

## Bactericidal, protistocidal, nematocidal properties and chemical composition of ethanol extract of *Punica granatum* peel

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We have studied the chemical composition and antibacterial profile of ethanolic extract of *Punica granatum* L. (Lythraceae) on strains of microorganisms in vitro. Analysis using GC-MS showed 5-hydroxymethylfurfural (36.6%), D-sucrose (23.2%), sorbitol (6.7%), palmitic acid  $\beta$ -monoglyceride (5.6%), 2-furancarboxaldehyde (3.5%) and  $\beta$ -D-glucopyranose (3.3%) as the major components of the title extract. The experiment revealed a positive antibacterial effect of extracts obtained from *P. granatum* on 14 strains specifically Enterobacteriaceae microorganisms (*Escherichia coli*, *Enterobacter aegorenes*, *Proteus vulgaris*, *Serratia marcescens*, *Klebsiella pneumonia*), Listeriaceae (*Listeria ivanovi*, *L. innocua*, *L. monocytogenes*) and yeasts from the family Saccharomycetaceae (*Candida albicans*). Our study showed that in many cases these extracts more intensively affect multi-resistant strains of microorganisms than macrolide antibiotic azithromycin and is therefore a source of molecules to be exploited in medicine or by the pharmaceutical industry. The investigated extracts of *P. granatum* can be recommended for further in-depth research against poly-resistant strains of the above-mentioned microorganisms. Effective drugs perform a leading role in providing stable veterinary well-being of livestock and healthcare of the population. The present study showed that the studied plant species more intensively affects multi-resistant strains of microorganisms than sodium salt of azithromycin. Lethal concentration (LC<sub>50</sub>) of ethanol extract from pomegranate for *Paramecium caudatum* Ehr. equaled 0.3%. Death of 100% of nematode larvae of *Strongyloides papillosus* (Ihle) was recorded during 24 h exposition in 20% extract of *P. granatum* peel.

**Keywords:** Pomegranate; bactericidal action; gas chromatography-mass spectrometry (GC-MS); 5-hydroxymethylfurfural; D-sucrose; sorbitol.

### Introduction

Pomegranate (*Punica granatum* L.) is a perennial fruit tree that originated in the Mediterranean region and is now cultivated worldwide. In the United States, commercial production of pomegranates is almost exclusively confined to California. *Punica granatum* and its evolutionary precursor *P. protopunica* Balf. (also known as the Socotran pomegranate) are the only two members of genus *Punica* (Holland et al., 2009). Pomegranate a long-established fruit in folk medicine (Schubert et al., 1999; Mehdi Talebi et al., 2018), has a protective and therapeutic effect in diseases such as coronary heart disease, arthritis, Alzheimer's disease, diabetes, obesity, cancer, infertility (Frawley, 1986; Cáceres et al., 1987; Schubert et al., 1999; Saxena & Vikram, 2004; Lansky & Newman, 2007). In addition to being a religious symbol and a culinary delight, the pomegranate's bark, roots, fruit peel, aril (juice sac covering the seed; sometimes used as a collective name for juice and seed), and seeds, have been used for medicinal purposes since ancient times. Several classes of natural product compounds have been identified from various pomegranate tissues, including phenolics, terpenoids, alkaloids and fatty acids/triglycerides, many of which account for the bioactivities of pomegranate constituents (Seeram et al., 2006). In addition, studies have shown the presence of antioxidants, such as steroids, flavonoids, polyphenols, saponins, alkaloids, triterpenoids and vitamin C in various extractions from whole fruits, juice, peel and pomegranate seeds (Bhandary et al., 2012).

Nuamsetti et al. (2012) also mentioned the effectiveness of extracts from *P. granatum* due to content of high levels of phenolics and exhibited antibacterial activity against all bacteria tested (*Bacillus subtilis*, *Sta-*

*phylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium*). Gram-positive bacteria were more sensitive to the extracts than Gram-negative ones. Pomegranate peel contains substantial amounts of polyphenols such as ellagic tannins, ellagic acid, and gallic acid (Negi et al., 2003). Kaur et al. (2006) found that the ethanol extract of pomegranate flowers contains a large amount of polyphenols and has a huge regenerating ability, which indicates a strong (81.6%) antioxidant capacity in the DPPH model system. Schubert et al. (1999) and Faisi et al. (2018) argue that the polyphenolic fraction of pomegranate juice can act as an anti-atherogenic supplement and natural preservative for meat and fatty foods by directly inhibiting the oxidation of LDL.

