

## Evaluation of Antibacterial Activity of *Salvia stepposa* Extracts Isolated using Microwave Extraction, Growing Wild in Kazakhstan

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Received: 4 November 2020, Revised: 21 May 2021, Accepted: 28 May 2021

### Abstract

The studies on antibacterial activity of 16 samples of *Salvia stepposa* (steppe sage) ethanolic extracts, an indigenous plant of Central Kazakhstan, against *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Candida albicans* (ATCC 10231) were assayed. Leaves and flowers of *Salvia stepposa* were extracted by ethanol 30, 40, 70 and 90 % using conventional and microwave extraction. Antibacterial screening performed by disc-diffusion method was conducted, the concentration of all extracts was 100 mg/mL. The results revealed that the highest activity against *Staphylococcus aureus* and *Bacillus subtilis* have leaves and flowers extract of steppe sage in all solvents except 70 % microwave leaf extract. The only extract that showed antibacterial activity against all strains of microorganisms used is 40 % microwave leaf extract of steppe sage with growth inhibition zones for *Staphylococcus aureus*  $23.5 \pm 0.65$  mm, for *Bacillus subtilis*  $38 \pm 1.3$  mm, for *Escherichia coli*  $16.5 \pm 1.72$  mm, for *Candida albicans*  $16 \pm 2.6$  mm, respectively. Was found that 70 % of leaf extract of the steppe sage obtained under microwave irradiation has the lowest activity. The MIC values for tested extracts were evaluated too.

**Keywords:** Steppe sage, Extract, Antibacterial activity, Microwave extraction, Disk diffusion method

### Introduction

In connection with the formation of strains of microorganisms with multiple antibiotic resistance, new drugs based on plant biologically active compounds (BAS) having antibacterial activity are of increasing interest. According to the literature, herb extracts are becoming more popular because they combine low toxicity with the ability to effectively act on pathogenic and conditionally pathogenic microflora and are also safe compared to products obtained chemically [1].

Nowadays, people are often infected with microorganisms such as bacteria, mold, viruses, etc. The use of antibacterial agents is important to ensure sterility and prevent the spreading of infection. In this regard, at the moment, there is an intensive search for new sources of plant raw materials that can potentially be used as highly effective antibacterial drugs.

Genus Sage (*Salvia L.*) - the largest in the family of *Labiatae* or *Lamiaceae*. There are about 700 - 900 species that are distributed in temperate and warm areas of both hemispheres. BAS of sage species have a potential application as anti-inflammatory, antiseptic, immunomodulating, hypoglycemic, antidiabetic, antioxidant, hepatoprotective, wound healing agents, as well as have the ability to prevent neurovegetative diseases and stimulate antitumor activity [3-9].

All types of sage are valuable for humans, each is individual in terms of the content of essential oil and, if possible, use as a medicinal raw material [10]. Additionally, a significant number of species of this genus are widely used in phytotherapy of our country and a several of countries of Western Europe and Central Asia [11,12]. One of them is medicinal sage (*Salvia officinalis*), which is included in State Pharmacopoeia of the Republic of Kazakhstan [13]. However, this species does not grow in Kazakhstan conditions due to cold weather, which complicates the use of this type of raw materials for medical purposes. On the territory of the Republic of Kazakhstan grows 8 species of plants of the genus sage. As an alternative for practical use of the species, we considered steppe sage (*Salvia stepposa* Des. - Shost., *Lamiaceae*), the properties of which still unexplored. Steppe sage has an extensive growing range in

Central Kazakhstan, mainly in the Karaganda region and has large raw materials. Earlier, we performed macro- and microscopic, histochemical analysis of steppe sage [14]. Studies have shown that the above-ground part of steppe sage contains essential oils, flavonoids and phenolic compounds which cause antibacterial activity [15,16]. Accordingly, determining the antibacterial properties of steppe sage is very important for the detection and use of new promising raw materials, as well as the development of antibacterial drugs based on it.

