

## PHYTOCHEMICAL STUDY OF *CRAMBE TATARICA* SEBEOK, A PROMISING SOURCE OF MEDICINAL PLANT EXTRACTS

TURALIN BAUYRZHAN<sup>1</sup>, KURBATOVA NATALYA<sup>1</sup>, INELOVA ZARINA<sup>1\*</sup>, KUPRIYANOV ANDREY<sup>2</sup>, KURMANBAYEVA MERUERT<sup>1</sup>, ABIDKULOVA KARIME<sup>1</sup> AND BAZARGALIYEVA ALIYA<sup>3</sup>

<sup>1</sup>Al-Farabi Kazakh National University, Department of Biology and Biotechnology, Republic of Kazakhstan, Almaty

<sup>2</sup>Kuzbass Botanical Garden of Institute of Human Ecology, Federal Research Center of Coal and Coal Chemistry, Siberian Branch of the Russian Academy of Sciences, Russia, Kemerovo

<sup>3</sup>Aktobe Regional State University named after K. Zhubanov, Kazakhstan, Aktobe

\*Corresponding author's email: [z.inelova2015@gmail.com](mailto:z.inelova2015@gmail.com)

### Abstract

The article presents the results of a phytochemical study of *Crambe tatarica* Sebeok, a promising plant of the family Brassicaceae growing in Western Kazakhstan. The results of qualitative and quantitative analyses confirmed that the aboveground parts of *C. tatarica* are richer in alkaloids, carotenoids, monosaccharides (glucose, galactose, rhamnose, mannose, xylose, arabinose, and rutinose) and phospholipids than the root system. In the roots of *C. tatarica*, a higher content of tannins has been recorded (2,3-*O*-hexaoxidifenoyl-4,6-*O*-sanguisorbil-*D*-glucose, 2,3-di-*O*-galloyl-*D*-glucose, 1,2,4-tri-*O*-galloyl- $\beta$ -*D*-glucose), coumarins (4,5-dioxycoumarin, 7-hydroxycoumarin, scopoletin), polysaccharides, triterpenoids, phenolic and hydroxycinnamic acids (gallic, caffeic, ferulic, lilac, gentisic, vanillic, o-cumaric) and flavonoids (kaempferol, quercetin, gossipetin, myricetin, (+)-catechin, 3-*O*  $\alpha$ -*L*-rhamnopyranoside kaempferol). Regardless of the place of growth, the Kazakh species *C. tatarica* contains alkaloids, tannins of the hydrolyzable type, carotenoids, coumarins, polysaccharides, triterpenoids, phenolic acids, flavonoids and phospholipids. We studied antibacterial and fungicidal activity of the aqueous, alcohol and acetone extracts of the aboveground parts and roots of *C. tatarica*. We found that certain concentrations of these extracts had both bactericidal and fungicidal activity. The highest activity of the tested samples of acetone extracts was shown against the test strain *S. aureus* ATCC 6538-P. Thus, acetone extracts of the aboveground parts and roots of *C. tatarica* were active against staphylococcus at a concentration of 0.78 mg / ml, where 0.9% saline was used as a solvent. When dissolved in 96% ethanol, acetone extracts also showed increased activity against the museum test strain of *S. aureus* ATCC 6538-P. Extracts from the aboveground parts and roots of *C. tatarica* had fungicidal activity against *Candida albicans* ATCC 10231 at concentrations of 0.78 mg / ml 12.5 mg / ml, respectively (96% ethanol was used as a solvent).

**Key words:** *Crambe tatarica*, Qualitative analysis, Quantitative analysis, Bactericidal activity, Fungicidal activity.

### Introduction

Comprehensive studies of promising medicinal plants currently play an important role in replenishing the range of species officially used in medicine. Phytochemical studies are important for the production of raw materials with the maximum amount of active ingredients. In Kazakhstan, researchers and scientists have been continuing their search for new species of the local flora.

*Crambe tatarica* is one of such promising medicinal plants. It is a Southern European-Mediterranean species. This is a perennial, up to 90 cm tall polycarpic plant. Roots are 60–120 cm long, fusiform and juicy. Plants are single- or multi-stemmed; stems are erect, with sparse, short, stiff hairs, later naked. Leaves are fleshy and grey-green in color; basal leaves are large, on petioles, up to 30 cm long and 20 cm wide, twice pinnate, with oblong-linear, toothed or incised lobes and stiff hairs, especially on the abaxial side, later on naked; the upper most leaves are linear and entire. Flowers are white, 4.5–5 mm in length, with a honey smell, arranged in dense panicle inflorescence. Pods are spherical; the upper segment of the pod is 4–5 mm in diameter, tetragonal, mesh-rugose, with four sharply prominent ribs, on the stalks up to 10 mm in length. The species grows in steppes, on stony-gravelly slopes, and on chalky outcrops. In Kazakhstan, it is found in the Northern Pre-Caspian, Aktobe, Atyrau and Kostanay regions, where the eastern border of its range is located. Outside Kazakhstan, the species is found in the south of the

European part of the former USSR, in the Crimea, in the Caucasus, in Western Siberia, and in Southern Europe (Pavlov, 1961; Red Data Book..., 2014; Gouz, 2016).

The floristic composition of plant communities with the participation of *C. tatarica* found on chalky outcrops in Western Kazakhstan has been studied in detail by Kupriyanov A.N. and others in 2020 (Kupriyanov *et al.*, 2020; Kupriyanov *et al.*, 2020). They described 141 species belonging to 33 families and 95 genera. The floristic composition of these plant communities reflects the ecological conditions of calcareous, partly clayey habitats in which *C. tatarica* populations are formed. Ecobiological analysis confirmed the desert-steppe nature of the cenoflora with a small participation of meadow species.

The knowledge of the chemical composition of the plant under study is limited, which justifies our research.

To date, only two species of the genus *Crambe* have been comprehensively studied: *Crambe kotschyana* Boiss. and *Crambe abyssinica* L. (Aguinalcalde & Del Pero Martinez, 1982; Sokolov, 1986; Budantsev & Lesiovskaya, 2001; Grudzinskaya *et al.*, 2014). The results of phytochemical study of *C. tatarica* are sketchy. We found the following information on the chemical composition of this plant. *C. tatarica* contains: kaempferol-3-(2-hydroxypropionyl) glucoside-4'-glucoside, kaempferol-3-(*p*-coumaroyl) glucoside-4'-glucoside, kaempferol-3-feruloylglucoside-7-glucoside, kaempferol-3-(*p*-coumaroyl)-glucoside-7,4'-diglucoside, kaempferol-3-(*p*-coumaroyl) feruloyl-diglucoside-7-

rhamnoside, quercetin-3-malonylglucoside-4'-glucoside, quercetin-3-feruloylglucoside-4'-glucoside, quercetin-3-feruloylglucoside-7-glucoside, quercetin-3-feruloylglucoside-7,4'-diglucoside (Aguinacalde & Del Pero Martinez, 1982), vitamin C, carotene (Amirkhanov et al., 1974; Sokolov, 1986; Budantsev & Lesiovskaya, 2001; Grudzinskaya et al., 2014) up to 43.40% of fatty oil (Grashchenkov, 1959; Miller & Earle, 1965; Sokolov, 1986; Budantsev & Lesiovskaya, 2001; Grudzinskaya et al., 2014), acids: oleic-28.69%, linoleic-22.17%, erucic-27.00%, palmitoleic-0.32%, eicosene-21.00%, linolenic-11.00%, palmitic-2.00%, eicosadienic-1.29%, stearic-0.80%, arachidic-0.50%, myristic-0.05% (Miller & Earle, 1965; Dolia et al., 1977; Sokolov, 1986; Pushkarova et al., 2016).

We also found information on the biological activity of phytopreparations of plants of the genus *Crambe* L. For example, an aqueous solution of *Crambe kotschyana* is used in folk medicine as an antiscorbutic and general tonic remedy (Sokolov, 1986).

**Aqueous infusions:** of *C. tatarica* roots have been used as a general tonic and vitamin supplementation for children (Sokolov, 1986; Budantsev & Lesiovskaya, 2001). In Indian medicine, a decoction of the roots of *Crambe cordifolia* Steven is used to treat scabies (Sokolov, 1986; Budantsev & Lesiovskaya, 2001; Chopra & Nayar, 2006; Grudzinskaya et al., 2014); a decoction of leaves of *Crambe orientalis* L. and roots of *Crambe pinnatifida* R. Br. is used to treat scurvy (Gammerman, 1982; Budantsev & Lesiovskaya, 2001; Grudzinskaya et al., 2014).

