



# Antimicrobial Activity of Ultrasonic Extracts of Two Chemotypes of *Thymus serpyllum* L. of Central Kazakhstan and their Polyphenolic Profiles

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## Abstract

**BACKGROUND:** The medicinal plant of *Thymus serpyllum* L. in nature, depending on the geographical region, climatic conditions, and growing environment, is represented with some chemotypes. Composition and quantitative content of the basic groups of the biologically active substances can be differed, and thus their biological properties are also various.

**AIM:** The aim of the study was to determine possibility of the using the ultrasonic extracts of two chemotypes of *T. serpyllum* L. of Central Kazakhstan as an antimicrobial agent against test strains of microorganisms.

**MATERIALS AND METHODS:** Two samples of *T. serpyllum* were extracted with 70% ethanol using ultrasound. The polyphenol content of the ultrasound extracts was determined using the LC-ultraviolet-ESI- tandem mass spectrometry technique. A study of an antimicrobial activity of the ultrasonic extracts was performed with eight strains of Gram-positive bacteria, six strains of Gram-negative bacteria, and four cultures of fungi.

**RESULTS:** The ultrasonic extracts of two chemotypes of *T. serpyllum* L. are similar in composition of phenolic compounds but differ in a quantitative content of phenolic acids and flavonoids, except for a rosmarinic acid. The ultrasonic extracts have a wide spectrum of antimicrobial activity, exhibit the bactericidal or bacteriostatic activity against all tested bacteria and fungi at a concentration of 0.0625–20 mg/ml, but differ in their strength of action against test strains of microorganisms.

**CONCLUSION:** The ultrasonic extracts of two chemotypes of *T. serpyllum* L. of Central Kazakhstan can be considered as a potential drug with a wide spectrum of antimicrobial activity. The results of chromatographic analysis will be used for standardization of a drug.

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## Introduction

*Thymus serpyllum* L. (creeping thyme, thyme) is a perennial shrub, Eurasian species, grows in the temperate climate of Eurasia, from Scandinavia to the Mediterranean and from the British Isles to Eastern Siberia.

The medicinal properties of *T. serpyllum* L. herb are known since ancient times and for many centuries used in traditional and folk medicine all over the world. *T. serpyllum* L. herb is included in the State Pharmacopoeia of the Republic of Kazakhstan, the Russian Federation, Ukraine, and other countries, in official medicine used as a medicinal plant material

with the antibacterial, astringent, anti-inflammatory, sedative, anticonvulsant, expectorant, antispasmodic, choleric, analgesic, diuretic, wound healing, and anthelmintic actions, applied as decoctions and infusions [1], [2], [3]. The British Herbal Pharmacopoeia classifies *T. serpyllum* as a medicinal plant and is recommended for the treatment of bronchitis, bronchial catarrh, whooping cough, and acute pharyngitis [4].

At present, *T. serpyllum* attracts the close attention of scientists all over the world due to its pharmacological properties. In recent years, interest in the ethnobotanical, phytochemical, and pharmacological studies of the medicinal properties of *T. serpyllum* is increased [5]. The systematic studies are performing on

the chemical composition and biological properties of *T. serpyllum* depending on the geographic region, climatic conditions, and growing environment, harvest season and vegetation phase. On their basis, it was determined that the essential oil and extracts of *T. serpyllum* are a promising natural resource for the pharmaceutical industry to develop and introduce the new effective herbal medicines in the traditional medicine [6], [7], [8], [9], [10], [11], [12], [13], [14], [15], [16], [17], [18].

In the world practice, it was proven that *T. serpyllum* in nature, depending on the geographic region, climatic conditions, and growing environment, is represented with some chemotypes, that is, changes in the qualitative composition and quantitative content of essential oil and the basic groups of the biologically active substances, therefore, the pharmacological properties also change [5]. Thus, the study of the chemical composition and the biological properties of *T. serpyllum*, depending on the territory and growing conditions, is of great importance to use this medicinal plant in the pharmaceutical industry and medicine.