In recent years, several new methods of extracting drugs to extract BAS from them were developed. Typically, conventional extraction (CE) methods such as Soxhlet extraction, maceration, reflux and hydrodistillation, which have been used for many decades are the standard and most popular choice for extracting phytochemicals. However, these methods also have serious disadvantages, such as long extraction time, low efficiency, especially when trace amounts of compounds are present. Moreover, many natural products are thermally unstable and can decompose when the temperature increases during extraction. The use of large volumes of organic solvents used in CE methods is detrimental to the environment and their subsequent removal also becomes a problem. In this regard, an ideal process would allow to produce the extracts with minimal process time, low production costs and minimal use of organic solvents. Modern extraction methods include microwave extraction (MAE) and ultrasound extraction (UE). One of the excellences of MAE is the ability to destroy structure of plant cell due to increase of internal pressure and thus release biologically active substances [17,18]. Compared to CE methods MAE has many advantages such as reduced extraction time, reduced power consumption, lower solvent consumption and a higher extraction rate. These advantages reduce production costs and consequently reduce the cost of the final product.

## Materials and methods

### Plant material

The plant raw material of study was the above-ground part of the steppe sage (*Salvia stepposa*) collected in the surrounding of the Karaganda region during the flowering phase in June - July 2020. Before extraction, fresh leaves and flowers were dried at 25 - 30 °C, avoiding exposure to direct sunlight, for 7 days. The moisture content of the steppe sage after drying was no more than 6.51 %.

### Preparation of extracts

Conventional extraction (CE). Leaves and flowers were immersed in 4 different solvents with concentrations of ethyl alcohol 30, 40, 70, 90 % (1:10 w/v). The plant material was heated to a slight boil and kept under reflux for 1 h. To completely deplete the raw material extraction for leaves was carried out 4 times, for flowers - 3 times until an almost transparent solution was obtained.

Microwave extraction (MAE). Leaves and flowers were immersed in 4 different solvents with concentrations of ethyl alcohol 30, 40, 70, 90 % (1:10 w/v). The flask with plant material and the solvent was immersed in a household microwave oven with a radiation frequency of 2.45 GHz and connected to a reflux condenser. The time of MW irradiation for leaves was 4 min, for flowers 3 min. The irradiation power was 300 W. Exposure time 10 s with an interval of 1 min, to avoid boiling away of the used solvents. The mixture temperature was recorded at 1 min intervals using an IR-T1 CONDROL infrared thermometer. To completely deplete the raw material extraction was carried out for leaves 4 times, for flowers - 3 times until an almost transparent solution was obtained.

### Concentration and drying of the obtained extracts

The resulting liquid extracts were left for 2 days at a temperature of 0 - 4 °C. The combined liquid extracts of each sample were filtered using a paper filter. Liquid extracts were evaporated under vacuum using a rotary evaporator at a temperature not higher than 60 °C. The residual solvent from the thick extract was evaporated in a water bath at a temperature of 60 °C and stored in a dry, dark, cool place until required. Received solid conventional and microwave extracts of steppe sage, which are a thick mass from dark brown to dark green, depending on the concentration of the solvent with a specific odour.

### Antimicrobial activity by disk diffusion method

The study of the antibacterial activity of the above samples was carried out in concerning to strains of gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), to a gram-negative strain of *Escherichia coli* (ATCC 25922) and to the yeast fungus *Candida albicans* (ATCC 10231) by the disk diffusion method [19]. Positive controls are benzylpenicillin for bacteria and nystatin for the yeasts. For the study, a suspension was prepared to contain a standard number of viable bacterial

cells, which was inoculated with a lawn on the surface of the nutrient medium in Petri dishes. 0.01 mL of the extract with a concentration of 100 mg/mL was applied to sterile filter paper disks. Discs with preparations were placed on the inoculation at a distance of 2.5 cm from the centre of the dish in a circle (4 discs per dish). The inoculations were incubated for 24 h at 36 °C for bacteria and 24 h at 28 °C for yeasts. 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. The antimicrobial activity of the samples was assessed by the diameter of the growth inhibition zones of the test strains. All tests were carried out in triplicate and expressed in millimetres. The diameter of the 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.

## Results and discussion

The results of the antimicrobial activity assay of all extracts obtained by various methods and using different solvents are presented in **Table 1**.

