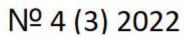


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5

STUDY OF ANTIBACTERIAL EFFECT OF SOME PLANT'S CANDIDATES ON GRAM-NEGATIVE BACTERIA

Sabitova S.G.¹, Baubekova A.S¹, Orazov A.E.^{2,3}, Tustubaeva Sh.T.³, Samarkhanova D.T.⁴

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The increase of the mortality after surgical operations is directly connected with opportunistic microorganisms. Long time rehabilitation and increasing demand for new generations of antibiotics is elevate the problem to new level. The pharmacological companies from around the world try to get solution to high level of resistance of opportunistic microorganisms. However, evolution in the genetic resistance of microorganisms is uncontrollable and the production of synthetic antibiotics is demanding a lot of struggle and cost billions of dollars. Nowadays, traditional medicine is familiar and popular among different countries. This work includes study on antimicrobial activity of extracts of endemic plants in Kazakhstan and several commercially available plants. The list of plants represented in the study are: Thuja occidentalis L., Amygdalus ledebouriana Schltdl., Ferula soongarica Pall. ex Spreng., Zingiber officinale Roscoe., Allium sativum L., Armoracia rusticana P.G. Gaertn., B. Mey. & Scherb., Apium graveolens L. Opportunistic microorganisms that was used during the study are: Escherichia coli and Pseudomonas aeruginosa. Thus, A. sativum revealed antibacterial effect against E. coli at concentration 25% which is the lowest concentration that was presented, while to the extracts of A. ledebouriana both of the strains were sensitive.

Key words*: Thuja occidentalis* L., *Amygdalus ledebouriana* Schltdl., *Ferula soongarica* Pall. ex Spreng., *Zingiber officinale* Roscoe., antimicrobial activity.

INTRODUCTION

Despite the fact that pharmacological ventures have created various new antibiotics over the most recent thirty years, protection from these medications by microorganisms has expanded. In most cases, microorganisms have the hereditary capacity to transmit and obtain resistance from drugs, which are used as part of treatment [1]. Such a reality is cause for concern, in view of the quantity of patients in medical clinics who have stifled invulnerability, and because of new bacterial strains, which are multi-resistant. Subsequently, new diseases can happen in emergency clinics bringing about high mortality.

The issue of microbial resistance is attracting more attention and the perspectives for the utilization of antimicrobial medications later on is as yet questionable [2]. Hence, the solution for the problem has to undertake, for instance, to control the use of antibiotics, discover deeply to more readily comprehend the genes coding resistance in microorganisms, and to proceed with studies to create new medications, either synthetic or natural. A definitive objective is to offer fitting and effective antimicrobial medications to the patient.

For a long time, plants have been an important source of natural products for keeping up human wellbeing, particularly somewhat recently, with more escalated discoveries for natural therapies. As per World Health Organization [3] medical plants would be the best source to acquire an assortment of medications. About 80% of people from leading countries use traditional type of medication, which has intensifies gotten from medical plants [4].

The use of plant extracts can be of great significance in therapeutic treatments, with known antimicrobial property [5]. Over the most recent couple of years, various investigations have been led in some countries to demonstrate such effectiveness [6]. Numerous plants have been utilized due to their antimicrobial qualities, which are due to compounds synthesized in the secondary metabolism of the plant. The medicinal value of plants lies in some chemical substances that produce a positive physiological activity on the human body. The main bioactive mixtures of plants are alkaloids [7], flavonoids, tannins and phenolic compounds [8].

A standout amongst other studied bacterial opportunistic microorganisms is *Pseudomonas aeruginosa* (*Ps. aeruginosa*). This microorganism is at present among the most common reasons for disease at medical clinics and is the significant reason for ongoing infections in cystic fibrosis patients. There are several times, when the single clone of *Ps. aeruginosa* is able to infect patients by maintaining and growing in lungs for a quite a long time [9].

Since *Ps. aeruginosa* is an environmental bacterium, it very well may be imagined that virulent strains establish a particular branch in the species, which is in its course to speciation as it has occurred with "proficient" microorganisms [10].

A couple of hours after infant birth *Escherichia coli* (*E. coli*) colonizes the gastrointestinal tract of human newborns. Generally, *E. coli* and its human host exist together healthy and with common advantage for quite a long time. The place of commensal *E. coli*, where conditions for replication is suitable, is the mucous layer of the mammalian colon. Despite the enormous literature devoted on the genetics and physiology of this species, the mechanisms whereby *E. coli* assures this auspicious

symbiosis in the colon are poorly characterized. In spite of the massive amounts of literature on the genetic qualities and physiology of this species, the components whereby *E. coli* guarantees this favorable beneficial interaction in the colon are not enough studied and presented [11].