In the laboratory conditions, *Crambe koktebelica* N. Busch exhibited antibacterial activity. A tincture of *Crambe kotschyana* seeds is used in Central Asia to treat respiratory tract infections (Sokolov, 1986; Sakhobiddinov, 1988).

**Nutritional uses** of etiolated leaves and roots of *Crambe aspera* M. Bieb. (Chopra & Nayar, 2006; Hostettmann & Marston, 1995), and stems and leaves of *Crambe cordifolia* (Sokolov, 1986; Medvedev, 1987; Budantsev & Lesiovskaya, 2001; Grudzinskaya et al., 2014), roots of *Crambe juncea* Bieb. and *Crambe pinnatifida*, roots and petioles of *Crambe litwinowii* H.Gross (Sokolov, 1986; Medvedev, 1987), roots and stems of *Crambe kotschyana* (Pavlov, 1947; Shalyt, 1951; Sokolov, 1986), etiolated shoots and leaves of *Crambe maritima* L. (Hoppe, 1975; Grudzinskaya et al., 2014;), **young leaves and shoots of *Crambe aspera***, shoots, leaves and fatty oil of *Crambe orientalis* (Kiryushina, 1971; Budantsev & Lesiovskaya, 2001), roots, young shoots and leaves of *C. tatarica* (Sokolov, 1986; Medvedev, 1987) have been described. **Shredded roots of *Crambe steveniana*** Rupr. have been used as a spicy food seasoning (Sokolov, 1986; Medvedev, 1987). *Crambe maritima* and *Crambe kotschyana* are honey bearing plants (Pavlov, 1947; Sokolov, 1986; Vulf & Maleeva, 1989; Budantsev & Lesiovskaya, 2001).

*Crambe aspera*, *C. cordifolia*, *C. koktebelica*, *C. litwinowii*, *C. maritima*, *C. orientalis*, *C. steveniana*, *C. tatarica*, *C. juncea*, and *C. kotschyana* have been used as fodder for domesticated livestock (Miller & Earle, 1965; Amirkhanov et al., 1970; Amirkhanov et al., 1973; Kondratyev, 1973; Kucherov, 1974a, 1974b; Mukumov,

1980; Sokolov, 1986; Taran & Nekrasova, 1986; Budantsev & Lesiovskaya, 2001; Pushkarova et al., 2016).

**The roots of *C. tatarica* can be used as a natural calcium accumulator.** *C. cordifolia*, *C. maritima* and *C. tatarica* can be used as decorative plants (Budantsev & Lesiovskaya, 2001).

Thus, the relevance of the study of the Kazakh species *C. tatarica* is obvious given practical applications of plants of the genus *Crambe* L. It is further justified by insufficient knowledge of this species and the need to expand the Kazakh plant material base for the production of valuable herbal remedies for medicine and agriculture.

## Material and Methods

In our study, we were guided by the following generally accepted methods of phytochemical analysis of plant materials.

The qualitative analysis of alkaloids was carried out [31]: 2 ml of a 1% solution of phosphor molybdic acid was added to 2 ml of a 50% aqueous-alcoholic extract, and a yellow precipitate formed, which turned blue after 20 minutes.

The quantitative determination of the content of alkaloids was carried out according to a modified method [32]. About 1 g (accurately weighed) of the crushed raw material was placed in a conical flask with a capacity of 100 ml. We then added 10 ml of 25% sodium hydroxide solution and stirred the mixture with a glass rod until a moistened mass formed; the latter was left at room temperature for two hours. Then, 50 ml of chloroform was added, and the mixture was left for one hour. Without stirring up the solution, 15 ml of the extract was transferred with a pipette into a separating funnel with a capacity of 100 ml, and the alkaloids were extracted three times with a 2% sulfuric acid solution in portions of 20, 10, and 10 ml until a negative reaction with silicotungstic acid was observed. The combined acid extracts were filtered into a volumetric flask with a capacity of 50 ml, the volume of the solution was brought to the mark with a 2% solution of sulfuric acid, and the optical density was measured at a wavelength of 420 nm in a cuvette with a layer thickness of 10 mm, using a 2% solution of sulfuric acid as a reference solution. The content (in percent, X) of alkaloids in terms of berberine bisulfate was calculated by the formula (1):

$$x = \frac{50 \times 50 \times D \times 100 \times 100}{15 \times 128 \times m \times (100 - W)} \quad (1)$$

where D is the optical density of sulfuric acid extraction at a wavelength of 420 nm;

128 is the specific absorption index of berberine bisulfate at a wavelength of 420 nm;

W is the loss in weight during drying of raw materials, %;

m is the weight of the sample of raw materials, g.

The qualitative analysis of tannins was carried out using the water extract of *Crambe tatarica* (Muzychkina et al., 2012). The quantitative evaluation of tannins was carried out using the pharmacopoeial method (State Pharmacopoeia., 2008). Isolation of the sum of all fractions of tannins and its

component analysis was carried out using a saturated solution of sodium bicarbonate (Rakhmadiyeva, 2000). Component analysis of tannins was carried out by HPLC using authentic samples in the system: LiChrospher-100 RP<sub>18</sub> (5 µm), PP -0.01M phosphoric acid - 0.01M potassium dihydrogen phosphate - acetonitrile (47.5:47.5:5), using a UV detector (254 nm) (Salminen, 1999).

The qualitative analysis of carotenoids was carried out according to the Carr-Price reaction (Muzychkina *et al.*, 2012). The quantitative evaluation of carotenoids was carried out using the spectrophotometric method (Kaczor & Baranska, 2016).

We carried out the qualitative analysis of coumarins (Kuznetsova, 1967). To 2 ml of aqueous-alcoholic extraction, 3 ml of pyridine was added, then 2 ml of 0.1 N sodium hydroxide solution were added dropwise. In this case, the color changed from yellow through green to blue in the presence of 3 drops of an alcohol solution of bromothymol blue.

The quantitative determination of the content of coumarins in plant material was carried out according to a modified method (Grinkevich & Safronich, 1983). An exact weighed portion of the crushed raw material was placed in a flask with a capacity of 100 ml, 50 ml of chloroform was added, and the mixture was heated with stirring for two hours in a boiling water bath with a reflux condenser; it was then filtered through a paper filter. Twenty milliliters of the filtrate was placed in a separatory funnel, and 1 g of sodium chloride was added, shaking for 5 minutes. The chloroform extract was evaporated to a dry state in a boiling water bath. The dry residue was dissolved in 10 ml of ethyl alcohol 96%, quantitatively transferred with 10 ml of ethyl alcohol 96% into a volumetric flask with a capacity of 25 ml, bringing the volume of the solution to the mark with ethyl alcohol 96%. The optical density of the solution was measured at a wavelength of 272 nm in a cuvette with a layer thickness of 10 mm, using 96% ethyl alcohol as a reference solution. The content (in percent) of coumarin derivatives in absolutely dry raw materials in terms of CO was calculated using the formula (2):

$$x = \frac{D \times 25 \times 100 \times 100}{734 \times 20 \times m \times (100-W)} \quad (2)$$

where D is the optical density of the test solution at a wavelength of 272 nm;

734 is the specific absorption index of CO of coumarin at a wavelength of 272 nm;

m is the weight of raw material sample, g;

W is the loss of mass during drying of raw materials, %.

To isolate the sum of all fractions of coumarins and to carry out its component analysis, the plant material was pretreated with petroleum ether and then exhaustively extracted with chloroform and ethyl alcohol (Muzychkina *et al.*, 2011). HPLC analysis of coumarins in the presence of authentic samples was carried out in the system: LiChrospher-100 RP<sub>18</sub> (5 µm), PP: dioxane - 0.01 M sodium hydrogen phosphate solution (36.3:63.7) (pH = 7.3) using a UV detector (340 nm) (Celeghini *et al.*, 2001).

We performed the qualitative analysis of polysaccharides (Grinkevich & Safronich, 1983). A four-fold excess of ethyl alcohol 95% was added to 5 ml of an aqueous extract of plant raw material, and a precipitate was observed.

The quantitative evaluation of polysaccharides was carried out according to a modified method of Bock K. (Bock, 1990).

The qualitative analysis of triterpenoids was carried out according to the Kedde method (Muzychkina *et al.*, 2012). For this, 2 ml of a 1% alcohol solution of m-dinitrobenzoic acid in an alkaline medium was added to 2 ml of the chloroform extract of the plant; the appearance of a bright green color was observed.