The above-stated is confirmed by our previously studies of *T. serpyllum* growing in populations of Central Kazakhstan. The results of a comparative pharmacognostic study determined that *T. serpyllum*, growing in the Karaganda region (Figure 1), is represented by two chemotypes. Two herbal samples of *T. serpyllum* have an external similarity and identical diagnostic features of the anatomical structure for a leaf, stem, calyx, and corolla. In addition, significant differences were found between two chemotypes of *T. serpyllum* on the yield and composition of essential oil. The yield of essential oil from *T. serpyllum* growing in the Karkaralinsk mountain-forest was 1.38%. The key components are carvacrol (37.44%), thymol (16.26%), O-cymene (16.11%), and  $\gamma$ -terpinene (11.85%) in compliance with requirements of the State Pharmacopoeia of the Republic of Kazakhstan. The yield of essential oil from *T. serpyllum* collected in the Korenevsky forests was 0.42%, the basic components are nerolidol (33.72%),  $\beta$ -linalool (6.21%), 1,8-cineole (5.92%), and  $\alpha$ -terpineol (5.40%), which does not meet the requirements State Pharmacopoeias of the Republic of Kazakhstan. The differences between two chemotypes of *T. serpyllum* by a quantitative content of the sum of flavonoids, phenol carboxylic acids, tannins, triterpene compounds, water-soluble polysaccharides, pectin substances, amino acids, and organic acids were also defined [19].

In this research, dry extracts from the aerial part of two chemotypes of *T. serpyllum* were first obtained



Figure 1: *Thymus serpyllum* L. in nature of Central Kazakhstan

not by traditional methods of extraction (maceration, percolation, Soxhlet apparatus), but by two-fold extraction of plant materials with 70% ethanol using ultrasound, at an ultrasonic frequency of 40 kHz. The study of antimicrobial activity and polyphenolic profiles of the obtained ultrasonic extracts was first performed. Thus, the aim of this research is to determine the possibility of using the ultrasonic extracts of two chemotypes of *T. serpyllum* of Central Kazakhstan as an antimicrobial agent against test strains of microorganisms.

## Materials and Methods

### Plant material

The aerial part of *T. serpyllum* L. was collected from populations of the Karaganda region of the Republic of Kazakhstan: Sample 1 in the Karkaralinsk mountain-forest (N 49°38395'; E 75°47577') and sample 2 in the Korenevsky forests (N 50°21652'; E 74°33377'), in July 2016, in the full flowering phase. The botanical identification is confirmed at the Institute of Botany and Phytointroduction of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (conclusion on the species of plant raw materials No. 01-04/358).

### The obtaining of ultrasonic extracts

Dry extracts from two samples of *T. serpyllum* L. were obtained by double extraction of air-dry raw materials (leaves, flower baskets, and thin stems) with 70% ethanol, without soaking, the ratio of raw material mass and extractant volume is 1:20, in an Ultrasonic Cleaner ultrasonic bath (China) with the frequency of the ultrasonic radiation (40 kHz,) at room temperature (20–22°C) for 30 min. After ultrasonic treatment, the liquid extracts were filtered and the extractant was evaporated on Rotavapor® R-100 (Buchi, Switzerland) to dryness at temperature of 50°C.

### LC- ultraviolet (UV)-ESI-tandem mass spectrometry (MS/MS) analysis

High-performance liquid chromatography (HPLC) combined with a UV detector and real-time ESI-MS/MS were used to analyze the polyphenolic compounds of ultrasonic extracts. The following reagents were used in this research: acetonitrile (ACN) for HPLC ( $\geq 99, 9\%$ , Sigma-Aldrich, France), formic acid (99–100%, AnalaR NORMAPUR®, VWR Chemicals, France), highly purified water was prepared with a Milli-Q (Millipore, France) water purification system. The 17 selected phenolic compounds and standards (gallic acid, caffeic acid, chlorogenic acid, ferulic acid, rosmarinic acid, catechin, epicatechin, naringin,

rutin, luteolin-7-O-glucoside, luteolin, quercetin, apigenin, kaempferol, dihydroquercetin, myricetin, and naringenin) were purchased from Sigma–Aldrich (USA).

