palustre, Mentha longifolia, Rosa canina, Johrenia paucijuga, Malabaila sulcata) on antibiotic-resistant pathogens was conducted. Medicinal plants were studied in the form of infusions, decoctions, essential oils, furthermore ready-made powdered medicinal forms obtained by solid-phase extraction were also used. Diagnosis of intestinal infections was carried out using the microbiological method (Endo, Mannitol Salt agar with egg yolk, blood agar, Sabouraud Dextrode agar), considering clinical, pathological and epidemiological data. Identification of microorganisms isolated during acute intestinal infections was carried out by latex agglutination (E. coli Latex Test), VITEK 2 Compact automated analyzer, Mari Pok diarrheal pannel enabling fast identification (2 hours). Microbicidal activity of extract of Mentha longifolia and essential oil of geranium against Staphylococcus aureus with inhibition zone 26±1.8 mm and 22±2.5 mm, Pseudomonas aeruginosa with inhibition zone 19±0.6 mm and 21±0.3 mm was detected. Essential oil of Geranium palustre had microbicidal properties against Candida albicans with inhibition zone 16±0.2 mm, and the extract of Mentha longifolia was active against Klebsiella pneumoniae (extended-spectrum β-lactamase-ESBL) with inhibition zone 15±1.8 mm. Carum carvi extract was active against Esherichia coli (inhibition zone 20±1.3 mm). The results of our study have revealed that medicinal plants have microbicidal activity and suggest possibility of their application in therapy of diarrheal diseases.

Keywords: antibiotic resistance, diarrheal diseases, microbicidality, plant extracts

INTRODUCTION

The use of plants, as well as their infusions and extracts for medicinal purposes in traditional medicine dates back to ancient times, coinciding with the emergence of the first civilizations [Bhat, 2022]. Knowledge about the beneficial properties and effects of plants has been collected, written and orally transmitted among ethnic groups for centuries [Saranraj, Sivasakthi, 2014; Ozturk et al., 2018]. The study of medicinal plants as a source of biologically active compounds for the treatment of common infectious diseases and for increasing the resistance of the immune system can be considered the best alternative because of their accessibility and effectiveness [Chandra et al., 2017; Roumy et al., 2020]. Natural plant products play an important role as a source of medicinal compounds, and many of the drugs obtained in this way are currently used in pharmacopeias.

Currently, there is a steady increase in human morbidity in the post-pandemic period, due to socio-economic and epidemiological conditions. Among them, intestinal microbiome disorder is one of the frequently occurring diseases. This pathology occupies one of the leading places and is accompanied by the development of various complications, often fatal, as well as the spread of antibiotic-resistant strains of microorganisms [Agayeva et al., 2020; Haziqah et al., 2021; Joakim, Carl-Fredrik, 2022].

The polyetiological of acute intestinal infections (AII), caused predominantly by opportunistic microorganisms (OPM), frequent changes in the etiological agent and its variability, the formation of atypical, uncultivable forms, the heterogeneity of populations of these microorganisms in many phenotypic properties and the low immune response

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of the microorganisms to OPM antigens cause changes in the intestinal microbiome and lead to various pathologies [Alexander, John, 2023; Franca et al, 2024; Shimaa et al., 2023;]. The heterogeneity of the population of OPM in terms of resistance to antibiotics is especially pronounced, which leads to the subsequent selection of antibiotic-resistant strains and their further spread.

Therefore, the study of intestinal infections, often caused by antibiotic-resistant microorganisms, becomes relevant. The resistance of OPM to antibiotics – the causative agents of many infectious diseases, as it previously noted, has reached the scale of a global "pandemic" and is considered as the largest threat to national security [Saranraj, Sivasakthi, 2014; Chandra et al., 2017; Emad et al., 2023]. As it was stated by K. Fukuda "The world is entering a period where antibiotics are becoming less effective, and infections that were once easily treatable for decades now pose a deadly threat once again [Hu et al., 2020; David et al., 2022; Safia et al., 2022].

Thus, the widespread use of chemotherapeutic drugs in medicine and veterinary medicine leads to the formation of resistance of microorganisms, changes in their phenotypic properties, difficulty and often impossibility of their identification [Sheveleva, 2018; Agayeva et al., 2020; Gostev et al., 2021; Lepper et al., 2022].