The quantitative evaluation of triterpenoids was carried out according to a modified method of Hostettmann K. and Marston A. (Hostettmann & Marston, 1995).

The qualitative analysis of phenolic acids was carried out using an acetone extract of the plants (Grinkevich & Safronich, 1983). The quantitative evaluation of phenolic acids was carried out according to a modified method of Schmuck C. And others (Schmuck *et al.*, 2004).

All fractions of phenolic and hydroxycinnamic acids were isolated and their component analyses carried out following Muzychkina R.A. and others (Muzychkina *et al.*, 2011). The obtained extracts were investigated by HPLC with authentic samples of phenolic and hydroxycinnamic acids on a LiChrospher RP-C<sub>18</sub> column using the following mobile phases: 0.1% trifluoroacetic acid - acetonitrile with an increase of the latter in the mixture from 10 to 100% in 45 min and methanol - acetic acid - water with a linear gradient of the mobile phase from (10:2:88) to (90:2:8) in 30 minutes, and using a UV detector at 276 nm (Javanmardi, 2002).

The qualitative analysis of flavonoids was carried out according to the reaction with the Martini-Bettolo reagent (Muzychkina *et al.*, 2004). The quantitative evaluation of flavonoids was carried out according to the method of The United States Pharmacopeia 33 - National Formulary 28. (The United States, 2010) with recalculation for the dominant quercetin in the raw material. The sum of all fractions of flavonoids was isolated and its component analysis was carried out (Korulkin *et al.*, 2007). The component analysis of flavonoids in the presence of authentic samples was carried out by HPLC on a column with m-Bondapak C<sub>18</sub> in the system: methanol-water-acetonitrile, with gradient elution from (6:90:4) to (17.4:71:11.6) for 30 minutes, and using a UV detector (280 nm) (Merken, 2000).

The qualitative analysis and quantitative evaluation of phospholipids was determined by the gravimetric method (Itavo *et al.*, 2015). Five grams of crushed raw material were placed in a conical flask with a capacity of 100 ml with a ground stopper, and 25 ml of a mixture of chloroform-ethyl alcohol 96% (2:1) was added and extracted for three hours with periodic stirring, then filtered. Extraction with a chloroform-alcohol mixture was repeated twice in portions of 25 ml. To the combined chloroform-alcohol extracts, 150 ml of acetone was added, stirred, and left to settle for 30 min. The formed precipitate (phospholipids) was filtered off through a paper filter and washed with 50 ml of acetone. The filter

with the precipitate was dried at a temperature of 50-60°C to a constant weight. The total content of phospholipids was calculated in terms of absolutely dry raw material.

To obtain preparations KTS-1 (*C. tatarica*, alcoholic extract, first population) and KTS-2 (*C. tatarica*, alcoholic extract, second population), shredded air-dry aboveground parts (KTS-1) and roots (KTS-2) of *C. tatarica* were infused with benzene to remove tar. The polyphenol complex was recovered with 50% aqueous alcohol. The aqueous-alcoholic extract was concentrated under mild conditions (vacuum of a water-jet pump, at a bath temperature not higher than 40°C), dried, and prepared for studying its biological activity.

To obtain preparations KTA-1 (*C. tatarica*, acetone extract, first population) and KTA-2 (*C. tatarica*, acetone extract, second population), shredded air-dry aboveground parts (KTA-1) and roots (KTA-2) of *C. tatarica* were infused with benzole to remove tar. The polyphenol complex was extracted with aqueous acetone (7:3 v/v). Concentrates of water-acetone extracts KTA-1 and KTA-2 were dried and prepared for their subsequent extensive bioscreening.

To obtain preparations KTV-1 (*C. tatarica*, aqueous extract, first population) and KTV-2 (*C. tatarica*, aqueous extract, second population), crushed air-dry aboveground parts (KTV-1) and roots (KTV-2) of *C. tatarica* were subjected to water extraction at a temperature of 60-70°C. The obtained aqueous decoctions of the aboveground parts and roots were concentrated under mild conditions (vacuum of a water-jet pump, at a bath temperature not higher than 40°C) and dried for the subsequent bio screening studies.

The aqueous, alcoholic and acetone extracts obtained from the *Crambe tatarica* aboveground parts and roots were studied for antibacterial and fungicidal activity with the aim of further introduction as medicines.

These studies were carried out in the Scientific Center for Anti-Infectious Drugs JSC.

The following test strains of microorganisms were used:

- 1) *Candida albicans* ATCC 10231 – a reference strain for testing fungicidal activity obtained from the American Type Culture Collection (ATCC), USA;
- 2) *Staphylococcus aureus* ATCC 6538-P – a reference strain for testing antibacterial activity obtained from the Republican Collection of Microorganisms (RCM), Astana, RK;
- 3) *Escherichia coli* ATCC 8739 – a reference strain for testing antibacterial activity obtained from the American Type Culture Collection (ATCC), USA;
- 4) *Pseudomonas aeruginosa* ATCC 9027 – a reference strain for testing antibacterial activity obtained from the American Type Culture Collection (ATCC), USA.

The procedure was carried out according to the methodological instruction CLSI (Methods for Dilution, 2015) and CLSI (Reference Method for., 2016). These research methods used are regulated by the Clinical and Laboratory Standards Institute, USA (CLSI - Clinical and Laboratory Standards Institute, USA).

## Methodology for preparation of agent stock solution

The concentration of the sample stock solution used in the study was 100 mg / ml.

The stock solution of the investigated extract was prepared according to the following formula (3):

$$mAA = \frac{C \times V}{a} \quad (3)$$

where mAA is the calculated weight of the test sample, mg; C is the required concentration of the agent, µg / ml; V is the volume of the solvent for a given sample of the agent, ml; a is the agent activity, µg / ml

The activity of the sample (value "a") was taken as 1000 µg / ml (100%) due to the lack of data on the amount of active substances in the extract.

The samples under study were dissolved in isotonic sodium chloride solution and 96% ethanol.

**Preparation of a suspension of microorganisms:** The suspension of each test microorganism was prepared in physiological saline (0.9% NaCl). For this, an aliquot of the daily cultured test strain was taken with a sterile loop, after which it was introduced into a sterile test tube with 5 ml of 0.9% NaCl. The turbidity control of the obtained inoculum was carried out by measuring the optical density on a DEN-1 densitometer. The density of the primary suspension was 0.5 units according to McFarland, which corresponds to  $1.5 \times 10^8$  CFU / ml. Further, the primary suspension in the amount of 0.05 ml was introduced into a test tube with 4.95 ml of isotonic solution to achieve a working concentration of  $1.5 \times 10^6$  CFU / ml.

**Testing the antimicrobial activity of the extract by the method of two-fold serial dilutions:** The procedure for testing antimicrobial activity was carried out by the method of two-fold serial dilutions in a liquid nutrient medium, Muller-Hinton broth (MHB). The procedure was carried out in sterile 48-well polystyrene culture plates (BIOLOGIX, China).

All wells of the plate were preliminarily filled with MHB medium in an amount of 0.5 ml. Then, in the first wells of each row (wells from A to F), 0.5 ml of the stock solution of the test sample was added. A number of two-fold serial dilutions were made as follows: after carefully pipetting the mixture from well 1 (0.5 ml of MHB + 0.5 ml of the sample), transferring 0.5 ml of this mixture into well 2 containing MHB; then 0.5 ml of the resulting mixture from well 2 was transferred to well 3. The action was repeated until the required number of two-fold dilutions was reached. From the last well (No. 8), 0.5 ml of the mixture was removed. Thus, in each row of the plate (wells A–F), serial dilutions from 50 mg / ml to 0.39 mg / ml were obtained in terms of the dry extract.

After dilutions, 0.05 ml of the corresponding test strain of the microorganism at a concentration of  $1.5 \times 10^6$  CFU / ml was added to all wells of the plate. The procedure was repeated for all test cultures.

The seeded plates were incubated for 18-24 hours at a temperature of  $37 \pm 1^\circ\text{C}$ . Petri dishes with Mueller-Hinton agar were preliminarily lined into eight sectors so that the well number (1–8) corresponded to the number of the well (1–8) in the row. That is, each row, consisting of eight holes, corresponds to one Petri dish with eight cells. After the incubation, 0.01 ml was inoculated from each well with a sterile loop. After seeding, the Petri dishes were placed in a thermostat for 18–24 hours, and cultivation was carried out at a temperature of  $37 \pm 1^\circ\text{C}$ .

The presence / absence of the growth of microorganisms on the surface of a dense nutrient medium was recorded. The minimum bactericidal / fungicidal concentration was considered the lowest concentration in the well, which completely suppressed the growth of the studied microorganism.