According to Mehru et al. (2008), methanol extract from peel of *P. granatum* exerted antimicrobial activity against enteropathogenic bacteria (*Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae*) with MIC equaling 12.5 mg/mL. The extract was tested on *Artemia salina* brine shrimps, but no reliable toxicity was observed, as LC<sub>50</sub> equaled 1.42 mg/mL, and preparations are considered toxic when they affect the shrimps in concentrations below 1 mg/mL.

In the literature one can regularly find data on the impact of aqueous and ethanol extracts of medical plants on the nematodes of different agricultural animals of different stages of the development (Rahmann et al., 2006; Burke et al., 2009; Lu et al., 2010; Ferreira et al., 2011; Boyko & Brygadyrenko, 2016). The effect of extracts from *P. granatum* on *S. papillosus* larvae is currently unstudied. Jahromi et al. (2015) studied the safety of the extract of pomegranate peel for mice. Doses of 0.5, 1.9 and 7.5 mg/kg of body weight introduced over 22 days displayed no toxic effect according to clinical signs and histopreparations.

Patel et al. (2008) determined no-observed-adverse-effect level (NOAEL) for standardized extract from fruits of pomegranate in dose up to 600 mg/kg of body weight a day, which is the highest tested dose. The extract was standardized according to punicalagins, the main phenols of *P. granatum* responsible for its antioxidant potential. Subchronic study on rats was carried out using a probe of 60, 240 and 600 mg/kg of body weight daily over 90 days.

During testing ethanol extract from fruits and seeds of *P. granatum* on white mice, Satheesh Kumar Bhandary et al. (2013) observed no death or changes in behaviour, impairments in parameters of blood and histological deviations in the liver and kidneys (the authors used extracts with internal administration by 2 g/kg of body weight daily over 28 days). Ethanol extract from seeds of *P. granatum* had no sharp systemic toxicity at consumption by mice in the dose of over 160 mg/kg of body weight during 7 days observation (Setiadhhi et al., 2017).

Therefore, it is practical to conduct complex *in vitro* study on the antibacterial effect of ethanol extracts of *P. granatum* on strains of microorganisms of Enterobacteriaceae, Pseudomonadaceae, Staphylococcaceae, Bacillaceae, Listeriaceae, Corynebacteriaceae and Saccharomycetaceae families, as well as *Paramecium caudatum* and nematode larvae of *Strongyloides papillosus* Wedl, 1956, parasites of the gastrointestinal tract of ruminants and rabbits.

## Materials and methods

*Gas chromatography-mass spectrometry (GC-MS) analysis of Punica granatum crude extract.* The GC-MS analysis of volatile components of extract was carried out on a Shimadzu GCMS-QP2010 mass spectrometer equipped with a Restek Rxi-5ms (Crossbond® 5% diphenyl/95% dimethyl polysiloxane) column (length 30 m, diameter 0.25 mm, thickness 0.25 µm) under the following conditions. The oven temperature was programmed from 50 to 300 °C at 10 °C/min, then held isothermally at 300 °C for 20 min (total GC time 45 min, solvent cut time 2.5 min). The injector temperature, interface temperature and ion source temperature were maintained at 250, 250 and 220 °C, respectively. The sample (1.0 µL) was injected manually in the split mode at a ratio of 1:50 using helium (99.996% purity) as carrier gas at 1.0 mL/min (pressure 53.5 kPa). Mass spectra were acquired over the range of m/z 25 to 500 using electron impact ionization (70 eV). The essential constituents were identified by comparing their mass spectra reported in the NIST (National Institute of Standards and Technology) mass spectral library (2014). We have taken into account only the strong levels of similarity of MS spectra more than 75%. Component relative percentages were calculated based on GC peak TIC areas without calculating the detector response factor.