The rich and diverse medicinal flora of Azerbaijan served as a reason for studying their antimicrobial properties with a purpose of their further application in the treatment and prevention of intestinal infections. The purpose of the study is to reveal a microbicidal properties of medicinal plants of Azerbaijan and the possibility of their use against the dominant antibiotic-resistant pathogens of diarrheal diseases.

MATERIAL AND METODS

Selected plant species. The research work was carried out in 2022-2023. The following plant species Mentha longifolia (L.) L. (Lamiaceae) were collected in Goygol, riverine aquatic vegetation; Carum carvi L. (Apiaceae) in Lankaran, forest edge meadow vegetation; Pastinaca umbrosa Steven ex DC. (Apiaceae) in Goygol, forest vegetation; Geranium palustre L. (Geraniaceae), in Guba, meadow vegetation; Johrenia paucijuga Bornm. (Apiaceae) in Julfa, scree vegetation; Malabaila sulcata Boiss. (Apiaceae) in Guba, shrub vegetation; Rosa canina L. (Rosaceae) in Shahbuz districts of the country. Preparation of medicinal plant substances. Medicinal plants were studied in the form of infusions, decoctions,

essential oils, and ready-made powdered medicinal forms obtained by solid-phase extraction were also used. Aqueous infusion and extract of *C. carvi*, powder form of *P. umbrosa*, essential oils of leaves *G. palustre*, extract of *M. longifolia*, extract of *R. canina*, extract of *J. paucijuga*, extract of *M. sulcata* were involved to the study. Plant raw materials were dried, their moisture content was determined, and infusions of medicinal plants were prepared in accordance with the requirements of the State Pharmacopoeia [State Pharmacopoeia, 1989].

The powdered dosage form of the plants (10 g) was diluted in the distilled water (100 ml) and boiled for 10 min. in a water bath. Then the liquid is cooled at room temperature, filtered through gauze-cotton filters. After it was sterilized by filtration and stored at 4 °C for no more than one day. The aboveground part of the plant (wet and dried) collected in different phases of vegetation was distilled for 3-6 hours by the hydrodistillation method [Ginzberq, 1932], and essential oils were obtained. Extraction, aqueous extracts and essential oils from plants were obtained in the Laboratory of Ethnobotany of the Institute of Botany, MSERA.

Determination of dominant pathogens of diarrheal diseases. Diagnosis of intestinal infections was carried out by microbiological methods. Selective media (Endo, Mannitol Salt agar with egg yolk, blood agar, Sabouraud Dextrode agar) were used taking into account clinical, pathological and epidemiological data. Identification of microorganisms isolated during AII was carried out using VITEK 2 Compact automated analyzer, Mari Pok diarrheal pannel, which allows fast simultaneous identification of several within two hours.

Herbal preparations provided by the Institute of Botany, MSERA were studied using the method of disk diffusion. Test cultures of *S. aureus*, *E. coli*, *C. albicans*, *P. aeruginosa*, *K. pneumoniae*, as well as clinical isolates isolated from diarrheal diseases in children and puppies were used (*E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *C. albicans*).

Serotype identification of *E. coli* was determined in AR with polyvalent OK serum, as well as by latex agglutination method (Latex Test kit (PN541010), PN5406). A suspension of daily cultures prepared in accordance with the turbidity standard 0.5 Mak Farland was used. Sterile disks or strips soaked in herbal preparations were prepared and placed in Petri dishes with Mueller Hinton medium with a microbial culture seeded into the lawn. As a control, 70% ethyl alcohol or petroleum jelly was used to test essential oils (1 ml of a suspension of each test strain from a dilution of 10-1

(108 CFU/ml) after 18-24 hours of incubation at 37°C. Cultivation of *C. albicans* was carried out on Sabouraud nutrient medium at 28°C. The antimicrobial effect of herbal preparations was determined by measuring the sterile area around the disc. The susceptibility testing was performed three times.

Clinical microbiological studies were carried out in the Panacea laboratory and at the Department of Microbiology and Immunology of AMU.

Application of antibiotics. Antimicrobial susceptibility testing was performed in accordance with EUCAST (European Committee on Antimicrobial Susceptibility Testing) guidelines and revealed resistance to Amoxicillin, Ampicillin and Chloramphenicol.