All experiments were performed in three replicates.

## Results and Discussion

To carry out the qualitative analysis of biologically active substances, the plant material was soaked in solvents of various concentrations and polarities: 10, 30, 50, and 70% aqueous acetone and 10, 30, 50, and 70% aqueous alcohol, hexane, benzene and chloroform. For isolation of mono- and polysaccharides, phenolic and hydroxycinnamic acids, 10% aqueous-alcoholic solution; for flavonoids, alkaloids, coumarins and tannins, 50 and 70% aqueous-alcoholic solution; and for triterpenoids and phospholipids, the chloroform solution has been proposed.

Three samples of *C. tatarica* were taken from different populations. Each of the obtained *C. tatarica* extracts was investigated by one- and two-dimensional chromatography on paper in various solvent systems using specific reagents (1% phosphoric-molybdic acid, 5% nitrosomethylurethane, 1% JAC, NaAc,  $\text{SbCl}_3$  in  $\text{CHCl}_3$ , pyridine, 0.1 N NaOH, bromothymol blue, 95% EtOH, 1% m-dinitrobenzoic acid in an alkaline

medium, 1%  $\text{K}_2\text{Fe}(\text{CN})_6$  and 2% solution of JAC,  $\text{SbCl}_5$  in  $\text{CCl}_4$ , precipitation with acetone from a chloroform-alcohol extract) (Kuznetsova, 1967; Grinkevich & Safronich, 1983; Muzychkina *et al.*, 2004; Muzychkina *et al.*, 2012; Itavo *et al.*, 2015). The results are presented in (Table 1).

Regardless of the place of growth, the Kazakh species *C. tatarica* contained alkaloids, tannins of the hydrolyzable type, carotenoids, coumarins, polysaccharides, triterpenoids, phenolic acids, flavonoids and phospholipids. At the same time, different color intensity of extracts with analytical reagents indicates a different quantitative structure of each of the identified types of biologically active substances (BAS).

Phytochemical and component analysis of biologically active substances of *C. tatarica*. To carry out phytochemical analysis of the identified groups of BAS, the crushed air-dried plant material was subjected to two-hour thermal extraction with solvents of different polarity according to the principle of structural affinity to the determined groups of natural metabolites. The quantitative analyses of alkaloids, hydrolyzable tannins, carotenoids, coumarins, polysaccharides, triterpenoids, phenolic acids, flavonoids and phospholipids were carried out according to pharmacopoeial and generally accepted methods (Grinkevich & Safronich, 1983; Bock, 1990; Hostettmann & Marston, 1995; Schmuck *et al.*, 2004; State Pharmacopoeia, 2008; **Kedik & Marakhova, 2010**; The United State, 2010; Itavo *et al.*, 2015; Kaczor & Baranska, 2016). Component analyses of flavonoids, tannins, coumarins, carbohydrates and phenolic acids were carried out by HPLC (Grinkevich & Safronich, 1983; Bock, 1990; Salminen, 1999; Merken, 2000; Rakhmadiyeva, 2000; Celeghini *et al.*, 2001; Javanmardi, 2002; Korulkin *et al.*, 2007; Muzychkina *et al.*, 2011) using authentic samples. The results obtained are presented in Tables 2 and 3.

**Table 1. Qualitative characteristics of biologically active substances in samples of *Crambe tatarica* Sebeok from different locations.**

Chemical	Qualitative evaluation result		
	Sample 1	Sample 2	Sample 3
1% phosphoric-molybdenum acid (alkaloids)	yellow→ intense blue	yellow→ blue	yellow→ blue
5% nitrosomethylurethane, 1% JAC, NaAc (hydrolyzed tannins)	Purple	Purple	Purple
$\text{SbCl}_3$ to $\text{CHCl}_3$ (carotenoids)	Greenish blue → brown	Green → brown	Greenish blue → brown
pyridine, 0.1 N NaOH, bromothymol blue (coumarins)	Yellow→ green→ blue	Yellow→ green→ blue	Yellow→ blue
95% EtOH (polysaccharides)	A white precipitate	Abundant white precipitate	Abundant white precipitate
1% m-dinitrobenzoic acid in an alkaline medium (triterpenoids)	Green	Green	Bright green
1% $\text{K}_2\text{Fe}(\text{CN})_6$ and 2% JAC (phenolic acid) solution	Dark blue	Blue	Dark blue
$\text{SbCl}_5$ to $\text{CCl}_4$ (flavonoids)	Orange	Bright orange	Orange
Precipitation with acetone from a chloroform-alcohol extract (phospholipids)	Cream precipitate	Beige precipitate	Cream precipitate

**Table 2. Results of phytochemical and qualitative component analysis of biologically active substances of the aboveground partsof *C. tatarica* from different locations.**

Identified compound	Biologically active substances, %		
	sample 02.05	sample 03.05	sample 04.05
<b>Alkaloids</b>	0.19	0.14	0.13
<b>Tannins</b>	3.91	4.77	4.98
2,3- <i>O</i> -hexaoxidifenoyl-4,6- <i>O</i> -sanguisorbil- <i>D</i> -glucose	-	+	+
2,3-di- <i>O</i> -galloyl- <i>D</i> -glucose	+	+	++
1,2,4-tri- <i>O</i> -galloyl- $\beta$ - <i>D</i> -glucose	-	-	-
1,3,4-tri- <i>O</i> -galloyl- $\beta$ - <i>D</i> -glucose	-	+	+
3,6- <i>O</i> -hexaoxidifenoyl- <i>D</i> -glucose	-	-	-
1,4-di- <i>O</i> -galloyl-3,6- <i>O</i> -hexaoxidifenoyl- $\beta$ - <i>D</i> -glucose	+	++	++
<b>Carotenoids</b>	1.84	0.92	1.65
<b>Coumarins</b>	1.35	1.23	0.89
coumarin	++	+	+
4,5-dioxycoumarin	+	+	-
7-oxycoumarin	+	+	+
scopoletin	-	-	-
<b>Polysaccharides</b>	7.41	13.54	11.23
<b>Monosaccharides</b>	0.31	0.36	0.38
glucose	+	+	+
galactose	-	+	+
rhamnose	+	+	+
mannose	+	-	-
xylose	-	-	+
arabinose	-	-	-
rutinose	-	+	+
<b>Triterpenoids</b>	2.32	1.96	3.88
<b>Phenolic and hydroxycinnamic acids</b>	0.15	0,19	0.20
gallic	+	+	+
caffeic	+	+	+
ferulic	+	+	+
lilac	+	-	-
gentisic	+	+	+
vanillic	-	+	+
o-kumaric	+	+	+
<b>Flavonoids</b>	1.55	2.01	2.25
kaempferol	±	+	+
quercetin	±	+	+
gossipetine	±	+	+
myricetin	±	-	-
(+)-catechin	±	+	+
(-) - epicatechin	±	-	trace
3- <i>O</i> - $\alpha$ - <i>L</i> -rhamnopyranosidekaempferol	±	+	+
3- <i>O</i> - $\alpha$ - <i>L</i> -rhamnopyranosidequercetin	±	+	+
3- <i>O</i> - $\beta$ - <i>D</i> -(2"- <i>O</i> -galloyl) glucopyranoside quercetin	±	-	-
3- <i>O</i> - $\beta$ - <i>D</i> -galactopyranosido-(6→1)- <i>O</i> - $\beta$ - <i>D</i> -xylopyranosidequercetin	±	-	-
3- <i>O</i> -rutinoside quercetin	±	+	+
3- <i>O</i> - $\beta$ - <i>D</i> -glucopyranoside quercetin	±	+	+
7- <i>O</i> - $\beta$ - <i>D</i> -xylopyranosidegossypetine	±	+	+
<b>Phospholipids</b>	6.44	3.93	5.71

**Table 3. Results of phytochemical and qualitative component analysis of BAS of *Crambe tatarica* Sebeok roots depending on the place of growth.**