**Table 1** Antimicrobial activity of the extracts against gram-positive, gram-negative bacteria and yeast fungus.

No.	Composition of extract	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
1	30 % ethanol extract of steppe sage (leaves) *	23±1.3	23±0	12.5±1.72	-
2	40 % ethanol extract of steppe sage (leaves) *	23.5±0.65	38±1.3	16.5±1.72	16±2.6
3	70 % ethanol extract of steppe sage (leaves) *	12±2.26	20.5±4.27	10±1.3	-
4	90 % ethanol extract of steppe sage (leaves) *	24.5±2.98	28±4.52	7.5±2.35	9.5±1.95
5	30 % ethanol extract of steppe sage (leaves)	26±6.9	31±3.45	11.5±1.72	9.5±1.13
6	40 % ethanol extract of steppe sage (leaves)	20.5±2.84	36±3.45	12.5±0.65	-
7	70 % ethanol extract of steppe sage (leaves)	24.5±4.07	30±5.68	12.5±1.95	-
8	90 % ethanol extract of steppe sage (leaves)	21.5±3.26	34.5±1.3	13.5±1,35	-
9	30 % ethanol extract of steppe sage (flowers) *	25±2.6	40.5± 3.45	6.5±1,13	-
10	40 % ethanol extract of steppe sage (flowers) *	24±3.45	39.5±2.84	9.5±1.13	8.5±0.65
11	70 % ethanol extract of steppe sage (flowers) *	23±2.6	38.5±1.95	7±1.3	7.5±1.13
12	90 % ethanol extract of steppe sage (flowers) *	21±3.45	21±3.91	7.5±1.72	7.5±1.13
13	30 % ethanol extract of steppe sage (flowers)	24±1.3	42.5±1.72	11.5±2.35	8.5±2.35
14	40 % ethanol extract of steppe sage (flowers)	30±1.3	38.5±1.72	8.5±1.13	9.5±1.72
15	70 % ethanol extract of steppe sage (flowers)	41±2.26	35±2.26	7.5±1.72	-
16	90 % ethanol extract of steppe sage (flowers)	27±1.3	35±3.45	7.5±1.72	7±1.72
<i>Positive controls</i>					
	Distilled water (sterile)	6±1	6±1	6±1	6±1
	Benzylpenicillin	16±1	14±1	21±1	-
	Nystatin	-	-	-	23±1

Note: \* - extracts obtained by MAE

**Table 2** The Minimal Inhibitory Concentration (MIC) of antimicrobial activity of the tested extracts.

No.	Composition of extract	MIC (mg/mL)			
		<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
1	30 % ethanol extract of steppe sage (leaves) *	50	50	50	-
2	40 % ethanol extract of steppe sage (leaves) *	25	25	50	100
3	70 % ethanol extract of steppe sage (leaves) *	50	25	100	-
4	90 % ethanol extract of steppe sage (leaves) *	50	25	100	100
5	30 % ethanol extract of steppe sage (leaves)	25	25	100	100
6	40 % ethanol extract of steppe sage (leaves)	50	25	100	-
7	70 % ethanol extract of steppe sage (leaves)	50	25	100	-
8	90 % ethanol extract of steppe sage (leaves)	25	25	100	-
9	30 % ethanol extract of steppe sage (flowers) *	25	25	100	-
10	40 % ethanol extract of steppe sage (flowers) *	25	25	100	100
11	70 % ethanol extract of steppe sage (flowers) *	25	25	100	100
12	90 % ethanol extract of steppe sage (flowers) *	25	25	100	100
13	30 % ethanol extract of steppe sage (flowers)	50	25	100	100
14	40 % ethanol extract of steppe sage (flowers)	50	25	100	100
15	70 % ethanol extract of steppe sage (flowers)	25	50	100	-
16	90 % ethanol extract of steppe sage (flowers)	25	25	100	100

Note: \* - extracts obtained by MAE

**Table 1** shows that 30, 40, 70, 90 % microwave and conventional extracts of steppe sage leaves and flowers showed strong antibacterial activity against the tested strains of microorganisms of gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. The exception was 70 % microwave extract of steppe sage leaves, which showed weak activity against *Staphylococcus aureus*.