Ethical approval. Ethical requirements in accordance with the law of the Republic of Azerbaijan on June 4, 1999 "On the Animal World" (No. 675-IQ).

RESULTS AND DISCUSSION

Microbial diversity. From examined 70 fecal samples of children with diarrhea 54 cultures of microorganisms were isolated, and 56 were isolated from 65 fecal samples of dog puppies. Of these, 49% were classified as bacteria of the Enterobacteriacea family and nonfermenting gram bacteria, 19% as fungi of the genus Candida, 32% as gram-positive microorganisms, among which S. aureus predominated (Fig. 1). It has been established that the most common causative agents of intestinal infections in children are E. coli, P. aeruginosa, K. pneumoniae. S. aureus, C. albicans.

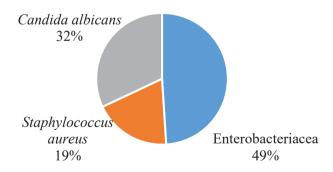


Figure 1. Results of identification of microorganisms in patients with diarrhea.

A diarrheal panel of bacterial etiology has been compiled. The dominant causative agents are *E. coli, K. pneumoniae, P. aeruginosa* and *S. aureus. E. coli* has been classified as EPEC. In case of serotyping, its relation to *E. coli* was determined as 0157:H7; H99; 0127; 0114; 08; 018; 039 serovars.

As a result of the study the composition of fecal microflora in children with diarrhea, a sharp increase in enterobacteria (92.82% versus 10.25% normal), a decrease in lactobacilli (2.47% versus 15.22%) and a significant decrease in bifidobacteria (4.71% versus 68.8%) were determined (Fig. 2).

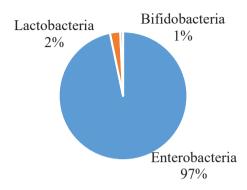


Figure 2. Composition of fecal mycoflora with clinical signs of diarrhea.

A sharp decrease in bifidobacteria and an increase in the number of enterobacteria due to the content in feces of *E. coli* from 103 to 106-8 was established.

Application of antibiotics. The sensitivity of isolated cultures to antibiotics, a tendency towards the spread of antibiotic-resistant strains was observed. Of the 110 strains examined concerning to *E. coli*, (8.2%) were resistant to amoxicillin, (5.5%) to ampicillin and (5.5%) to chloramphenicol. Studies conducted to determine the sensitivity and resistance of *E. coli* to antibiotics in recent years have shown that the number of resistant and polyresistant strains continues to increase. Therefore, the most promising, in our opinion, is the use of medicinal plants (Tab. 1, 2).

Microbicidal properties of medicinal plants. Bactericidal properties were established in relation to the dominant pathogens of diarrhea agents (Tab. 1).

As can be seen from Table 2, the most pronounced bactericidal effect on *S. aureus* is exerted by mint extract (*Mentha longifolia* with a maximum growth retardation zone - 25±1.3 mm), essential oil of geranium (*Geranium palustre* - 20±1.1 mm), extract of *Johrenia paucijuga* - 18±1.6 mm). The most pronounced bactericidal effect for *E. coli* was found in caraway extract (20±1.3 mm).

The most effective against P. aeruginosa were extracts of M. longifolia (22±0.1 mm) and essential oil (20±1.3 mm), of J. paucijuga (16±1.5 mm). When studying the antimicrobial effect of the presented plant extracts on C. albicans, essential oil of geranium had the most pronounced antimicrobial effect (Tab. 3).

The zone of inhibition of *C. albicans* by *Geranium* palustre essential oil was 16 ± 1.7 mm, *Johrenia* paucijuga – 14 ± 1.9 mm, and against *K. pneumonia*e the most active were *M. longifolia* extract – 16 ± 1.7 mm, the zone of inhibition of *G. palustre* essential oil – 11 ± 0.5 mm and *C. carvi* extract – 10 ± 0.5 mm. Thus, a comparative study of the presented medicinal plants showed that mint extract, geranium essential oil, caraway extract had the most pronounced bactericidal effect. So, *S. aureus*, *P. aeruginosa* were most sensitive

to mint extract with growth retardation zone 25 ± 1.3 and 22 ± 0.1 mm respectively and to essential oil of geranium with growth retardation zone 20 ± 1.1 and 20 ± 1.3 mm respectively. Essential oil of geranium also had a pronounced bactericidal effect on *C. albicans* (16 ± 1.7 mm). Caraway extract had the strongest effect on *E. coli* (20 ± 1.3 mm), and mint extract had the most pronounced effect on *K. pneumoniae* (16 ± 1.7 mm).