The analysis was performed on an “Agilent 1260 Infinity HPLC system” liquid chromatograph (Agilent Technologies, USA), equipped with G1311C 1260 Pump VL, autosampler G1329B 1260 ALS, thermostatted column compartment G1316A 1260 TCC; variable wavelength detector G1314C 1260 VWD VL+ and mass-spectrometer G6130A Quadrupole LC-MS/MS. Operated by Windows NT based ChemStation software was used.

Chromatographic separations were performed on a column with a “Zorbax Eclipse Plus C18” reversed-phase sorbent (150 mm × 4.6 mm, 3.5 μm, Agilent Technologies, USA). For separations, a gradient of mobile phase A (2.5% (v/v) formic acid in water) and mobile phase B (2.5% (v/v) formic acid in ACN) was used. The gradient profile was set as follows: 0.00 min 3% B eluent, 7.00 min 20% B eluent, 7.10 min 30% B eluent, 27.00 min 40% B eluent, 35.00 min 50% B eluent, 35.10 min 20% B eluent, and 40.00 min 3% B eluent. The flow rate was 0.4 mL/min, the column temperature was 30°C. The ultrasonic extracts and standards were dissolved in a mixture of solvents ACN:water = 1:1 (v/v). The injection volume was 20 μL for the ultrasound extracts and standards. The column effluent passed through a UV detector before arriving in the MS interface. UV detection wavelengths were 280 nm and 360 nm. The electrospray ionization mass spectrometry detection was performed in negative mode with the following optimized parameters: Capillary temperature 350°C; drying gas N<sub>2</sub> 8 L/min; and nebulizer pressure 45 psi. Data gaining was performed using multiple reactions monitoring method that only monitors specific mass transitions during preset retention times.

The identification of each compound was performed by comparing their retention times to authentic standards and also confirmed by an Agilent G6130A LC-MS/MS spectrometer equipped with an electrospray ionization source. The quantitative content of phenolic compounds in ultrasonic extracts was calculated by the external standard method.

### Study of antimicrobial activity

The ultrasound extracts were screened for the antibacterial and antifungal activities by microdilution method using Mueller–Hinton (MH) broth and MH broth with 5% lysed sheep/horse blood for growth of non-fastidious and fastidious bacteria, respectively, or MH broth with 2% glucose for growth of fungi. Minimal Inhibitory Concentration (MIC) of the tested extract was evaluated for the panel of the reference microorganisms from American Type Culture Collection (ATCC), including Gram-positive bacteria (*Staphylococcus aureus* ATCC6538, *Staphylococcus epidermidis* ATCC12228, *Micrococcus luteus* ATCC10240, *Bacillus subtilis* ATCC6633, *Bacillus cereus* ATCC10876, *Streptococcus pneumoniae* ATCC49619, *Streptococcus*

*pyogenes* ATCC19615, and *Streptococcus mutans* ATCC25175), Gram-negative bacteria (*Salmonella typhimurium* ATCC14028, *Klebsiella pneumoniae* ATCC13883, *Escherichia coli* ATCC25922, *Proteus mirabilis* ATCC12453, *Pseudomonas aeruginosa* ATCC9027, and *Helicobacter pylori* ATCC43504), and fungi (*Candida albicans* ATCC102231, *Candida parapsilosis* ATCC22019, *Candida glabrata* ATCC 90030, and *Candida krusei* ATCC 14243).

The ultrasound extracts dissolved in dimethyl sulfoxide (DMSO) were first diluted to concentration (20 g/mL) in an appropriate broth medium recommended for bacteria or yeasts. Then, using the same media, serial two-fold dilutions were made to obtain the final concentrations of the tested extracts ranged from 20 to 0.156 mg/mL. The sterile 96-well polystyrene microtiter plates (Nunc, Denmark) were prepared by dispensing 200 μL of appropriate dilution of the tested extracts in broth medium per well. The inocula were prepared with fresh microbial cultures in sterile 0.85% NaCl to match the turbidity of 0.5 McFarland standard and 2 μL were added to wells to obtain final density of 1.5 × 10<sup>6</sup> colony forming units (CFU)/ml for bacteria and 5 × 10<sup>4</sup> CFU/ml for yeasts; CFU. After incubation (35°C for 24 h), the MICs were assessed visually as the lowest concentration of the extracts showing complete growth inhibition of the reference microbial strains. Appropriate DMSO control (at a final concentration of 10%), a positive control (containing inoculum without the tested extracts), and negative control (containing the tested extracts without inoculum) were included on each microplate [20].