Antibacterial activity of extract of *P. granatum* peel was determined by the method of agar disc diffusion. A suspension was prepared from a daily culture of reference cryogenic strains of 14 microorganisms according to the turbidity standard of a bacterial suspension of 0.5 units according to Mac Farland  $1.5 \times 10^8$  CFU, which was determined using Densimeter II. The resulting suspension was subcultured on Müller-Hinton agar (Himedia), followed by cultivation in a TCO-80/1 thermostat for 24 and 48 hours at 37 °C. After the time necessary for the cultivation of the studied microorganisms, we assessed the number of the microorganisms that grew in the Petri dish. The cultivated colonies underwent microscopy. If necessary, an additional identification of microorganisms was conducted in accordance with EN ISO 11133:2014, IDT.

Discs with 15.0 µg azithromycin served as positive control (Valle et al., 2015). Azithromycin is a white crystalline powder. It is a broad-spectrum antibiotic; antibiotic-azalide, a representative of a new subgroup of macrolide antibiotics. Discs with 15.0 µg amphotericin were also used as a second control against *Candida albicans*.

After 24 and 48 hours of incubation, the diameter of the culture growth inhibition zone was measured using the template for measuring the size of microorganism growth inhibition zones (Antibiotic Zone Scale-C, model PW297, India) and TpsDig2 program (2016, F. James Rohlf).

An analysis of the extract of pomegranate on *P. caudatum* was performed according to the generally accepted methods (Kotsumbas et al., 2006; Zazharskiy et al., 2018). Cultivation of *P. caudatum* was carried

out in lactic media. The culture was maintained at the room temperature (+18...+20 °C). For the biotesting, we used 24h culture which was in the phase of exponential (active) growth. For conducting toxicologic study, a series of dilutions of extract of pomegranate measuring 0.05%, 0.10%, 0.20%, 0.50% and 1.00% was prepared. Into the micro-aquarium cavities (here micro-aquarium cavity is understood as a 20 µL depression in a microscope slide), 20 µL of media with ciliates (10–20 individuals) were added. Then 20 µL of aqueous solution of the studied preparation of different concentration were added to each of the capacities, and the number of ciliates in the mixture was counted. After an hour's exposure, the number of *P. caudatum* in each micro-aquarium was counted again for assessing the level of their survival.

The larvae of nematodes *Strongyloides papillosus* Wedl, 1956 in feces of ruminants were found using the Baermann test (Zajac et al., 2011). Then, 1 mL of the extract of *P. granatum* in different concentrations (1%, 5%, 10%, 15%, 20%, 25%) was added to each culture of *S. papillosus* nematode larvae (in five replications). The experimental exposure equaled 24 hours. We determined the number of vital and dead larvae.

Data on acute toxicity of the studied concentrations of the pomegranate extract were obtained in accordance with the recommended methods of express-biotesting. Value of LC<sub>50</sub> for ciliates was determined using probit-analysis of curves of lethality (Recommendations of OECD Guidelines for the Testing of Chemicals for evaluation of toxic effect of toxicants). The data in the tables are presented in the form  $x \pm SD$ . The differences between the values in the control and experimental groups were determined using the Tukey test, where the differences were considered significant at  $P < 0.05$  (taking into account the Bonferroni correction).