During the study of the bactericidal properties of seven medicinal plants against antibiotic-resistant pathogens of

Table 1. Plant materials used in this study.

	Study samples of herbal preparations (zone of growth retardation, mm)								Control
Clinical isolates	Carum carvi L. (aqueous infusion)	Pastinaca umbrosa Steven ex DC. (powder)	Carum carvi L. (extract)	Geranium palustre L. (essential oil)	Mentha longifolia (L.) L. (extract)	Rosa canina L. (extract)	Johrenia paucijuga Bornm. (extract)	Malabaila sulcata Boiss. (extract)	Vaseline
S. aureus (MRSA)	0	9±0.1	6±0.3	22±2.5	26±1.8	10±1.1	18±2.2	10±1.1	0
E. coli (GSBL)	12 ± 1.3	5 ± 0.4	19 ± 2.4	10 ± 1.1	11 ± 1.2	8 ± 0.6	17 ± 0.7	5 ± 0.4	0
P. aeruginosa	10 ± 1.1	8 ± 0.6	$8\pm0,6$	19 ± 0.6	21 ± 0.3	10 ± 1.1	16 ± 0.2	8 ± 0.6	0
C. albicans	5 ± 0.4	0	0	16 ± 0.2	0	0	14 ± 2.7	0	0
K. pneumoniae (GSBL)	4 ± 0.2	9 ± 0.1	8 ± 0.6	10 ± 1.1	15 ± 1.8	0	0	10 ± 1.1	0

Table 2. Results of the antimicrobial effect of medicinal plants on test cultures using the disk diffusion method.

	Study samples of herbal preparations (zone of growth retardation, mm)								
Test cultures under study	Carum carvi L. (aqueous infusion)	Pastinaca umbrosa Steven ex DC. (powder)	Carum carvi L. (extract)	Geranium palustre L. (essential oil)	Mentha longifolia (L.) L. (extract)	Rosa canina L. (extract)	Johrenia paucijuga Bornm. (extract)	Malabaila sulcata Boiss. (extract)	Vaseline
S. aureus	0	10±1.4	5±0.1	20±1.1	25±1.3	10± 0.6	18±1.6	10 ± 0.5	0
E. coli	20 ± 0.4	5 ± 0.1	20 ± 1.3	10 ± 0.6	11 ± 0.2	8 ± 1.4	17 ± 0.1	5 ± 0.2	0
P. aeruginosa	10 ± 0.6	8 ± 0.3	8 ± 0.3	20 ± 1.3	22 ± 0.1	10 ± 0.6	16 ± 1.5	8 ± 0.2	0
C. albicans	5 ± 0.2	0	0	16 ± 1.7	0	0	14 ± 1.9	0	0
K. pneumoniae	5±0.3	10±0.5	10±0.5	11 ± 0.3	16 ± 1.7	0	0	9±2.1	0

Table 3. Results of the antimicrobial effect of medicinal plants on clinical isolates using the disk diffusion
method.

	Study samples of herbal preparations (zone of growth retardation, mm)								Control
Clinical isolates	Carum carvi L. (aqueous infusion)	Pastinaca umbrosa Steven ex DC. (powder)	Carum carvi L. (extract)	Geranium palustre L. (essential oil)	Mentha longifolia (L.) L. (extract)	Rosa canina L. (extract)	Johrenia paucijuga Bornm. (extract)	Malabaila sulcata Boiss. (extract)	Vaseline
S. aureus (MRSA)	0	9±0.1	6±0.3	22±2.5	26±1.8	10±1.1	18±2.2	10±1.1	0
E. coli (GSBL)	12 ± 1.3	5 ± 0.4	19 ± 2.4	10 ± 1.1	11 ± 1.2	8 ± 0.6	$17\pm0,7$	5 ± 0.4	0
P. aeruginosa	10 ± 1.1	8 ± 0.6	$8\pm0,6$	19 ± 0.6	21 ± 0.3	10 ± 1.1	16 ± 0.2	8 ± 0.6	0
C. albicans	5 ± 0.4	0	0	16 ± 0.2	0	0	14 ± 2.7	0	0
K. pneumoniae (GSBL)	4 ± 0.2	9 ± 0.1	8 ± 0.6	10 ± 1.1	15 ± 1.8	0	0	10 ± 1.1	0