The MIC of *H. pylori* was determined using two-fold microdilution method in MH broth with 7% of lysed horse blood at extracts concentration ranging from 20 to 0.156 mg/mL with bacterial inocula of 3 McFarland standard. After incubation at 35°C for 72 h under microaerophilic conditions (5% O<sub>2</sub>, 15% CO<sub>2</sub>, and 80% N<sub>2</sub>), the growth of *H. pylori* was visualized by the addition of resazurin. The MIC endpoint was recorded as the lowest concentration of extracts that completely inhibit growth [20].

Minimal bactericidal concentration (MBC) or minimal fungicidal concentration (MFC) were obtained by subculturing 5 ml from each well that showed through growth inhibition, from the last positive one and the growth control onto recommended agar plates. The plates were incubated at 35° for 24 h for all microorganisms but *H. pylori* which was incubated for 72 h in microaerophilic conditions [20], [21]. The MBC/MFC was defined as the lowest concentration of extracts without the growth of microorganisms. The MBC/MIC ratios were calculated to determine the bactericidal or bacteriostatic effect of the tested extracts [22]. Experiment was repeated in triplicate. Representative data are presented.

## Results

### Results of extraction

It was found that under similar extraction conditions, the yield of dry ultrasonic extract from *T. serpyllum* (sample 1) was  $14.1 \pm 0.04\%$ , which significantly more than the yield of dry ultrasonic extract from *T. serpyllum* (sample 2)  $6.3 \pm 0.08\%$  ( $p < 0.001$ ).

### Results of LC-UV-ESI-MS/MS analysis

The phenolic profiles of the obtained ultrasonic extracts of two chemotypes of *T. serpyllum* and mass spectra for the identified compounds in negative ionization mode are listed in Table 1.

In total, 15 phenolic compounds were identified and quantified, five of which are phenolic acids, and ten are flavonoids. In the obtained ultrasonic extracts, we found a similarity in the qualitative composition of the phenolic compounds but determined the significant differences in the quantitative content of phenolic acids and flavonoids, except for a rosmarinic acid. The dominant polyphenolic compounds in the tested ultrasonic extracts are luteolin 7-O-glucoside with a content of 94.69 and 119.26 mg/g, rosmarinic acid (32.61 and 32.62 mg/g), naringenin (7.69 and 17.82 mg/g),

and epicatechin (9.03 and 11.74 mg/g). The ultrasonic extract of *T. serpyllum* (sample 1) contains a relatively high amount of catechin, myricetin, quercetin, apigenin, and kaempferol. In the ultrasonic extract of *T. serpyllum* (sample 2), a relatively higher content of caffeic acid, gallic acid, chlorogenic acid, ferulic acid, epicatechin, rutin, luteolin 7-O-glucoside, naringenin, and luteolin.

### Research results of antimicrobial activity

The research results in Table 2 demonstrate that the ultrasonic extracts of two chemotypes of *T. serpyllum* have a broad spectrum of antimicrobial activity, exhibit the bactericidal (MBC/MIC  $\leq 4$ ) or bacteriostatic (MBC/MIC  $> 4$ ) activity against all test strains.