Plant extracts and plant-based products are still main source of pharmaceutical agents which are used in traditional medicine. As of late interest in a massive number of traditional natural products has expanded [12]. Microorganisms have developed resistance from numerous antibiotic agents and this has made huge clinical issue in the treatment of infectious disease [13]. Likewise, antibiotics and other non-natural drugs show unfavorable impacts on host like ulcers, hypersensitivity and so on other than their high price. The effects of plant extracts on microorganisms have been discovered by different specialists in various pieces growing all over the world [14, 15, 16].

Thuja occidentalis L. (*T. occidentalis*) and *Thuja orientalis* L. (*T. orientalis*) Franco are two unique species having a place with a similar family Cupresseaceae. *T. occidentalis* are trees that grow in South Eastern Canada and the North Western United States densely and they are native to North America, in addition thay are slow-growing trees. The essential oil from seed layers of *T. orientalis* was evaluated for antimicrobial ability by Jain and Garg et al. (1997) while its cancer prevention agent action was ascribed by Duhan et al. (2013). In view of the above reality, in the current investigation, different concentrates of stem and leaf of *T.occidentalis* were evaluated for their antimicrobial action. The consequences of this examination may additionally fortify the proposal for the utilization of ethno medication in the treatment and control of microbial contaminations.

Ferula soongarica Pall. ex Spreng (*F. soongarica*) is the plant used in medicine as a gum-resin. A perpetual herbaceous plant of the class ferula has a place with the group of celery (umbrella) – Apiaceae (Umbliferae). There are in excess of 160 species of Ferula on the planet, 104 species in the Central Asian Republics, and in excess of 50 species in Uzbekistan. The the Dzungarian ferula is native to Iran, India and Afghanistan. Species of the genus Ferula, the neighborhood populace is called sassik kovrak, rova, ravshan, kamol, murcha kamol and others. For the production of gum tar, 10 sorts of ferula are basically utilized, these are stinky ferula, Kuhistan ferula, rova and others. These species are fundamentally the same as in appearance and morphological highlights to one another, yet in nature, the stinky (Ferula assa-foetida) is broadly disseminated in the primary gum-pitch is acquired from this species. The piece of gum tar is for the most part made out of essential oils, organic sulfides that give them the smell of garlic, pinenes, coumarins and different mixtures. Ferula gum-resin in non- medical and it is utilized for the treatment of convulsions, tuberculosis, plague, syphilis, challenging hack, toothache, sensory system illnesses and different diseases, utilized as a tonic, expectorant and anthelmintic [17]. Youthful shoots of ferula, squashed and blended in with sour milk are utilized for the treatment of harmful tumors and syphilis [18].

Liqueur gum-resin ferules and emulsions are utilized in the treatment of asthma, seizures and apprehensive illnesses. In scientific medicine, gum-resin called "assa-foetida" as powder, emulsion and liquor color, is utilized as a analgesic, narcotic and entered the pharmacopeia of numerous states [15].

Ginger is an individual from the family *Zingiberaceae*; a little family with in excess of 45 genera, and 800 species; its Latin name is *Zingiber officinale* Roscoe (*Z. officinale*) [16]. It is an erect lasting plant developing from one to three feet in stature; its stem is encircled by the shealthing bases of the two positioned leaves. A clublike spike of yellowish, purple lipped blossoms has greenish yellow brackswhich seldom blossoms in cultivation. Ginger is genuinely a world homegrown cure. It is additionally utilized in India and different spots like the antiquated Chinese where the new and dried roots were viewed as particular therapeutic items.

Ginger has been utilized for cold-incited sicknesses, queasiness, asthma, hack, colic, heart palpitation, growing, dyspepsia, less of craving, and ailment [16]. In nineteenth century ginger fills in as a famous solution for hack and asthma when the juice of new ginger was blended in with a little squeeze of new garlic and honey[16].