Identified compound	BAS, %		
	sample 02.05	sample 03.05	sample 04.05
<b>Alkaloids</b>	0.16	0.12	0.09
<b>Tannins</b>	6.21	5.60	7.14
2,3- <i>O</i> -hexaoxidifenoyl-4,6- <i>O</i> -sanguisorbil- <i>D</i> -glucose	+	+	+
2,3-di- <i>O</i> -galloyl- <i>D</i> -glucose	++	+	++
1,2,4-tri- <i>O</i> -galloyl- $\beta$ - <i>D</i> -glucose	-	-	-
1,3,4- tri- <i>O</i> -galloyl- $\beta$ - <i>D</i> -glucose	+	+	+
3,6- <i>O</i> -hexaoxidifenoyl- <i>D</i> -glucose	-	-	-
1,4-di- <i>O</i> -galloyl-3,6- <i>O</i> -hexaoxidifenoyl- $\beta$ - <i>D</i> -glucose	++	++	++
<b>Carotenoids</b>	1.31	0.72	1.20
<b>Coumarins</b>	1.72	1.56	1.39
coumarin	++	+	+
4,5-dioxycoumarin	++	++	-
7-oxycoumarin	+	+	+
scopoletin	-	-	-
<b>Polysaccharides</b>	9.59	16.03	14.11
<b>Monosaccharides</b>	0.17	0.22	0.15
glucose	+	+	+
galactose	-	+	-
rhamnose	+	+	+
mannose	-	-	-
xylose	-	-	-
arabinose	-	-	-
rutinose	+	+	+
<b>Triterpenoids</b>	4.61	3.75	5.26
<b>Phenolic and hydroxycinnamic acids</b>	0.21	0.27	0.23
gallic	+	++	+
caffeic	++	+	++
ferulic	+	+	+
lilac	+	-	-
gentisic	+	+	+
vanillic	-	+	+
o-kumaric	+	+	+
<b>Flavonoids</b>	1.63	1.47	1.82
kaempferol	±	+	++
quercetin	++	+	++
gossipetine	±	+	+
myricetin	±	-	-
(+)-catechin	±	+	+
(-) - epicatechin	±	-	+
3- <i>O</i> - $\alpha$ - <i>L</i> -rhamnopyranosidekaempferol	±	+	+
3- <i>O</i> - $\alpha$ - <i>L</i> -rhamnopyranosidequercetin	±	+	+
3- <i>O</i> - $\beta$ - <i>D</i> -(2"- <i>O</i> -galloyl) glucopyranoside quercetin	±	-	-
3- <i>O</i> - $\beta$ - <i>D</i> -galactopyranosido-(6→1)- <i>O</i> - $\beta$ - <i>D</i> -xylopyranosidequercetin	±	-	-
3- <i>O</i> -rutinoside quercetin	±	+	+
3- <i>O</i> - $\beta$ - <i>D</i> -glucopyranoside quercetin	±	++	+
7- <i>O</i> - $\beta$ - <i>D</i> -xylopyranosidegossypetine	±	+	+
<b>Phospholipids</b>	2.13	1.75	2.38

From the analysis of the data in Tables 2 and 3, it follows that the aboveground parts of *C. Tatarica* are richer in alkaloids, carotenoids, monosaccharides and phospholipids than the root system; in the roots, larger amounts of tannins, coumarins, polysaccharides, triterpenoids, phenolic and hydroxycinnamic acids and flavonoids have been recorded.

The composition of the samples from different locations is also different. Sample 1 is characterized by a large, in comparison with the rest of the samples, amount of alkaloids, carotenoids, coumarins, and phospholipids in the aboveground parts, as well as alkaloids, carotenoids and coumarins in the root system. The aboveground parts of sample 2 are the most promising for the isolation of polysaccharides, and the root system, of mono- and polysaccharides, phenolic and hydroxycinnamic acids. Sample 3 is characterized by a larger amount of hydrolyzable tannins, monosaccharides, triterpenoids, phenolic and hydroxycinnamic acids, and flavonoids in the aboveground parts, and tannins, triterpenoids, flavonoids, and phospholipids in the roots.

We also assessed the antibacterial and fungicidal activity of aqueous, alcoholic and acetone extracts of the aboveground parts and roots of *C. tatarica* in relation to museum strains of microorganisms.

The results of testing the antimicrobial activity of acetone extracts of the aboveground parts (KTA-1) and roots (KTA-2) of *C. tatarica* against museum microorganisms by the method of two-fold serial dilutions are presented in Tables 4-8.

Table 4 presents data on the antimicrobial activity of KTA-1 and KTA-2, where saline solution (0.9%) was used as a solvent.

The samples tested were most effective against the *S. aureus* ATCC 6538-P test strain. The acetone extract of the

aboveground parts (KTA-1) and the roots were effective against staphylococcus at a concentration of 0.78 mg / ml. The extract from the aboveground parts (KTA-1) exhibited antimicrobial activity against *E. coli* ATCC 8739 and *P. aeruginosa* ATCC 9027 at concentrations of 12.5 mg / ml and 6.25 mg / ml, respectively. Acetone extract from the roots (KTA-2) was active against *E. coli* ATCC 8739 at a concentration of 25 mg / ml and against *P. aeruginosa* ATCC 9027 at a concentration of 12.5 mg / ml.

Extracts from the aboveground parts (KTA-1) and roots (KTA-2) exhibited fungicidal activity against *C. albicans* ATCC 10231 at a concentration of 6.25 mg / ml and 25 mg / ml, respectively.

Tables 5-8 show data on the antimicrobial activity of KTA-1 and KTA-2 against the reference strains, where ethanol (96%) was used as a solvent.

The samples tested were most effective against the *S. aureus* ATCC 6538-P test strain. The acetone extract of the aboveground parts (KTA-1) and the roots were effective against staphylococcus at a concentration of 0.78 mg / ml. The extract from the aboveground parts (KTA-1) exhibited antimicrobial activity against *E. coli* ATCC 8739 and *P. aeruginosa* ATCC 9027 at concentrations of 12.5 mg / ml and 6.25 mg / ml, respectively. Acetone extract from the roots (KTA-2) was active against *E. coli* ATCC 8739 at a concentration of 25 mg / ml and against *P. aeruginosa* ATCC 9027 at a concentration of 12.5 mg / ml.

Extracts from the aboveground parts (KTA-1) and roots (KTA-2) exhibited fungicidal activity against *C. albicans* ATCC 10231 at a concentration of 6.25 mg / ml and 25 mg / ml, respectively.

Tables 5-8 show data on the antimicrobial activity of KTA-1 and KTA-2 against the reference strains, where ethanol (96%) was used as a solvent.

**Table 4. The results of testing the antimicrobial activity of acetone extracts of the aboveground parts (KTA-1) and roots (KTA-2) of *C. tatarica* (0.9% saline used as a solvent), mg / ml.**

Concentration of the samples studied, mg / ml	Minimal bactericidal / fungicidal concentration, mg / ml								
	<i>S. aureus</i> ATCC 6538-P		<i>E. coli</i> ATCC 8739		<i>P. aeruginosa</i> ATCC 9027		<i>C. albicans</i> ATCC 10231		
	KTA-1	KTA-2	KTA-1	KTA-2	KTA-1	KTA-2	KTA-1	KTA-2	
50	-	-	-	-	-	-	-	-	-
25	-	-	-	+	-	-	-	-	+
12.5	-	-	+	+	-	+	-	-	+
6.25	-	-	+	+	+	+	+	+	+
3.125	-	-	+	+	+	+	+	+	+
1.56	-	-	+	+	+	+	+	+	+
0.78	+	+	+	+	+	+	+	+	+
0.39	+	+	+	+	+	+	+	+	+

1. «+» – growth observed; 2. «-» – growth not observed

**Table 5. The results of testing the antimicrobial activity of acetone extracts of the aboveground parts (KTA-1) and roots (KTA-2) of *C. tatarica* (ethanol 96% used as a solvent) against *Staphylococcus aureus* ATCC6538-P, mg / ml.**

Concentration of the samples studied, mg / ml	Minimal bactericidal concentration, mg / ml		Ethanol 96% (solvent control)	
	KTA - 1	KTA - 2	Effectiveness	Concentration, %
50	-	-	-	48
25	-	-	-	24
12.5	-	-	+	12
6.25	-	-	+	6
3.125	-	-	+	3
1.56	-	-	+	1,5
0.78	-	+	+	0,75
0.39	+	+	+	0,375

1. «+» – growth observed; 2. «-» – growth not observed



**Table 6. The results of testing the antimicrobial activity of acetone extracts of the aboveground parts (KTA-1) and roots (KTA-2) of *C. tatarica* (ethanol 96% used as a solvent) against *Escherichia coli* ATCC 8739, mg / ml.**

Concentration of the samples studied, mg / ml	Minimal bactericidal concentration, mg / ml		Ethanol 96% (solvent control)	
	KTA-1	KTA-2	Effectiveness	Concentration, %
50	-	-	-	48
25	-	-	-	24
12,5	-	+	+	12
6,25	+	+	+	6
3,125	+	+	+	3
1,56	+	+	+	1,5
0,78	+	+	+	0,75
0,39	+	+	+	0,375