However, the ultrasonic extract of *T. serpyllum* (sample 1) showed the maximum bactericidal activity against *H. pylori* with MIC = 0.625 mg/ml and MBC = 1.25 mg/ml (MBC/MIC = 2). It has a stronger bactericidal effect against 5 strains of Gram-positive bacteria of *S. aureus*, *S. epidermidis*, *M. luteus*, and *B. subtilis* at a concentration of 1.25–2.5 mg/ml (MBC/MIC = 1), and causes growth inhibition of cultures of *B. cereus* at a concentration of MIC = 2.5 mg/ml. Furthermore, at a concentration of 1.25–2.5 mg/ml can be observed a bactericidal effect (MBC/MIC = 1) against Gram-negative strains of *K. pneumoniae* and *P. mirabilis*.

**Table 1: Identification and content of phenolic compounds of ultrasound extracts two chemotypes of *T. serpyllum* L**

Peak	Retention time (min)	M-H <sup>+</sup> (m/z)	Compound identity	Quantification (mg/g dry extract)	
				Ultrasonic extract <i>T. serpyllum</i> (sample 1)	Ultrasonic extract <i>T. serpyllum</i> (sample 2)
1	3.928	179	Caffeic acid	0.07	0.53
2	4.932	169	Gallic acid	3.89	4.18
3	12.485	353	Chlorogenic acid	0.46	0.92
4	13.089	289	Catechin	1.74	1.30
5	13.945	289	Epicatechin	9.03	11.74
6	14.112	609	Rutin	2.54	2.94
7	14.600	447	Luteolin 7-O-glucoside	94.69	119.26
8	16.299	193	Ferulic acid	2.64	3.03
9	16.986	359	Rosmarinic acid	32.61	32.62
10	17.448	317	Myricetin	3.08	2.95
11	22.303	301	Quercetin	0.65	0.47
12	25.267	271	Naringenin	7.69	17.82
13	27.350	269	Apigenin	1.16	0.72
14	28.350	285	Luteolin	0.87	1.30
15	28.806	285	Kaempferol	0.53	0.19

*T. serpyllum*: *Thymus serpyllum*.

**Table 2: Research results of antimicrobial activity of ultrasonic extracts of two chemotypes of *T. serpyllum* L**

Microorganism	Ultrasonic extract <i>T. serpyllum</i> (sample 1)			Ultrasonic extract <i>T. serpyllum</i> (sample 2)		
	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC ratio	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC ratio
Gram-positive bacteria						
<i>Staphylococcus aureus</i>	1.25	1.25	1	2.5	2.5	1
<i>Staphylococcus epidermidis</i>	1.25	1.25	1	1.25	1.25	1
<i>Micrococcus luteus</i>	2.5	2.5	1	2.5	5	2
<i>Bacillus subtilis</i>	1.25	1.25	1	1.25	5	4
<i>Bacillus cereus</i>	2.5	>20	>8	2.5	>20	>8
<i>Streptococcus pneumoniae</i>	5	10	2	2.5	5	2
<i>Streptococcus pyogenes</i>	5	10	2	2.5	10	4
<i>Streptococcus mutans</i>	5	20	4	5	5	1
Gram-negative bacteria						
<i>Salmonella typhimurium</i>	10	10	1	5	5	1
<i>Klebsiella pneumoniae</i>	1.25	1.25	1	1.25	1.25	1
<i>Proteus mirabilis</i>	2.5	2.5	1	2.5	2.5	1
<i>Escherichia coli</i>	10	10	1	5	5	1
<i>Pseudomonas aeruginosa</i>	5	10	2	5	10	2
<i>Helicobacter pylori</i>	0.625	1.25	2	0.0625	0.250	4
Yeasts						
<i>Candida albicans</i>	10	10	1	2.5	10	4
<i>Candida parapsilosis</i>	10	10	1	5	10	2
<i>Candida glabrata</i>	10	>20	>2	5	10	2
<i>Candida krusei</i>	10	10	1	5	10	2

*T. serpyllum*: *Thymus serpyllum*.