Moderate activity against gram-negative *Escherichia coli* microorganisms showed only 40 % microwave extract of steppe sage leaves with growth inhibition zone  $16.5 \pm 1.72$  mm and is presented in **Table 1**. Weak antibacterial activity against this strain was shown by 30 and 40 % ethanol extracts of steppe sage leaf obtained by 2 methods 70 and 90 % ethanol extract of leaves and 30 % ethanol extract of flowers obtained by the traditional method. The remaining extracts were not active against *Escherichia coli*.

In the same way, the moderate antibacterial activity of 40 % ethanol extract of steppe sage leaves with concerning to the yeast fungus *Candida albicans* was revealed, the inhibition zone approximately the same as to was *Escherichia coli*  $16 \pm 2.6$  mm. The results against yeast fungus *Candida albicans* is shown in **Table 1**, other steppe sage extracts did not show activity against.

Minimal inhibitory concentration of the tested extracts was evaluated against above described strains. MICs were assessed visually as the lowest concentration of the extracts showing complete growth

inhibition of the reference microbial strains. From **Table 2** it can be clearly seen that 40 % ethanol extract of steppe sage leave isolated using MAE has the lowest MIC values against all tested strains. However, for 7 extracts MIC values have not been identified because the highest tested concentration was 100 mg/mL. Only 30 and 40 % ethanol extracts of steppe sage leave obtained by MAE cause growth inhibition of cultures of *Escherichia coli* at a concentration of MIC = 50 mg/mL, other at 100 mg/mL. It is noticeable that the resulting extracts inhibit the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Candida albicans* at different concentration of extracts. The lowest MIC values were determined for *Staphylococcus aureus* and *Bacillus subtilis*.

According to the results of study MAE is an interesting alternative to CE of extracting plant raw materials, which can reduce extraction time and energy consumption. This study demonstrated that, some microwave extracts showed more pronounced antibacterial activity than those obtained by the conventional method. It can be concluded that the direct interaction of microwaves with free water molecules present in the raw material causes a huge increase in internal pressure inside the plant cell due to evaporation of internal moisture, which leads to the subsequent rupture of the plant tissue and the release of BAS into the solvent.

In this regard, the current task and topic for future research may be the development of technology for the production of plant extracts isolated using MAE and the study of the activity of the obtained extracts to further develop drugs based on them.

## Conclusions

For the first time, MAE was used to extract BAS from the leaves and flowers of steppe sage and the antibacterial properties of steppe sage extracts growing wild in Kazakhstan were studied. This work demonstrated that *in vitro* experiment the power of antibacterial activity of *Salvia stepposa* extracts showed strong activity against gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* as described above. All findings of this study suggest that 40 % microwave leaf extract of *Salvia stepposa* contain some active components which possess antibacterial properties against all 4 strains of the studied microorganisms with growth inhibition zones for *Staphylococcus aureus*  $23.5 \pm 0.65$  mm, for *Bacillus subtilis*  $38 \pm 1.3$  mm, for *Escherichia coli*  $16.5 \pm 1.72$  mm, for *Candida albicans*  $16 \pm 2.6$  mm, respectively. The study shows that 40 % microwave leaf extract isolated using MAE also inhibit growth of using strains at lowest concentration of all the investigated extracts. The results revealed that the rest of the extracts showed no activity or showed weak activity against gram-negative *Escherichia coli* and yeast fungus *Candida albicans*.

## Acknowledgements

The authors are grateful to the researchers of the Microbiology Department, Medical University of Karaganda, Kazakhstan.