Allium sativum L. (Garlic) (A.sativum) has a place with the family Liliaceae and sort Allium. It is a hard and perennial plant. It has a strong odor, incredible dry entering smell and is utilized as [17]. A. sativum (garlic) has been broadly found in India, China, Asia, Southern Europe, North America and the other Northern piece of Nigeria. Garlic has a place with the family Allium which is an overall name for all substances which structure allicins through enzymatic activity. At the point when new garlic is squashed, a trademark scent is given-off which is chiefly because of the disulphides compound allicin. Allins are scentless natural disulphides or their oxides and allicins are framed from allin by the activity of catalyst allinase. Allicins have been accounted for to have anti-infection movement and it is viewed as liable for a large portion of garlic's pharmacological exercises [17].

Horseradish (*Armoracia rusticana* P.G. Gaertn., B. Mey. & Scherb.) (*A.rusticana*) is a perennial crop having a place with the Brassicaceae family. Because of its extremely pungent root. (*A. rusticana*) is grown especially for the root to be used as a spice for groceries, and once in the past utilized therapeutically, especially as an antiscorbutic [18]. At the point when horseradish tissues are harmed by cutting, chemical from the harmed horseradish plant cells breakdown sinigrin (a glucosinolate) to create isothiocyantes (ITCs). The trademark scent and taste of horseradish is because of ITCs shaped by the activity of myrosinase on glucosinolate when plant tissues is damaged [18]. The significant segments for antimicrobial activity in horseradish are isothiocyantes. Allyl isothiocyantes [18], phenylethyl isothiocyanate, and other isothiocyantes are contained in ITCs removed from horseradish root. Despite the fact that usage of ITCs are restricted

because of a extreme volatility and not appropriate water solubility, ITCs have antimicrobial effect against microorganisms and quite high toxisity [19, 20].

Apium graveolens L. (Root of Celery) (A.graveolens) (Apiaceae) A. graveolens (Apiaceae) grows wild at the base of the North Western Himalyas and remote slopes in Punjab and in Western India. Celery seeds or celery seed extracts are used as flavoring agents and also in anti rheumatic formulations as the seeds have significance as arthritic pain relief, for treating rheumatic conditions and gout. Aside from the part in rheumatism, celery seeds demonstrated its usage in asthma, bronchitis and incendiary conditions [22].

The *Amygdalus ledebouriana* Schltdl. (*A.ledebouriana*) is a very rare and endemic species of Eastern Kazakhstan, that has been used basically in ornamental purposes. It is assumed that it contains antibacterial compounds. *A. ledebouriana* is listed in the Red Book of Kazakhstan. In the blossoming stage, it frames a delicate pink viewpoint. It fills in the grass-knoll steppe, on mountain steppe slants and levels, in stream valleys and in glade valleys. It is found in the lower regions of the southwestern Altai, Tarbagatai, in the Dzungarian Alatau [23].

MATERIALS AND METHODS

Microbial samples

Microorganisms were cultivated in specified agar for each microorganism for 24 hours at 37°C. The authenticity of microorganisms checked by Gram-staining, microscopic evaluation methods. Then prepared nutrient broth and sterilize tubes and probes, petri dishes. Re-cultivation of microorganisms to nutrient broth for and transferring to Eppendorf tubes for long storage was done. Finally, preparation of bacterial dilution 1*10^5 CFU per ml was performed.

Culture media, antibiotic

Different culture media were used in study. Firstly, for cultivation and election of desired microorganisms were used Endo agar (Titan Biotech Ltd., India) for *E. coli* and *Pseudomonas* isolation agar (Titan Biotech Ltd., India) for *Ps. aeruginosa*. Following, Nutrient Broth (HiMedia Laboratories Pvt. Ltd., India) and Nutrient Agar (Titan Biotech Ltd., India) were used to cultivate microorganisms and microorganisms with plant extracts. Ceftriaxone (Open Joint Stock Company "Borisov Plant of Medical Preparations"., Republic of Belarus) is an antibiotic classified to Cephalosporins and against Gram-negative bacteria.

Plant extracts preparation

The plant materials leaves of *T.occidentalis, A.ledebouriana* and root of *F.soongarica* were obtained from different institutions and researchers, who are working with certain plant material. Plant materials cut to small pieces to increase surface area and grinded. 5

g of plant materials are soaked into 500 ml ethanol (96%) as a solvent material and covered with parafilm properly. Mixture of solvent and plant material was agitated using shaker for 3 days by 210rpm. Mixtures kept in room temperature in dark place for 7 days and filtered through filter paper and collected in a sterile flask for further use. Rotary evaporator was used remove ethanol and to get pure extract. The bath temp 40°C, rotation 180 rpm, 500 mPa for 3-3.5 hours for each plant extract. Obtained extracts were kept under fume hood to further additional evaporation of ethanol. Stock extract solutions were mixed with DMSO 10%. The dilution of plant materials was done by the following equation.