The ultrasonic extract of *T. serpyllum* (sample 2) showed the maximum bactericidal activity against *H. pylori* with the lowest MIC = 0.0625 mg/ml and MBC = 0.250 mg/ml (MBC/MIC = 4). This ultrasound extract has stronger bactericidal activity against only 2 strains of Gram-positive bacteria of *S. aureus* and *S. epidermidis* at a concentration of 1.25-2.5 mg/ml (MBC/MIC = 1), while inhibiting the growth of cultures of 5 strains of *M. luteus*, *B. subtilis*, *B. cereus*, *S. pneumoniae*, and *S. pyogenes* at a concentration of 1.25-2.5 mg/ml. Furthermore, at a concentration of 1.25-2.5 mg/ml, it has a bactericidal effect (MBC/MIC = 1) against 2 strains of gram-negative bacteria of *K. pneumoniae* and *P. mirabilis*. In addition, at a concentration of 2.5 mg/ml, it causes growth inhibition of the culture of *C. albicans* fungus.

## Discussion

Our results confirm that *T. serpyllum* L. in nature, depending on the geographic region, climatic conditions, and growing environment, is represented by some chemotypes, their composition, quantitative content of the biologically active substances, and biological properties, in particular, antimicrobial action is differ.

In addition, the method of extracting of the biologically active substances from plant raw materials of *T. serpyllum* is important. The basic biologically active substances of *T. serpyllum*, besides an essential oil, are phenolic compounds. These secondary metabolites play an important biological role and have a wide range of pharmacological properties, including antimicrobial action [23], [24]. The content of polyphenols and their structure (number and position of the hydroxyl groups in a molecule) significantly influence on the pharmacological properties of the medicinal plants. Water, methanol, and ethanol are the most commonly used solvents to extract polyphenols from plant materials. As a rule, the standard methods of extraction are used to extract polyphenols (maceration, percolation, and Soxhlet apparatus). Leaves of *T. serpyllum* (Morocco) were extracted with 90% ethanol for 4 days at room temperature. The resulting extract has antibacterial activity against *Listeria monocytogenes* (MIC = 2.15 mg/ml) and also inhibits the growth of *S. aureus*, *E. coli*, *Enterococcus faecalis*, *K. pneumoniae*, *Enterobacter cloacae*, and *Acinetobacter baumannii* [25]. The buffered methanol leaf extract of *T. serpyllum* growing in Yemen has antimicrobial activity against *Listeria monocytogenes*, *S. aureus*, and *B. cereus* with MIC = 660-1320 mg/l. At a maximum concentration of 2640 mg/l, it does not exhibit an antimicrobial action against *E. coli* and *Salmonella infantis* [26]. Fresh leaves of *T. serpyllum* collected at the edge of a pine forest (Baymaky village, Bilohirya district, Khmelnytsky region, Ukraine) were washed,

weighed, ground, and homogenized in 96% ethanol (1:19 ratio) at room temperature. The resulting extract exhibits an antimicrobial action against *Acinetobacter baumannii*, the average diameter of the zone of inhibition is  $10.45 \pm 0.81$  mm [27]. The plant *T. serpyllum* used in this research was obtained commercially (Aboca, SpA, S. Sepolcro, Arezzo, Italy). The plant was extracted with distilled water (aqueous extract) or 95% ethanol (ethanol extract) at room temperature for 24 h. The extracts have an antibacterial effect against the control and eleven clinical strains of *H. pylori*, for the aqueous extract of MIC = 1.25-10 mg/mL and for ethanol extract o MIC = 1.25–2.5 mg/mL [28].

In this research, the sum of the polyphenolic compounds from the dry aerial part of two chemotypes of *T. serpyllum* was extracted by not traditional methods of extraction (maceration, percolation, and Soxhlet apparatus) but using ultrasound. A method for obtaining of dry extract from *T. serpyllum*, due to the use of the ultrasonic extraction, is characterized by high productivity of the technological process, low consumption of extractant, elimination of labor-intensive and time-consuming procedures, which makes it affordable, rational, and economical. This method can find its application in the pharmaceutical industry to produce new medicines with an antimicrobial action of the plant origin [29], [30].

The obtained ultrasonic extracts of two chemotypes of *T. serpyllum* growing in the territory of Central Kazakhstan have a wide spectrum of antimicrobial activity, exhibit the bactericidal or bacteriostatic activity against all tested bacteria and fungi at a concentration of 0.0625 to 20 mg/ml, but differ in their effect against the test strains of microorganisms.

