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Antibacterial activity of ultrasonic extracts of *Salvia stepposa* growing in Kazakhstan

This article presents data on the studies on antibacterial activity of ultrasonic extracts of *Salvia stepposa* (steppe sage) growing wild on the territory of the Republic of Kazakhstan. Screening for the antibacterial activity of the presented samples was carried out by the disc-diffusion method against strains of gram-positive bacteria *Staphylococus aureus*, *Bacillus subtilis*, gram-negative strains *Escherichia coli* and the yeast *Candida albicans*. The results showed that in relation to *Staphylococus aureus* the most pronounced antibacterial activity with growth inhibition zones 35 ± 1 mm is possessed by 30 % ultrasonic extract of steppe sage flowers, in relation to *Bacillus subtilis* 49 ± 1 mm — 40 % ultrasonic extract of steppe sage leaves, for *Escherichia coli* 24 ± 1 mm — 70 % ultrasonic extract of steppe sage leaves. This study demonstrated that, 30 % ultrasonic extract of flowers and 90 % ultrasonic extract of flowers and leaves of steppe sage showed weak activity against yeast fungus *Candida albicans* with growth inhibition zones 12 ± 1 mm, 12 ± 1 mm and 11 mm, respectively. As a result, out of 8 extracts isolated by ultrasonic activation only 2 extracts showed antibacterial activity against 4 studied bacteria. Additionally, it was found that the use of ultrasonic extraction can reduce the extraction time of biologically active substances in 8–9 times compared to conventional extraction methods.

Keywords: antibacterial activity, plant extracts, ultrasound extraction, bacteria, disc-diffusion method.

Introduction

Nowadays, in connection with the formation of microorganism strains with multiple antibiotic resistances, new drugs based on plant biologically active substances (BAS) with antibacterial activity are of increasing interest among scientists. According to the literature, plant extracts are becoming more and more popular because they combine low toxicity with the ability to effectively act on pathogenic and opportunistic micro flora and are also safe in comparison with chemically obtained products [1].

Currently, 8 species of plants of the genus Sage grow in the Republic of Kazakhstan. The State Pharmacopoeia of the Republic of Kazakhstan includes medicinal sage (*Salvia officinalis* L., *Lamiaceae* family), which is recommended for use as an anti-inflammatory and antiseptic agent, is also used for diseases of the upper respiratory tract, wound healing agent for burns or skin diseases [2–5]. However, other species of the genus *Salvia* L. are still insufficiently studied and are not used in medicine. Thus, the steppe sage (*Salvia stepposa* Des.-Shost.) grows practically on all territory of the Republic of Kazakhstan [6]. The chemical composition of this species and biological activities has not been completely studied [7]. Some research is conducted in Russia [8, 9], but in Kazakhstan plant raw material of *Salvia stepposa* is not studied. This species is the potential source of the medicinal raw material for pharmacy [10].

Accordingly, the study of promising plant raw materials of steppe sage with the aim of using it for the production of domestic medicines indicates the relevance of this work and its practical importance for the pharmaceutical industry.

At the moment, there are many ways to extract plants in order to isolate BAS from them. Traditional extraction methods, such as Soxhlet extraction, solid-liquid extraction or liquid extraction, are characterized by low process selectivity, the use of a large amount of solvents, long extraction timeand these methods also make it possible to obtain products with a low yield of BAS [11]. Moreover, many natural products are thermally unstable and can decompose when the temperature rises during extraction. Modern extraction methods include microwave-assisted extraction and ultrasound-assisted extraction. The use of ultrasound has significant advantages over traditional technologies for the isolation of BAS. Ultrasonic extraction is used in a variety of chemical processes because it is a fast method that consumes little energy and reduces solvent consumption, resulting in a cleaner product and higher yields of the final product. The extraction mechanism of ultrasound-assisted extraction includes two types of physical phenomena: diffusion through the cell walls and leaching of the cell contents after the destruction of the walls [12]. Ultrasonic waves change their physi-

cal and chemical properties after interacting with the exposed plant material and their cavitation effects promote the release of BAS and increase mass transfer, destroying the walls of plant cells [13]. The advantage of using ultrasound is the reduction in extraction time and the amount of solvent used, as well as an increase in the yield of the final product. The authors [14] carried out the extraction of polyphenols from *Salvia officinalis* using ultrasonic extraction. In comparison with traditional extraction methods, the reaction time was reduced by 20 %. Thus, these advantages allow for lower manufacturing costs and correspondingly lower cost of the final product.

Materials and methods

As a plant material, we used the above-ground part of the steppe sage (*Salvia stepposa*), which is represented by leaves and flowers, collected in the surrounding of the Karaganda city during the flowering phase (June-July, 2020). Before extraction, fresh raw materials were dried at 25–30 ºC, avoiding exposure to direct sunlight for 7 days.