C (%) =V(extract)*100%/V(DMSO)

<u>Note</u>:

 $C (\%) = \frac{V(extract)}{V(DMSO)} \times 100\%$ 25%=1 ml/4ml (DMSO) 50%=2ml/4ml (DMSO) 75%=3ml/4ml (DMSO) 100%=4ml/4ml (DMSO)

A.sativum (Garlic) and roots of *A.graveolens* (Horseradish), *A. rusticana* (Celery), *Z.officinale* (Ginger) used in this study were purchased from the local market of Almaty city. These plant extracts were obtained by soaking 50 g of grinded plant materials in 100 ml of ethanol and water for garlic especially [24]. The different solvents as ethanol and garlic used for this study, in the purpose of solubility of the components of plant materials. For example: ginger has essential oils that are not solubilize in the water. These plant extracts not evaporated using rotary evaporator and without using DMSO 10% solvent.

Antimicrobial activity of the plant extracts

After incubation of microorganisms with plant extract, the inhibition zone of each disk measured with ruler.

As DMSO is used to increase antimicrobial activity of compound in the pharmacy (sometimes in the preparation of some antibiotics), in this experiment also was used DMSO to enhance antimicrobial activity. DMSO taken as a negative control and to subtract the average zone of inhibition of DMSO to the average zone of inhibition of plant extract dissolved in DMSO, to get the antibacterial activity of plant extract alone. The sum of the 3 repetitions for each type of microorganism have to be summed and average measure is obtained. Thus, average zone of inhibition for 25%, 50%, 75%, 100% was estimated. In addition, Ceftriaxone average zone of inhibition was used as positive control.

Disk diffusion method

The method of dick diffusion involves the preparation of a Petri dish containing 15–25 ml agar, bacteria of fixed volume are then swabbed across the agar surface [25]. *T.occidentalis, F.soongarica, A.ledebouriana* were used in disk diffusion method. One size paper disk from soaked with 25%, 50%, 75% 100% extracts and then placed on agar at equivalence distance in petri dishes with 25 ml of Nutrient agar and 1 ml 10⁻⁵cfu/ml bacterial suspension. After, dishes incubated for 24h at 37°C. After proper incubation, the "cleared" zone (zone of inhibition) surrounding the disk is measured and compared with zones for standard antibiotics or literature values of isolated chemicals or similar extracts.

Agar well diffusion method

Agar well diffusion is another method similar with disk diffusion method can be employed for determining antimicrobial activity [26]. A standardized concentration of inoculums with 0.5 ml is spread evenly on the surface of Nutrient agar plate. Four wells, ranges from 4 - 5 mm in diameter, can be punched with a sterile cork borer aseptically on solid surface of agar. 0.5 ml of plant extracts should be introduced into the 4 bored agar well. The 2 remaining well should be introduced with fixed volume of positive control and negative control. Then plates are incubated at optimum temperature and duration depending upon the test microorganism. The interpretation of results is performed by measuring the diameter of zone of inhibition by comparing those formed by positive control around each well.

RESULTS AND DISCUSSION

The antimicrobial activity of plant extracts varied depending on the species used antimicrobial activity of different plant extracts through two methods against *E. coli* and *Ps. aeruginosa* (Table 1). Antimicrobial activity of plant extracts was observed from 1 mm to 8.5 mm. Garlic showed antimicrobial activity against *E. coli*, while ginger, horseradish, root of celery was effective against *Ps. aeruginosa*. However, among all studied plants *A.ledebouriana* showed positive results against both microorganisms.

Microogani	T.occident	А.	F.soongari	Ζ.	A. rusticana/	A.graveolens	А.
sms	alis	sativum	са	officinale/Gi	Horseradish	/Celery	ledebouri
	(Leaves)	(Garlic)	(Root)	nger (Root)	(Root)	(Root)	ana
							(Leaves)
E. coli	-	+	-	-	-	-	+
Ps.	-	-	-	+	+	+	+
aeruginosa							

Table 1 - Antimicrobial activity of plant extracts through disk diffusion method and agar well diffusion method

<u>Note</u>: (+) susceptibility (inhibition zone \geq 1 mm), (-) absence of susceptibility

Figure 1 and 2 represents positive result of the study. Extracts of *A.ledebouriana* cultivated with *E. coli* and *Ps. aeruginosa* show slightly significant results.