intestinal infections, a pronounced antibacterial activity of mint extract and geranium extract was established against Methicillin-resistant *S. aureus* (MRSA) strains in the growth retardation zone - 26±1.8 mm, 22±2.5 mm respectively.

Geranium extract also showed the most active effect on *C. albicans* with growth inhibition zone was 16±0.2 mm. However, *C. albicans* was resistant to many herbal preparations. Also, a high bactericidal effect of geranium essential oil was noted against *P. aeruginosa* with 21±0.3 mm and mint extract 19±0.6 mm, respectively. Mint extract had high bactericidal activity against *K. pneumoniae* with 15±1.8 mm, against *C. albicans* the same drug was not effective. For *E. coli* the most active bactericidal property was observed for caraway extract with 19±2.4 mm and *J. paucijuga* with 17±0.7 mm. Thus, of the seven herbal preparations studied, the most pronounced antibacterial activity was established for geranium essential oil, mint and cumin extract.

Often the causative agents of AII are already antibiotic-resistant strains that cause pathologies in association. It is impossible to overcome the resistance of microorganisms to antibiotics, since the latter are a selective marker for the emergence of mutants resistant to chemotherapeutic preparation. Thus, according to the literature, the frequency of detection of penicillin-resistant staphylococci in some regions of the world is 80-90%, streptomycin-resistant - 60-70%, *Escherichia* resistant to penicillin - 89%, laevomycetin - 72.3% and

tetracycline-64.2%, *Shigella* resistant to ampicillin -20%, to tetracycline and streptomycin more than 50% [Antimic. Res, 2014; Gostev et al., 2021; Alexander, John, 2023].

Currently, the most effective and safe method for treating infectious diseases is the search for medicinal plants with a wide spectrum of antimicrobial action. Medicinal plants favorably differ from antibiotics and other chemotherapy drugs in their low cost, lack of side effects, and most importantly, the lack of development of resistance to etiological agents [Ozturk et al., 2018; Ibadullayeva, 2021; Ibadullayeva et al., 2023].

The etiology of diarrheal diseases has been studied, and their dominant pathogens have been identified as E. coli, S. aureus, P. aeruginosa, K. pneumoniae and C. albicans. Antibiotic-resistant pathogens were monitored, and isolates of E. coli and K. pneumoniae producing extended-spectrum β-lactamase (ESBL), as well as methicillin-resistant S. aureus (MRSA) were identified. A comparative study of aqueous infusion, extract and essential oil of the medicinal plants such as C. carvi, P. umbrosa, G. palustre, M. longifolia, R. canina, J. paucijuga, M. sulcata presented in relation to the dominant pathogens of diarrhea, including antibiotic-resistant pathogens, showed that mint extract, geranium essential oil, and caraway extract had the most pronounced bactericidal effect. Thus, S. aureus, P. aeruginosa were the most sensitive to mint extract and geranium essential oil. Geranium essential oil also had a pronounced bactericidal effect on *C. albicans*. Caraway extract had the strongest effect on *E. coli*, and mint extract had the most pronounced effect on *K. pneumonia*.

In conclusion the dominant pathogens of *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *C. albicans* diarrheal diseases have been identified. A comparative study of eight medicinal plants in relation to the main causative agents of diarrheal diseases is presented and the pronounced microbicidal activity of mint extract and *Geranium* essential oil against *S. aureus*, *P. aeruginosa*, *K. pneumoniae* is determined. Essential oil of geranium had microbicidal properties against *C. albicans*, and caraway extract was most active against *E. coli*.