## References

- [1] M Cioch, P Satora, M Skotniczny D Semik-Szczurak and T Tarko. Characterisation of antimicrobial properties of extracts of selected medicinal plants. *Pol. J. Microbiol.* 2017; **66**, 463-72.
- [2] S Nabavi, A di Lorenzo, M Izadi, E Sobarzo-Sánchez, M Daglia and SM Nabavi. Antibacterial effects of cinnamon: From farm to food, cosmetic and pharmaceutical industries. *Nutrients* 2015; **7**, 7729-48.
- [3] CSC Garcia, C Menti, APF Lambert, T Barcellos, S Moura, C Calloni, CS Branco, M Salvador, M Roesch-Ely and JAP Henriques. Pharmacological perspectives from Brazilian *Salvia officinalis* (Lamiaceae): Antioxidant, and antitumor in mammalian cells. *An. Acad. Bras. Ciências* 2016; **88**, 281-92.
- [4] RM Harfouch, M Darwish, W Al-Asadi, AF Mohammad, NM Gharib and M Haroun. Antibacterial activity of essential oils of *Rosmarinus officinalis*, *Salvia officinalis* and *Anthemis nobilis* widespread in the Syrian coast. *Res. J. Pharm. Tech.* 2019; **12**, 3410-2.
- [5] K Gayathiri, JGS Kumar and S Kavimani. Evaluation of immunomodulatory activity of *Salvia hispanica*. L seeds. *Res. J. Pharm. Tech.* 2017; **10**, 4255-60.
- [6] Vidyasabbani, A Ramesh, Snehalatha, B Rahul, Sriharitha, Sanjayvarma and Aparna. *In-vitro* and *in-vivo* anti-inflammatory activities of *Salvia hispanica* and *Linum usitatissimum* seeds in Swiss Albino rats. *Res. J. Pharm. Tech.* 2015; **8**, 1438-44.

- [7] R Sawant, A Baghkar, S Jagtap, L Harad, A Chavan, NA Khan, RP Yevale and MK Kale. A review on - herbs in anticancer. *Asian J. Res. Pharm. Sci.* 2018; **8**, 179-84.
- [8] N Muthukumar and NS Veerappa. Powder microscopic analysis on leaf, petiole, stem and root on *Thespesia populnea* (L.) Soland ex Correa. *Res. J. Pharmacognosy Phytochem.* 2019; **11**, 189-92.
- [9] A Nayak, RR Rao, PJ Shenoy, H Sindhu, S Teerthanath and Bhuvaneshwari. *Salvia hispanica* (Chia) seeds afford hepatoprotection against isoniazid and rifampicin induced toxicity in a murine model. *Res. J. Pharm. Tech.* 2020; **13**, 4805-10.
- [10] M Acimovic, B Kiproviski, M Rat and MD Brdar-Jokanovic. *Salvia sclarea*: Chemical composition and biological activity. *J. Agron. Tech. Eng. Manag.* 2018; **1**, 18-28.
- [11] I Sengun, E Yucel, G Kilic and B Ozturk. Antimicrobial activities of *Salvia sclarea* L. and *Salvia aethiopsis* L. essential oils. In: Proceedings of the International Conference on Agronomy and Food Science & Technology, Istanbul, Turkey. 2019.
- [12] H Cui, X Zhang, H Zhou, C Zhao and L Lin. Antimicrobial activity and mechanisms of *Salvia sclarea* essential oil. *Bot Stud.* 2015; **56**, 16.
- [13] *The state pharmacopoeia of the Republic of Kazakhstan*. Vol III. Zhibek zholy, Almaty, Kazakhstan, 2014, p. 801.
- [14] Ya Levaya, G Atazhanova and M Ishmuratova. Macroscopic analysis of *Salvia stepposa*. In: Proceedings of the 8<sup>th</sup> International Scientific and Practical Conference, Poltava, Ukraine. 2020, p. 174-7.
- [15] B Tyagi, A Dubey, AK Verma and S Tiwari. Antibacterial activity of phenolics compounds against pathogenic bacteria. *Int. J. Pharm. Sci. Rev. Res.* 2015; **35**, 16-8.
- [16] L Bouarab-Chibane, V Forquet, P Lantéri, Y Clément, L Léonard-Akkari, N Oulahal, P Degraeve and C Bordes. Antibacterial properties of polyphenols: Characterization and QSAR (quantitative structure - activity relationship). *Front. Microbiol.* 2019; **10**, 829.
- [17] PJ Desai and P Parikh, Extraction of natural products using microwaves as a heat source. *Sep. Purif. Rev.* 2010; **39**, 1-32.
- [18] P Tatke and Y Jaiswal. An overview of microwave assisted extraction and its applications in herbal drug research. *Res. J. Med. Plant.* 2011; **5**, 21-31.
- [19] DJ Alderman and P Smith. Development of draft protocols of standard reference methods for antimicrobial agent susceptibility testing of bacteria associated with fish diseases. *Aquaculture* 2001; **232**, 511-24.