For ultrasonic extraction of steppe sage, aqueous-ethanol solutions of the following concentrations of 30 %, 40 %, 70 %, and 90 % were used as solvent. For the extraction, the above-ground part of the steppe sage was used; the extraction of flowers and leaves was carried out separately. Air-dry raw materials of the steppe sage (*Salvia stepposa*) leaves and flowers were separately immersed in a flat-bottomed flask and the necessary solvent was added. The ratio of the mass of raw materials to the volume of the solvent was 1:10, respectively. The flask with the raw material and the solvent was immersed in an ultrasonic bath Digital Ultrasonic Cleaner VGT 1200, with an ultrasonic frequency of 40,000 Hz [15]. Extraction of each sample of raw materials for leaves was carried out 4 times, for flowers — 3 times until an almost transparent solution was obtained. The ultrasonic irradiation time for leaves and flowers was 30 minutes. The extracts of each sample were filtered using a paper filter. The obtained extracts were combined and evaporated under vacuum using a rotary evaporator at a temperature not exceeding 60 ºC. Residual solvent from the thick extract was evaporated in a water bath at a temperature of 60 ºC and stored in a dry, dark, cool place until required. The received solid ultrasonic extracts of steppe sage were from dark brown to dark green color, which depending on the concentration of the solvent with a specific odor.

The study of the antibacterial activity of the above samples was carried out against two strains of gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), against the gram-negative strain *Escherichia coli* (ATCC 25922) and against the yeast *Candida albicans* (ATCC 10231). These bacterial strains are part of the collection of the Department of Microbiology of the Medical University of Karaganda, Kazakhstan. The study of antibacterial activity was carried out using the disc-diffusion method [16]. For the study, a suspension was prepared containing a standard number of viable bacterial cells, which was inoculated with a lawn on the surface of the nutrient medium in Petri dishes. On sterile filter paper disks, 0.01 ml of extract at a concentration of 100 mg/ml was applied. Discs with preparations were placed on the inoculation at a distance of 2.5 cm from the center of the dish in a circle (4 disks per dish). The inoculations were incubated for 24 h at 36 ºC for bacteria and 24 h at 28 ºC for fungi. After incubation against the background of a uniform bacterial lawn around the discs, zones of complete and partial suppression of bacterial growth were formed. The results were taken into account by measuring the diameter of the growth inhibition zones. Comparators are benzyl penicillin for bacteria and nystatin for yeast. Each sample was tested in three parallel runs.

Results and Discussion

The antibacterial activity of the samples was assessed by the diameter of the growth inhibition zones of the test strains (mm). The diameter of growth inhibition zones is less than 10 mm and continuous growth in the dish was assessed as the absence of antibacterial activity, 10–15 mm — weak activity, 15–20 mm — moderate activity, over 20 mm — strong. The results of the study of the antibacterial activity of the samples are shown in Table 1.

As can be seen from the data in Table 1, it follows that ultrasonic 30 %, 40 %, 70 % and 90 % extracts of both leaves and flowers of steppe sage have strong activity in relation to gram-positive strains *Staphylococus aureus* and *Bacillus subtilis*. As a result of the experiment, it was found that 70 % of the leaf extract and 90 % of the flower extract demonstrated strong activity against the gram-negative strain *Escherichia coli*. In addition, a weak antimicrobial activity of 90 % alcoholic extract of the leaves and 30 %, 90 % of alcoholic extract of flowers was revealed against strains of yeast fungus *Candida albicans*, the rest of the extracts showed no activity against yeast.

Table 1

Antibacterial activity of the studied samples of ultrasonic extracts

№	Sample	*Staphylococus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Candida albicans*
1	30 % ethanol extract of steppe sage (leaves)	28±1	32±1	9±1	8±1
2	40 % ethanol extract of steppe sage (leaves)	33 ±1	49±1	16±1	8±1
3	70 % ethanol extract of steppe sage (leaves)	20±1	29±1	24±1	8±1
4	90 % ethanol extract of steppe sage (leaves)	26±1	28±1	10±1	11±1
5	30 % ethanol extract of steppe sage (flowers)	35±1	44±1	12±1	12±1
6	40 % ethanol extract of steppe sage (flowers)	30±1	29±1	11±1	9±1
7	70 % ethanol extract of steppe sage (flowers)	25±1	32±1	11±1	7±1
8	90 % ethanol extract of steppe sage (flowers)	26±1	30±1	21±1	12±1

Conclusions

For the first time, ultrasonic extraction was used to extract biologically active substances from the leaves and flowers of steppe sage; the antibacterial activity of the obtained extracts was assessed in an *in vitro* experiment. The use of ultrasonic extraction provides a reduction extraction time of biologically active substances by 8–9 times in comparison with conventional methods. Both flower and leaf extracts showed strong antibacterial activity against the gram-positive bacteria *Staphylococus aureus* and *Bacillus subtilis*, as described above. In addition, two steppe sage extracts showed the highest activity against the gram-negative strain *Escherichia coli*. The study showed that for the most part, with the exception of a few, ultrasonic extracts of steppe sage were not active against the yeast *Candida albicans*.