Figure 1 - A. ledebouriana/ E. coli



Figure 2 - A. ledebouriana/ Ps.aeruginosa

Figure 3 and 4 demonstrate cultivation of garlic extracts with test microorganisms of the study. As can be seen on the pictures, isolated transparent area around well with diluted extracts show the inhibition zone. All four diluted extracts have clear inhibition zone.



Figure 3 - Garlic/ Ps. aeruginosa



Figure 4 - Garlic/ E. coli

The diameter of zone of inhibition against *E. coli* varied ranging from 1 mm to 8.5 mm for plant extracts comparing to antibiotic from 20 mm to 30 mm. Among 7 plant extracts antimicrobial activity show garlic and *A.ledebouriana*. Nevertheless, for the solvents (ethanol and water) there was no inhibition zone obviously. The inhibition zone of plant extracts shows only antimicrobial activity of plant, without any assistive compounds. The highest inhibition zone against *E. coli* presented by garlic at 100% (Fig. 5).

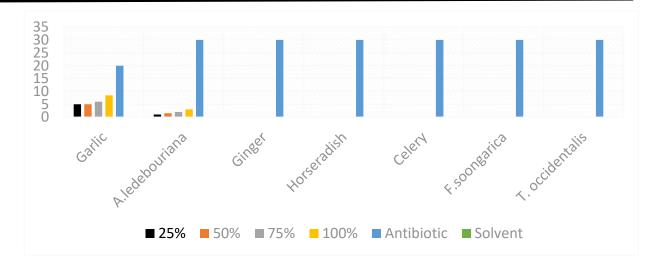


Figure 5 - The representation of plant extracts from plant materials with cultivated E. coli

The diameter of zone of inhibition against *Ps. aeruginosa* varied ranging from 1 mm to 3mm for plant extracts in comparison with antibiotic ranging from 22.5 mm to 30 mm. Among 7 plant extracts 3 of them show antimicrobial activity against *Ps. aeruginosa*. *A. ledebouriana* and celery root at concentration 100% are effective against multi-resistant microorganism. Compared to these two extracts ginger has antimicrobial activity, but inhibition zone is not significant (Fig.6).

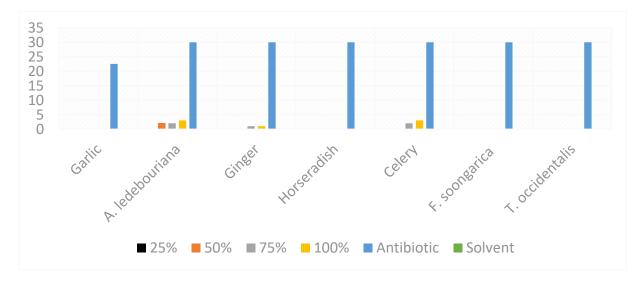


Figure 6 - The representation of plant extracts from plant materials with cultivated Ps. aeruginosa

It was established that *A. ledebouriana possessed* antimicrobial activity against Gramnegative (*E. coli, Ps. aeruginosa*) microorganisms. Previously there was no study about antibiotic effect of this plant. *A. ledebouriana* known as decorative plant, the other reason this plant is endemic in Kazakhstan.

According to Cheesbrough (1984) an antimicrobial agent which the diameter of the zone of inhibition is above 3mm, the organism is said to be sensitive but if it is 2mm or less than that, the organism is said to have resistant against the particular agent.

Therefore, considering the statement of Cheesbrough and comparing results from Figure 5-6, *E. coli* and *Ps. aeruginosa* are sensitive to *A. ledebouriana* at concentration 100%. So, for ginger and celery root *Ps. aeruginosa* is resistant.

Regardless, garlic extract is possessed antibiotic effect against Escherichia coli at low concentration (25%) compared to *A. ledebouriana*. The sensitivity of microorganisms was significantly increased with the increase of garlic extract concentration.

There are several factors that effect on reduction of antimicrobial activity and the presence of phytoncides in the plant. The season and time of collection of plant materials, storage conditions, extraction methods, types of solvents are directly influence on the presence or absence of phytoncides, which is the main indicator of antibiotic activity. For example: the volatilization of some compounds can appear during inappropriate drying of the plant materials in drying oven or during usage of rotary evaporator with increase in temperature of water bath. Furthermore, according to antibiotic activity data of garlic and ginger is revealed that even high temperature garlic preserve antibiotic properties [27]. However, ginger totally lost antimicrobial effect against tested microorganisms. Consequently, further research is required to provide more information and data.