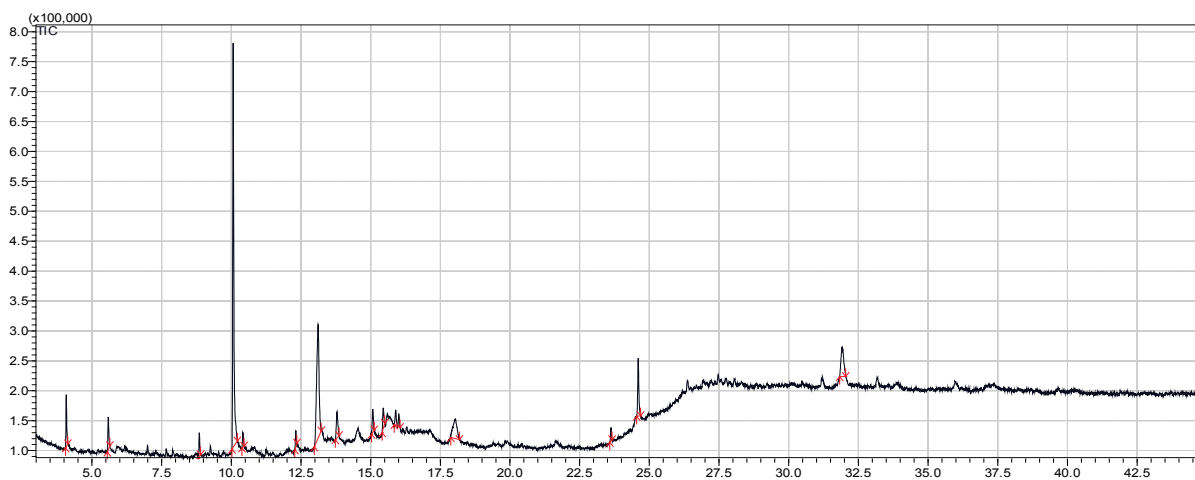
## Results

The chemical composition of the volatile part of the *P. granatum* crude extract was analyzed by GC-MS method (Fig. 1). Identified compounds are summarized in Table 1 and structures of major components are depicted in Figure 2. As presented in Table 1, about 15 different components were identified. Major compounds (>3%) in the sample are 5-hydroxymethylfurfural (36.6%), D-sucrose (23.2%), sorbitol (6.7%), palmitic acid β-monoglyceride (5.6%), 2-furancarboxaldehyde (3.5%) and β-D-glucopyranose (3.3%). These 6 components are representing ca. 79% of sample in total. Unfortunately, one component in crude extract has not been identified.

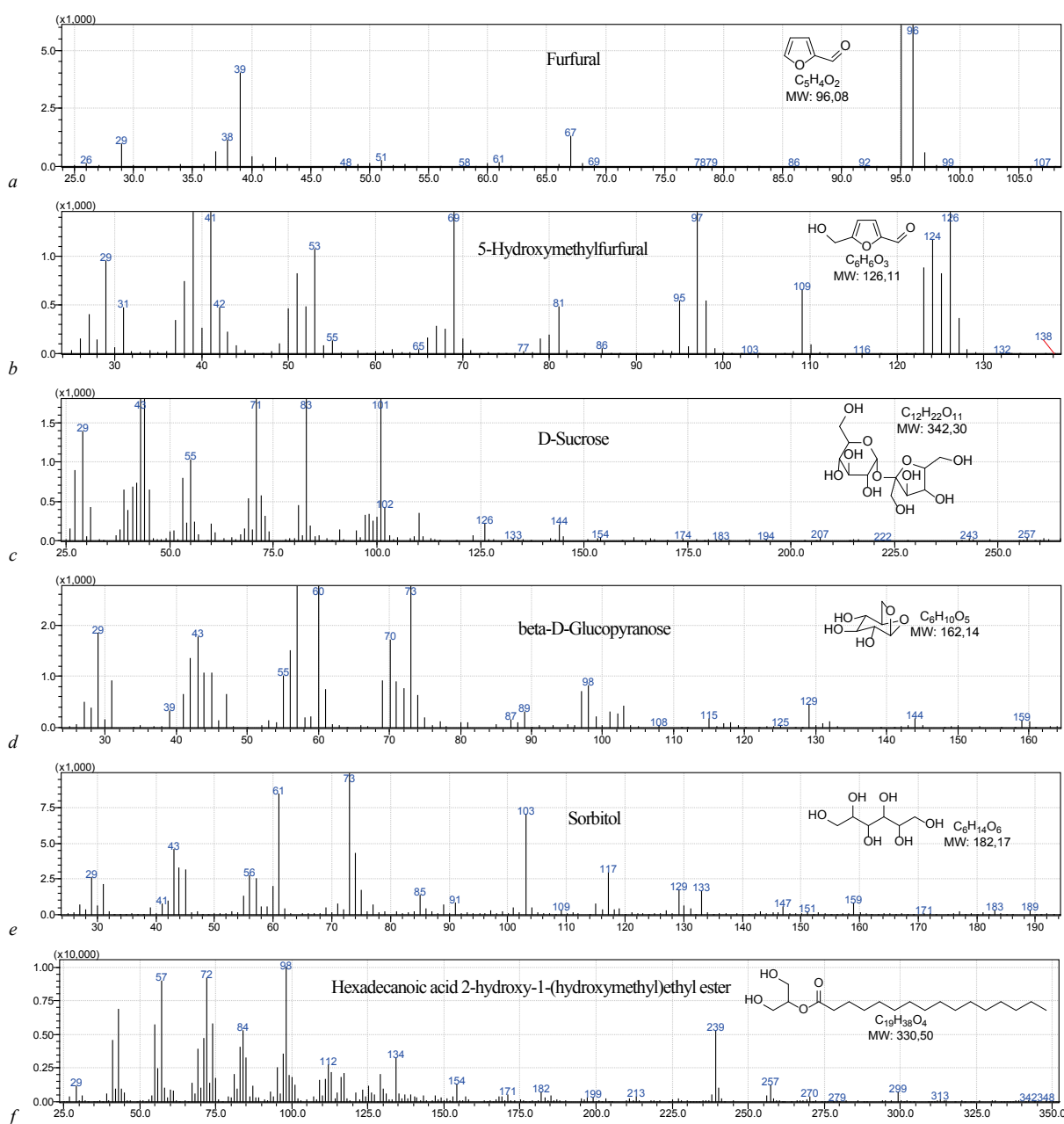
We determined the antibacterial effect of the extract from *P. granatum* peels towards *Escherichia coli* (Table 2): if after 24 hour of exposure it gave a zone of growth inhibition of less than the control by 2.11 mm, then after 48 h this parameter exceeded the control by 0.83 mm. The zone of inhibition of growth of *P. vulgaris* after 24 h of exposure in the experimental group was higher than the control by 17.22 mm, and after 48 h – by 18.44 mm. We observed antibacterial effect of extract from *P. granatum* peels on *S. marcescens*: after 24 h of cultivation of microorganisms, the zone of inhibition of growth in the experimental group was higher than the control by 5.46 mm, and after 48 h – by 7.13 mm.