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Azərbaycanın bəzi dərman bitkilərinin mikrobisid xüsusiyyətlərinin müqayisəli qiymətləndirilməsi

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Məqalədə yeddi dərman bitkisinin (*Carum carvi*, *Pastinaca umbrosa*, Geranium palustre, *Mentha longifolia*, *Rosa canina*, *Johrenia paucijuga*, *Malabaila sulcata*) antibiotiklərə rezistent mikroorqanizmlərə qarşı mikrobosid təsiri müqayisəli öyrənilmişdir. Dərman bitkiləri ekstrakt, efir yağı, dəmləmə, eyni zamanda bərk fazalı ekstraksiya ilə alınmış hazır toz şəklində istifadə olunmuşdur. Bağırsaq infeksiyalarının diaqnostikası klinik, patoloji və epidemioloji məlumatlar nəzərə alınmaqla mikrobioloji üsullarla (Endo, yumurta sarılı mannitol duzlu aqar, Sabouraud dekstroz aqar) aparılmışdır. Kəskin bağırsaq infeksiyaları zamanı təcrid olunmuş mikroorqanizmlərin identifikasiyası lateks

agglütinasiya (E. coli Latex Test), VITEK 2 kompakt avtomatlaşdırılmış analizator, sürətli identifikasiyaya imkan verən Mari Pok ishal paneli (2 saat) vasitəsilə həyata keçirilmişdir. Mentha longifolia ekstraktı və ətirşah bitkisinin efir yağının Staphylococcus aureus (inhibisiya zonası 26±1.8 mm və 22±2.5 mm), Pseudomonas aeruginosa növünə (inhibisiya zonası 19±0.6 mm və 21±0.3 mm) qarşı mikrobosid aktivliyi askar edilmisdir. Geranium palustre bitkisindən alınan efir yağı Candida albicans növünə qarşı fungisid (inhibisiya zonası 16±0.2 mm) xüsusiyyətə malikdir. Mentha longifolia ekstraktı Klebsiella pneumoniae (GSBL(+)) əleyhinə (inhibisiya zonası 15±1.8 mm), Carum carvi ekstraktı isə Esherichia coli növünə garsı (inhibisiya zonası 20±1.3 mm) aktiv olmuşdur. Tədqiqat nəticəsində dərman bitkilərinin mikrobosid fəallığa malik olduğu müəyyənləsmis və onların ishal zamanı istifadəsi tövsiyə edilmişdir.

Açar sözlər: antibiotik rezistentliyi, ishal, mikrobosidiallıq, bitki ekstraktları

Сравнительная оценка микробицидных свойств некоторых лекарственных растений Азербайджана

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Проведено сравнительное изучение микробицидной активности семи лекарственных растений (Carum carvi, Pastinaca umbrosa, Geranium palustre, Mentha longifolia, Rosa canina, Johrenia paucijuga, Malabaila sulcata) на антибиотикорезистентных возбудителях. Лекарственные растения изучались в виде настоев, отваров, эфирных масел, а также использовались готовые порошкообразные лекарственные формы,

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полученные методом твердофазной экстракции. Диагностику кишечных инфекций проводили микробиологическим методом (среда Эндо, маннит-солевой агар с яичным желтком, кровяной агар, агар Сабуродекстроде) с учетом клинических, патологических и эпидемиологических данных. Идентификацию микроорганизмов, выделенных при острых кишечных инфекциях, проводили методом латексной агглютинации (комплект для определения латекса Е. coli Latex Test), автоматический анализатор VITEK 2 Сотраст, диарейная панель Mari Pok, обеспечивающая быструю идентификацию (2 часа). Выявлена микробицидная активность экстракта Mentha longifolia и эфирного масла герани в отношении Staphylococcus aureus (зона задержки роста 26±1.8 мм и 22±2.5 мм), Pseudomonas aeruginosa (зона задержки роста 19±0.6 мм и 21±0.3 мм). Эфирное масло Geranium palustre обладало микробицидными свойствами в отношении Candida albicans (зона задержки роста 16±0.2 мм), а экстракт Mentha longifolia был активен в отношении Klebsiella pneumoniae (БЛРС) (зона задержки роста 15±1.8 мм). Экстракт Carum carvi проявил активность в отношении Esherichia coli (зона ингибирования 20±1.3 мм). Результаты наших исследований свидетельствуют о наличии у лекарственных растений микробицидной активности и предполагают возможность их применения в терапии диарейных заболеваний.

Ключевые слова: устойчивость к антибиотикам, диарейные заболевания, микробицидность, растительные экстракты