CONCLUSION

Garlic (*A. sativum*) is the only extract which has antibacterial activity against *E. coli*. The antimicrobial effect of Garlic was studied in a wide variety of research. The effect of the Garlic extract was proved. Application of plant extracts as antibiotics in modern medicine have to be considered into account.

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Кейбір өсімдік кандидаттарының антибактериялық әсерін граммтеріс бактерияға зерттеу

Хирургиялық операциялардан кейінгі өлім-жітімнің артуы шартты патогенді микроорганизмдермен тікелей байланысты. Ұзақ оңалту және жаңа буын антибиотиктеріне сұраныстың артуы мәселені жаңа деңгейге көтереді. Әлемнің түкпір-түкпірінен фармакологиялық компаниялар оппортунистік микроорганизмдердің жоғары қарсыласу деңгейінің мәселесін шешуге тырысады. Алайда, микроорганизмдердің генетикалық тұрақтылығының эволюциясын бақылау мүмкін емес және синтетикалық антибиотиктерді өндіру үлкен күресті қажет етеді. Қазіргі уақытта дәстүрлі медицина әртүрлі елдерде танымал. Бұл жұмыс Қазақстанның эндемик өсімдіктері және бірқатар нарықтық өсімдіктердің экстракттарының микроорганизмдерге қарсы белсенділігін зерттеуді қамтиды. Эксперименттік бөлім алдыңғы зерттеулерге негізделген және шолуға енгізілген. Зерттеуде ұсынылған өсімдіктер тізімі: *Thuja occidentalis* L., *Amygdalus ledebouriana* Schltdl., *Ferula soongarica* Pall. ex Spreng., *Zingiber officinale* Roscoe., *Allium sativum* L., *Armoracia rusticana* P. G. Gaertn., B. Mey. & Scherb., *Apium graveolens* L. Зерттеу барысында пайдаланылған оппортунистік микроорганизмдер: *Escherichia coli* және *Pseudomonas aeruginosa*. Нәтижесінде, *A. sativum* 25% концентрациясында *E. coli*-ге қарсы бактерияға қарсы әсер еткенін көрсетеді. Бұл *A. ledebouriana* экстрактарына сезімтал болған кезде ұсынылған ең төменгі концентрация.

Кілт сөздер: *Thuja occidentalis* L., *Amygdalus ledebouriana* Schltdl., *Ferula soongarica* Pall. ex Spreng., *Zingiber officinale* Roscoe., микробқа қарсы белсенділік.

Изучение антибактериального действия некоторых растенийкандидатов на грамотрицательные бактерии

Увеличение смертности после хирургических операций напрямую связано с условнопатогенными микроорганизмами. Длительная реабилитация и растущий спрос на антибиотики нового поколения поднимают проблему на новый уровень. Фармакологические компании со всего мира пытаются получить решение проблемы высокого уровня резистентности условнопатогенных микроорганизмов. Однако эволюция генетической устойчивости микроорганизмов не поддается контролю, и производство синтетических антибиотиков требует большой борьбы. В настоящее время традиционная медицина хорошо известна и популярна в разных странах. Данная работа включает в себя изучение антимикробной активности экстрактов эндемичных растений Казахстана и ряда рыночных растений. Экспериментальная часть была основана на предыдущих исследованиях и включена в обзор. Список растений, представленных в исследовании: Thuja occidentalis L., Amygdalus ledebouriana Schltdl., Ferula soongarica Pall. ex Spreng., Zingiber officinale Roscoe., Allium sativum L., Armoracia rusticana P. G. Gaertn., B. Mey. & Scherb., Apium graveolens L. Условно-патогенными микроорганизмами, которые были использованы в ходе исследования, являются: Escherichia coli и Pseudomonas aeruginosa. Результаты показывают, что A. sativum проявлял антибактериальный эффект против E. coli в концентрации 25%. Это самая низкая концентрация, которая была представлена, в то время как к экстрактам A. ledebouriana оба штамма были чувствительны.

Ключевые слова: Thuja occidentalis L., Amygdalus ledebouriana Schltdl., Ferula soongarica Pall. ex Spreng., Zingiber officinale Roscoe., антимикробная активность.