An ultrasonic extract of creeping thyme collected in the Karkaralinsk mountain-forest showed maximum bacteriostatic activity against *H. pylori* with MIC = 0.625 mg/ml and bactericidal at a concentration of 1.25 mg/ml. It has a stronger bactericidal effect against 5 strains of gram-positive bacteria of *S. aureus*, *S. epidermidis*, *M. luteus*, and *B. subtilis* at a concentration of 1.25-2.5 mg/ml, and only bacteriostatic action against *B. cereus* MIC = 2.5 mg/ml. Furthermore, at a concentration of 1.25-2.5 mg/ml, it exhibits a bactericidal effect against Gram-negative strains of *K. pneumoniae* and *P. mirabilis*.

For an ultrasonic extract of *T. serpyllum* collected in the Korenevsky forests, the maximum bacteriostatic and bactericidal activity against *H. pylori* was defined with the lowest MIC = 0.0625 mg/ml and MBC = 0.250 mg/ml. This ultrasonic extract demonstrates a stronger bactericidal activity against only 2 strains of gram-positive bacteria of *S. aureus* and *S. epidermidis* at a concentration of 1.25-2.5 mg/ml (MBC/MIC = 1), but at the same concentration inhibits the growth of cultures of 5 strains *M. luteus*, *B. subtilis*, *B. cereus*, *S. pneumoniae*, and *S. pyogenes*. Furthermore, at a concentration of 1.25–2.5 mg/ml, it has a bactericidal effect (MBC/

MIC = 1) against 2 strains of Gram-negative bacteria of *K. pneumoniae* and *P. mirabilis*. In addition, at a concentration of 2.5 mg/ml, it causes growth retardation of the culture of the fungus *C. albicans*.

There are also differences in the bactericidal and bacteriostatic effects of the tested ultrasonic extracts against the tested bacteria and fungi at a concentration of 5–20 mg/ml.

It can be explained by the difference in the quantitative content of the identified phenolic acids and flavonoids. The dominant phenolic compounds in the studied ultrasonic extracts are luteolin 7-O-glucoside with a content of 94.69 and 119.26 mg/g, rosmarinic acid (32.61 and 32.62 mg/g), naringenin (7.69 and 17.82 mg/g), and epicatechin (9.03 and 11.74 mg/g). The ultrasonic extract of *T. serpyllum* collected in the Karkaralinsk mountain-forest, contains a relatively high amount of catechin, myricetin, quercetin, apigenin, and kaempferol. In an ultrasonic extract of *T. serpyllum* collected in the Korenevsky forests, a relatively higher content of caffeic acid, gallic acid, chlorogenic acid, ferulic acid, epicatechin, rutin, luteolin 7-O-glucoside, naringenin, and luteolin was determined. Research of the composition of the phenolic compounds by ultrasonic extracts of two chemotypes of *T. serpyllum* L. of Central Kazakhstan was first performed. Our obtained results proved that depending on the territory and growing conditions in nature, the composition and quantitative content of biologically active substances of *T. serpyllum* L. change. The obtained ultrasonic extracts have significant differences in the qualitative composition of phenolic compounds and the quantitative content of phenolic acids and flavonoids, from the data previously published in the world literature [24], [31], [32], [33], [34], [35].

Thus, it was first established that ultrasonic extracts of the two chemotypes of *T. serpyllum* growing in the territory of Central Kazakhstan have a wide spectrum of antimicrobial activity, exhibit the bactericidal or bacteriostatic activity against all tested bacteria and fungi at a concentration of 0.0625–20 mg/ml, but differ in their effect against test strains of microorganisms. Since, they contain various amounts of the identified phenolic acids and flavonoids, except for rosmarinic acid.

## Conclusions

Ultrasonic extracts of two chemotypes of *T. serpyllum* L. of Central Kazakhstan can be considered as a potential drug with a wide spectrum of antimicrobial activity. The results of chromatographic analysis will be used for standardization of a drug.

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