1. «+» – growth observed; 2. «-» – growth not observed

**Table 7. The results of testing the antimicrobial activity of acetone extracts of the aboveground parts (KTA-1) and roots (KTA-2) of *C. tatarica* (solvent ethanol 96%) against *Pseudomonas aeruginosa* ATCC 9027, mg / ml.**

Concentration of the samples studied, mg / ml	Minimal bactericidal concentration, mg / ml		Ethanol 96% (solvent control)	
	KTA-1	KTA-2	Effectiveness	Concentration, %
50	-	-	-	48
25	-	-	-	24
12,5	-	-	+	12
6,25	-	+	+	6
3,125	-	+	+	3
1,56	+	+	+	1,5
0,78	+	+	+	0,75
0,39	+	+	+	0,375

1. «+» – growth observed; 2. «-» – growth not observed

**Table 8. The results of testing the fungicidal activity of acetone extracts of the aboveground parts (KTA-1) and roots (KTA-2) of *C. tatarica* (solvent ethanol 96%) against *Candida albicans* ATCC 10231, mg / ml.**

Concentration of the samples studied, mg / ml	Minimal fungicidal concentration, mg / ml		Ethanol 96% (solvent control)	
	KTA-1	KTA-2	Effectiveness	Concentration, %
50	-	-	-	24
25	-	+	+	12
12,5	-	+	+	6
6,25	-	+	+	3
3,125	-	+	+	1,5
1,56	+	+	+	0,75
0,78	+	+	+	0,375
0,39	-	-	-	24

1. «+» – growth observed; 2. «-» – growth not observed

The acetone extract of the aboveground parts (KTA-1) was active against *E. coli* ATCC 8739 at a concentration of 6.25 mg / ml, while the extract from the roots was active at a concentration of 12.5 mg / ml (Table 6).

Extracts from the aboveground parts (KTA-1) and roots (KTA-2) had bactericidal activity against the museum test strain *Pseudomonas aeruginosa* ATCC 9027 at concentrations of 1.56 mg/ml and 6.25 mg / ml, respectively.

Table 8 shows the results of testing the fungicidal activity of acetone extracts of *C. tatarica* against *Candida albicans* ATCC 10231.

The extract from the aboveground parts (KTA-1) had fungicidal activity against *Candida albicans* ATCC 10231 at a concentration of 0.78 mg / ml. The extract from the roots (KTA-2) was active against *Candida albicans* ATCC 10231 at a concentration of 12.5 mg / ml.

The results of testing the antimicrobial activity of aqueous extracts from the aboveground parts (KTV-1) and roots (KTV-2) of *C. tatarica* obtained by the method of two-fold serial dilutions in relation to museum microorganisms are presented in Tables 9-13.

Table 9 presents data on the antimicrobial activity of aqueous extracts from the aboveground parts (KTV-1) and roots (KTV-2) of *C. tatarica*, where 0.9% saline was used as a solvent.

The highest antimicrobial activity against the test strain of *S. aureus* ATCC 6538-P had the sample obtained from the roots of *C. tatarica* (KTV-2). The aqueous extract of roots (KTV-2) was effective against staphylococcus at a concentration of 1.56 mg / ml, while the extract obtained from the aboveground parts (KTV-1) was effective at a concentration of 12.5 mg / ml. The extract from the aboveground parts (KTV-1) exhibited the same activity against *E. coli* ATCC 8739 and *C.*

*albicans* ATCC 10231; the values of the minimal bactericidal concentration were 25 mg / ml. An aqueous extract from the aboveground parts of the plant was active against *P. aeruginosa* ATCC 9027 at a concentration of 12.5 mg / ml, whereas the extract from the roots (KTV-2) exhibited bactericidal and fungicidal activity against *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027 and *C. albicans* ATCC 10231 at a concentration of 12.5 mg / ml.

Tables 10-13 show data on the antimicrobial activity of KTV-1 and KTV-2 against the reference strains, where ethanol (96%) was used as a solvent.

An aqueous extract from the roots (KTV-2) had antimicrobial activity in relation to the museum test strain *S. aureus* ATCC 6538-P at a concentration of 0.78 mg/ml. Whereas the aqueous extract from the aboveground parts (KTV-1) was active only at a concentration of 6.25 mg / ml.

Table 11 shows the data on bactericidal activity of aqueous extracts KTV-1 and KTV-2 (96% ethanol used as a solvent) against *Escherichia coli* ATCC 8739.

The data in table 11 show that the aqueous extract from the roots (KTV-2) was active against *E. coli* ATCC 8739 at a concentration of 3.125 mg / ml, while the extract from the aboveground parts was active at a concentration of 6.25 mg / ml.

Extracts from the roots (KTV-2) and the aboveground parts (KTV-1) had bactericidal activity against the museum test strain *Pseudomonas aeruginosa* ATCC 9027 at concentrations of 3.125 mg / ml and 6.25 mg / ml, respectively.

Table 13 shows the results of testing the fungicidal activity of aqueous extracts of *C. tatarica* against *Candida albicans* ATCC 10231.

**Table 9. The results of testing the antimicrobial activity of aqueous extracts of the aboveground parts (KTV-1) and roots (KTV-2) of *C. tatarica* (0.9% saline used as a solvent), mg / ml.**

Concentration of the samples studied, mg / ml	Minimal bactericidal / fungicidal concentration, mg / ml							
	<i>S. aureus</i> ATCC 6538-P		<i>E. coli</i> ATCC 8739		<i>P. aeruginosa</i> ATCC 9027		<i>C. albicans</i> ATCC 10231	
	KTV-1	KTV-2	KTV-1	KTV-2	KTV-1	KTV-2	KTV-1	KTV-2
50	-	-	-	-	-	-	-	-
25	-	-	+	-	-	-	+	-
12.5	+	-	+	+	+	+	+	+
6.25	+	-	+	+	+	+	+	+
3.125	+	-	+	+	+	+	+	+
1.56	+	+	+	+	+	+	+	+
0.78	+	+	+	+	+	+	+	+
0.39	+	+	+	+	+	+	+	+

1. «+» – growth observed; 2. «-» – growth not observed

**Table 10. The results of testing the antimicrobial activity of aqueous extracts of the aboveground parts (KTA-1) and roots (KTA-2) of *C. tatarica* (ethanol 96% used as a solvent) against *Staphylococcus aureus* ATCC6538-P, mg / ml.**

Concentration of the samples studied, mg / ml	Minimal bactericidal concentration, mg / ml		Ethanol 96% (solvent control)	
	KTA-1	KTA-2	Effectiveness	Concentration, %
50	-	-	-	48
25	-	-	-	24
12,5	-	-	+	12
6,25	+	-	+	6
3,125	+	-	+	3
1,56	+	-	+	1,5
0,78	+	+	+	0,75
0,39	+	+	+	0,375

1. «+» – growth observed; 2. «-» – growth not observed

**Table 11. The results of testing the antimicrobial activity of aqueous extracts of the aboveground parts (KTA-1) and roots (KTA-2) of *C. tatarica* (ethanol 96% used as a solvent) against *Escherichia coli* ATCC 8739, mg / ml.**

Concentration of the samples studied, mg / ml	Minimal bactericidal concentration, mg / ml		Ethanol 96% (solvent control)	
	KTA-1	KTA-2	Effectiveness	Concentration, %
50	-	-	-	48
25	-	-	-	24
12,5	-	-	+	12
6,25	+	-	+	6
3,125	+	+	+	3
1,56	+	+	+	1,5
0,78	+	+	+	0,75
0,39	+	+	+	0,375

1. «+» – growth observed; 2. «-» – growth not observed

**Table 12. The results of testing the antimicrobial activity of aqueous extracts of the aboveground parts (KTV-1) and roots (KTV-2) of *C. tatarica* (ethanol 96% used as a solvent) against *Pseudomonas aeruginosa* ATCC 9027, mg/ml.**

Concentration of the samples studied, mg / ml	Minimal bactericidal concentration, mg / ml		Ethanol 96% (solvent control)	
	KTA-1	KTA-2	Effectiveness	Concentration, %
50	-	-	-	48
25	-	-	-	24
12,5	-	-	+	12
6,25	+	-	+	6
3,125	+	+	+	3
1,56	+	+	+	1,5
0,78	+	+	+	0,75
0,39	+	+	+	0,375