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Қазақстанда өсетін *Salvia stepposa* ультрадыбыстық экстрактарының бактерияғақарсы белсенділігі

Мақалада Қазақстан Республикасының аумағында өсетін *Salvia stepposa* (дала шалфейі) ультрадыбыстық экстрактарының бактерияғақарсы белсенділігін анықтау бойынша деректер ұсынылған. Үлгілердің бактерияғақарсы белсенділігін зерттеу скринингі *Staphylococus aureus*, *Bacillus subtilis* грамоң бактериялардың штамдарына, *Escherichia coli* грамтеріс штамдарына және *Candida albicans* ашытқы саңырауқұлақтарына қатысты диско-диффузиялық әдіспен жүргізілді. Нәтижелер *Staphylococus aureus* қатысты өсу кідірісі 35 ± 1 мм болатын 30 пайыздық дала шалфей гүлінің ультрадыбыстық экстрактісі, *Bacillus subtilis* қатысты 49 ± 1 мм — дала шалфейінің 40 пайыздық ультрадыбыстық экстрактісі, *Escherichia coli* қатысты 24 ± 1 мм — далалық шалфей жапырағының 70 пайыздық ультрадыбыстық экстрактісі барынша айқын бактерияғақарсы белсенділікке ие екенін көрсетті. *Candida albicans*-қа қатысты 30 пайыздық ультрадыбыстық гүл экстрактісі және 90 пайыздық ультрадыбыстық дала шалфейінің гүлдерімен жапырақтарының экстрактісі сәйкесінше 12 ± 1 мм, 12 ± 1 мм және 11 мм өсу кідірісі бар әлсіз белсенділікті көрсетті. Нәтижесінде ультрадыбыстық активтендіру арқылы бөлінген 8 экстрактінің 2-і зерттелетін 4 микроағзалар дақылдарына қатысты бактерияғақарсы белсенділікті көрсетті. Ультрадыбыстық экстракцияны қолдану дәстүрлі экстракция әдістерімен салыстырғанда биологиялық белсенді заттардың экстракция уақытын 8–9 есе қысқартуға мүмкіндік беретіні анықталды.

Кілт сөздер: бактерияғақарсы белсенділік, экстрактілер, ультрадыбыстық экстракция, бактериялар, диско-диффузиялық әдіс.

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Антибактериальная активность ультразвуковых экстрактов *Salvia stepposa*, произрастающего в Казахстане

В статье представлены данные по выявлению антибактериальных свойств ультразвуковых экстрактов *Salvia stepposa* (шалфей степной), произрастающего на территории Республики Казахстан. Скрининг по изучению антибактериальной активности образцов проводился диско-диффузионным методом в отношении штаммов грамположительных бактерий *Staphylococus aureus*, *Bacillus subtilis*, грамотрицательных штаммов *Escherichia coli* и к дрожжевому грибку *Candida albicans*. Результаты показали, что в отношении *Staphylococusaureus* максимально выраженной антибактериальной активностью с зонами задержки роста 35 ± 1 мм обладает 30 %-ный ультразвуковой экстракт цветков шалфея степного; в отношении *Bacillus subtilis* 49 ± 1 мм — 40 %-ный ультразвуковой экстракт листьев шалфея степного; в отношении *Escherichia coli* 24 ± 1 мм — 70 %-ный ультразвуковой экстракт листьев шалфея степного. В отношении *Candida albicans* 30 %-ный ультразвуковой экстракт цветков и 90 %-ный ультразвуковой экстракт цветков и листьев шалфея степного проявили слабую активность с зонами задержки роста 12 ± 1 мм, 12 ± 1 мм и 11 мм, соответственно. В результате из 8 экстрактов, выделенных ультразвуковой активацией, 2 показали антибактериальную активность в отношении 4-х исследуемых культур микроорганизмов. Было установлено, что использование ультразвуковой экстракции позволяет сократить время экстракции биологически активных веществ в 8–9 раз по сравнению с традиционными способами экстракции.

Ключевые слова: антибактериальная активность, экстракты, ультразвуковая экстракция, бактерии, диско-диффузионный метод.

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