1. «+» – growth observed; 2. «-» – growth not observed

**Table 13. The results of testing the fungicidal activity of aqueous extracts of the aboveground parts (KTV-1) and roots (KTV-2) of *C. tatarica* (ethanol 96% used as a solvent) against *Candida albicans* ATCC 10231, mg / ml.**

Concentration of the samples studied, mg / ml	Minimal fungicidal concentration, mg / ml		Ethanol 96% (solvent control)	
	KTA-1	KTA-2	Effectiveness	Concentration, %
50	-	-	-	48
25	-	-	-	24
12,5	-	-	+	12
6,25	-	-	+	6
3,125	-	-	+	3
1,56	+	+	+	1,5
0,78	+	+	+	0,75
0,39	+	+	+	0,375

1. «+» – growth observed; 2. «-» – growth not observed

**Table 14. The results of testing the antimicrobial activity of alcohol extracts of the aboveground parts (KTS-1) and roots (KTS-2) of *C. tatarica* (0.9% saline used as a solvent), mg / ml.**

Concentration of the samples studied, mg / ml	Minimal bactericidal / fungicidal concentration, mg / ml							
	<i>S. aureus</i> ATCC 6538-P		<i>E. coli</i> ATCC 8739		<i>P. aeruginosa</i> ATCC 9027		<i>C. albicans</i> ATCC 10231	
	KTS-1	KTS-2	KTS-1	KTS-2	KTS-1	KTS-2	KTS-1	KTS-2
50	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-
12.5	-	-	+	+	+	+	+	+
6.25	-	+	+	+	+	+	+	+
3.125	-	+	+	+	+	+	+	+
1.56	+	+	+	+	+	+	+	+
0.78	+	+	+	+	+	+	+	+
0.39	+	+	+	+	+	+	+	+

1. «+» – growth observed; 2. «-» – growth not observed

Aqueous extracts of the roots (KTV-2) and the aboveground parts (KTV-1) demonstrated fungicidal activity against *Candida albicans* ATCC 10231 at a concentration of 0.78 mg / ml (96% ethanol used as a solvent).

The results of testing the antimicrobial activity of alcohol extracts from the aboveground parts (KTS-1) and roots (KTS-2) of *C. tatarica* obtained by the method of two-fold serial dilutions against the museum microorganisms are presented in Tables 14-18

Table 14 presents the data on the antimicrobial activity of alcohol extracts from the aboveground parts (KTS-1) and roots (KTS-2) of *C. tatarica*, where 0.9% saline was used as a solvent.

The sample from the aboveground parts of *C. tatarica* (KTS-1) had the highest antimicrobial activity against the *S. aureus* ATCC 6538-P test strain at a concentration of 1.56 mg / ml. However, the extract obtained from the roots (KTS-2) was effective at a concentration of 6.25 mg / ml. In relation to the test strains of *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027 and *C. albicans* ATCC 10231, alcohol extracts from the aboveground parts and roots exhibited bactericidal and fungicidal activity at the same concentration of 12.5 µg / ml.

Tables 15-18 show the data on the antimicrobial and fungicidal activity of KTS-1 and KTS-2 in relation to the reference strains, where ethanol (96%) was used as a solvent.

The alcohol extract from the roots (KTS-2) had antimicrobial activity against the museum test strain *S. aureus* ATCC 6538-P at a concentration of 25 mg / ml, whereas the alcohol extract from the aboveground parts (KTS-1) was active at a concentration of 1.56 mg / ml.

Table 16 shows the data on bactericidal activity of aqueous extracts KTS-1 and KTS-2 (96% ethanol used as a solvent) against *Escherichia coli* ATCC 8739.

From the data in Table 16, it can be seen that the alcohol extract of the aboveground parts (KTS-1) was active against *E. coli* ATCC 8739 at a concentration of 3.125 mg / ml, while the extract from the roots was active at a concentration of 12.5 mg / ml.

Alcoholic extracts from the roots (KTS-2) and the aboveground parts (KTS-1) had the same bactericidal activity against the museum test strain *Pseudomonas aeruginosa* ATCC 9027, 6.25 mg / ml.

Table 18 shows the results of testing the fungicidal activity of alcohol extracts of *C. tatarica* against *Candida albicans* ATCC 10231.

The extract from the aboveground parts (KTS-1) had fungicidal activity against *Candida albicans* ATCC 10231 at a concentration of 1.56 mg / ml, while the alcohol extract (KTS-2) inhibited the growth of *Candida albicans* ATCC 10231 at a concentration of 6.25 mg / ml.

The results of the phytochemical study, as well as the evaluation of the antibacterial and fungicidal activities of aqueous, alcoholic and acetone extracts of the aboveground parts and roots of *Crambe tatarica* indicate that this species is a promising medicinal plant, due to the fact that in certain concentrations it has both bactericidal and fungicidal activity.

**Table 15. The results of testing the antimicrobial activity of alcohol extracts of the aboveground parts (KTS-1) and roots (KTS-2) of *C. tatarica* (ethanol 96% used as a solvent) against *Staphylococcus aureus* ATCC6538-P, mg / ml.**

Concentration of the samples studied, mg / ml	Minimal bactericidal concentration, mg / ml		Ethanol 96% (solvent control)	
	KTA-1	KTA-2	Effectiveness	Concentration, %
50	-	-	-	48
25	-	-	-	24
12,5	-	+	+	12
6,25	-	+	+	6
3,125	-	+	+	3
1,56	+	+	+	1,5
0,78	+	+	+	0,75
0,39	+	+	+	0,375

1. «+» – growth observed; 2. «-» – growth not observed

**Table 16. The results of testing the antimicrobial activity of alcohol extracts of the aboveground parts (KTS-1) and roots (KTS-2) of *C. tatarica* (ethanol 96% used as a solvent) against *Escherichia coli* ATCC 8739, mg / ml.**

Concentration of the samples studied, mg / ml	Minimal bactericidal concentration, mg / ml		Ethanol 96% (solvent control)	
	KTA-1	KTA-2	Effectiveness	Concentration, %
50	-	-	-	48
25	-	-	-	24
12,5	-	+	+	12
6,25	-	+	+	6
3,125	+	+	+	3
1,56	+	+	+	1,5
0,78	+	+	+	0,75
0,39	+	+	+	0,375

1. «+» – growth observed; 2. «-» – growth not observed

**Table 17. The results of testing the antimicrobial activity of the alcohol extracts of the aboveground parts (KTS-1) and roots (KTS-2) of *C. tatarica* (solvent ethanol 96%) against *Pseudomonas aeruginosa* ATCC 9027, mg / ml.**

Concentration of the samples studied, mg / ml	Minimal bactericidal concentration, mg / ml		Ethanol 96% (solvent control)	
	KTA-1	KTA-2	Effectiveness	Concentration, %
50	-	-	-	48
25	-	-	-	24
12,5	-	-	+	12
6,25	+	+	+	6
3,125	+	+	+	3
1,56	+	+	+	1,5
0,78	+	+	+	0,75
0,39	+	+	+	0,375

1. «+» – growth observed; 2. «-» – growth not observed

**Table 18. The results of testing the fungicidal activity of alcohol extracts of the aboveground parts (KTS-1) and roots (KTS-2) of *C. tatarica* (solvent ethanol 96%) against *Candida albicans* ATCC 10231, mg / ml.**

Concentration of the samples studied, mg / ml	Minimal fungicidal concentration, mg / ml		Ethanol 96% (solvent control)	
	KTA-1	KTA-2	Effectiveness	Concentration, %
50	-	-	-	48
25	-	-	-	24
12,5	-	-	+	12
6,25	-	+	+	6
3,125	-	+	+	3
1,56	+	+	+	1,5
0,78	+	+	+	0,75
0,39	+	+	+	0,375

1. «+» – growth observed; 2. «-» – growth not observed

## References

- Aguinacalde, I. and M.A. Del Pero Martinez. 1982. The occurrence of acylated flavonol glycosides in the *Cruciferae*. *Phytochem.* 21(12): 2875-2878.
- Amirkhanov, N.A., K.R. Mukumov and S.S. Khamrakulov. 1974. The content of vitamins and trace elements in some species of *Crambe* L. *Grows. Resour.*, 10(3): 422- 426.
- Amirkhanov, N.A., N. Zhanonov and N. Ibadov. 1970. Pleasant Katran is a new forage silage plant in the conditions of the foothill semi-desert of Uzbekistan. *Mater. of 5 Sim. for New Silage Plants*, 2: 32-33.
- Amirkhanov, N.A., S.S. Khamrakulov and K.R. Mukumov. 1973. Experience of introduction and chemical composition of some species of the genus *Katran* in the conditions of meadow-boggy soils of Uzbekistan. *Mater. of 6 Sim. for New Silage Plants*, 183-184.
- Bock, K. 1990. *The carbohydrates*. Pergamon, New York.
- Budantsev, A.L. and E.E. Lesiovskaia. 2001. *Wild useful plants of Russia*. SPHFA publishing house. St. Petersburg.
- Celeghini, R.M.S., J.H.Y. Vilegas and F.M. Lanças. 2001. Extraction and quantitative HPLC analysis of coumarin in hydroalcoholic extracts of *Mikania glomerata* Spreng. leaves. *J. Braz. Chem. Soc.*, 12: 706-709. <https://doi.org/10.1590/S0103-50532001000600003>
- Chopra, R.N. and S.L. Nayar. 2006. Glossary of Indian medicinal plants. *New Delhi*. p. 330.
- CLSI. 2015. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard. Tenth Edition. CLSI M07-A10. 32(2).
- CLSI. 2016. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast: Approved Standard. CLSI Document M27-A2. 22(15).
- Dolia, V.S., E.N. Shkurupiy, N.A. Kaminskiy and E.D. Megerya. 1977. Seed oils of nine species of the genus *Crambe*. *Chem. Nature. Con.*, 1: 18-20.
- Gammerman, A.F. 1982. *Overview of medicinal plants in Turkmenistan*. Turkm. Phil. Acad. Sci. of the USSR, 2: 55-92.
- Gouz, G.V. 2016. The spatial distribution of *Crambe tatarica* (Brassicaceae) across Striltsivsky steppe. In: Proceedings of the 4<sup>th</sup> International Conference “Rare Plants and Fungi of Ukraine and Adjacent Areas: Implementing Conservation Strategies”. Palyvoda, Kyiv, pp. 67-69.
- Grashchenkov, A.E. 1959. Katrans, their biology and introduction to culture near Leningrad. *Tr. Bot. Inst. of the USSR Acad. Sci.: Introduction and green building*, 7: 124-127.
- Grinkevich, N.I. and L.I. Safronich. 1983. *Chemical analysis of medicinal plants*. High school, Moscow.
- Grudzinskaya, L.M., N.G. Gemedzhieva, N.V. Nelina and Zh. Karzhaubekova. 2014. *Annotated list of medicinal plants in Kazakhstan*. Almaty.
- Hoppe, H. 1975. *Drogen kunde. Band 1. Walter de Gruyter, Berlin-New-York*.
- Hostettmann, K. and A. Marston. 1995. *Saponins*. Cambridge: Cambridge University Press, Britain.
- Itavo, L.C.V., C.M. Soares, C.C. Ítavo B., A.M. Dias, H.V. Petit, E.S. Leal and A.D. de Souza. 2015. Calorimetry, chemical Composition and *in vitro* digestibility of oilseeds. *Food Chem.*, 185: 219-225.
- Javanmardi, J. 2002. The HPLC chromatography of hydrocinnamic acids of cider. *J. Agric. Food Chem.*, 50: 5878-5883.
- Kaczor, A. and M. Baranska. 2016. *Carotenoids: Nutrition, Analysis and Technology*. Wiley, London.
- Kedik, S.A. and A.I. Marakhova. 2010. Alkaloids: synthesis, isolation and analysis methods. Institute of Pharmaceutical Technology, Moscow.
- Kiryushina, E.I. 1971. Oil-bearing plants of the Chirchik river basin. Author's abstract. *diss. Cand. Biol. Sciences. Tashkent*.
- Kondratyev, E.K. 1973. Culture of new fodder plants on irrigated lands of the Prut river in the south of Moldova. *Mater. of 6 Sim. for New Silage Plants*, 32-33.
- Korulkin, D.Y., Z.A. Abilov, R.A. Muzychkina and G.A. Tolstikov. 2007. *Natural flavonoids*. Teo, Novosibirsk.
- Kucherov, E.V. 1974a. Resources and introduction of medicinal, fodder and fatty oil plants in the Bashkir ASSR *Author's abstract. diss., Cand. Biol. Sci., Sverdlovsk*.
- Kucherov, E.V. 1974b. Some questions of biology of new silage plants in the forest-steppe of the Bashkir ASSR. *Wild-growing and introduced useful plants of Bashkiria*, 4: 163-211.
- Kupriyanov, A.N., B.A. Turalin, N.V. Kurbatova, M.S. Kurmanbaeva, K.T. Abidkulova and A.A. Bazargaliev. 2020. Features of age-related conditions of the *Crambe tatarica* Sebeok in Western Kazakhstan. *Eur-Asian J. Biosci.*, 14: 177-182.
- Kupriyanov, A.N., B.A. Turalin, N.V. Kurbatova, M.S. Kurmanbaeva, K.T. Abidkulova and A.A. Bazargaliev. 2020. Cenoflora of the Tatar katran (*Crambe tatarica* Sebeok) in Western Kazakhstan. *Bull. of KazNU, Ser: Biol.*, 1(82): 52-62.
- Kuznetsova, G.A. 1967. *Natural coumarins and furocoumarins*. Science. Leningrad.
- Medvedev, P.F. 1987. Food plants of the USSR. In: Vegetable raw materials of the USSR. *Moscow*, 2: 5-151.
- Merken, H.M. 2000. Liquid chromatographic method for the separation and quantification of prominent flavonoid aglycones. *J. Chromatogr. A.*, 897: 177-184.
- Miller, R.W. and F.R. Earle. 1965. Search for new industrial oils: Oils from 102 species of *Cruciferae*. *J. Amer. Oil Chem. Soc.*, 42(10): 817-821.
- Mukumov, K.R. 1980. Biological and ecological substantiation of the introduction of Litvinov's katran and rough katran as new fodder plants in the cultural conditions of the Samarkand region. *Author's abstract. diss. Cand. Biol. Sci., Samarkand*.

- Muzychkina, R.A., D.Y. Korulkin and Z.A. Abilov. 2004. Qualitative and quantitative analysis of the main groups of biologically active substances in medicinal plant raw materials and phytopreparations. Kazakh university, Almaty.
- Muzychkina, R.A., D.Y. Korulkin and Z.A. Abilov. 2011. *Production technology and analysis of phyto preparations*. Kazakh university, Almaty.
- Muzychkina, R.A., D.Y. Korulkin and Z.A. Abilov. 2012. Methodology for the study of plant metabolites. MV-Print, Almaty.
- Pavlov, N.V. (Ed.). 1961. Flora of Kazakhstan. Vol: 4. *Academy of Sciences Kaz SSR publishing house, Alma-Ata*.
- Pavlov, N.V. 1947. Vegetable raw materials of Kazakhstan. *Publishing house of USSR Academy of Sciences, M.-L.*
- Pushkarova, N., M. Kalista, M. Kharkhota, D. Rakhmetov and M. Kuchuk. 2016. *Crambe tataria* Sebeok seeds and plants grown *In vitro* and *In vivo* fatty acid Composition Comparison. *Potravinarstvo*, 10(1): 494-498.
- Rakhmadiyeva, S.B. 2000. *Hydrolyzed tannins of plants of the genus Euphorbia L. and their biological activity*. Elorda. Astana.
- Red Data Book of Kazakhstan. 2014. Vol. 2. Part 1. Plants. 2nd ed. Astana.
- Sakhobiddinov, S.S. 1988. Wild medicinal plants of Central Asia. *Tashkent*.
- Salminen, J.P. 1999. Characterization of hydrolysable tannins from leaves of *Betula pubescens* by HPLC-mass spectrometry. *J. Chromatogr.*, 864(2): 283-291.
- Schmuck, C., H. Wennemers and R. Breslow. 2004. *Highlights in bioorganic chemistry: Methods and applications*. Wiley, London.
- Shalyt, M.S. 1951. Wild-growing useful plants of the Turkmen SSR. *Publishing house of the Moscow Society of Naturalists, M.*
- Sokolov, P.D. 1986. *Plant resources of the USSR. Flowering plants, their chemical composition, use. Families Paeoniaceae - Thymelaeaceae*. Science, Leningrad, p. 336.
- State Pharmacopoeia of the Republic of Kazakhstan. 2008. *Zhibek zholy*, Almaty. 1: 592.
- Taran, P.F. and L.F. Nekrasova. 1986. Test results of the cornelian katran and Sosnovsky hogweed in the conditions of the Kiev region. In: *New silage plants*. Syktyvkar, pp. 193-198.

(Received for publication 2 September 2020)