Moderate antibacterial activity was observed against *K. pneumoniae*: it was lower than the control by 3.75 mm after 24 h, and by 2.42 mm after two days. Lower effect was demonstrated after impact of extract of *P. granatum* peels on *E. aegorenes*: growth inhibition zone equaled 6.32–8.13 mm, which was lower than the control values. Strains of *E. faecalis* and *S. typhimurium* were resistant to the extract of *P. granatum* peels and azithromycin. No effect was exerted by the extract from peels of *P. granatum* against microorganisms of Pseudomonadaceae families: *P. aeruginosa* ATCC 2853 (F) and *P. aeruginosa* 27/99 were resistant to the tested extract.

Moderate resistance of *S. aureus* and *B. cereus* to the tested preparation was observed: the inhibition zone grew by 2.84 and 2.54 mm respectively, though this parameter was lower than the control by 15.25 and 8.17 mm. High antimicrobial efficiency was displayed against microorganisms of the Listeriaceae family. If the growth inhibition zone of *L. ivanovi* was lower than the control by 3.97 mm after 48 h, for *L. innocua* and *L. monocytogenes* it exceeded control by 1.92 and 7.66 mm.



**Fig. 1.** Total ion current (TIC) chromatogram of the crude ethanol extract of *P. granatum*: abscissa axis (X) is time in minutes, ordinate axis (Y) is signal intensity in relative units)



**Fig. 2.** Major components *a-f* (>3% each) found in the crude ethanol extract of *Punica granatum* with their experimentally measured MS-spectra: abscissa axis (X) is mass-to-charge ratio (m/z), ordinate axis (Y) is signal intensity in relative units; molecular weight (MW) is given in Da

**Table 1**  
Chemical composition of the crude ethanol extract of *P. granatum* by GC-MS method

Entry	Retention time, min	Compound name (synonyms)	Formula	Area, %
1	4.080	Furfural (2-Furancarboxaldehyde)	C <sub>5</sub> H <sub>4</sub> O <sub>2</sub>	3.49
2	5.586	3-methyl-2,5-furandione (Citriconic anhydride)	C <sub>5</sub> H <sub>6</sub> O <sub>3</sub>	2.67
3	8.854	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	1.75
4	10.070	5-Hydroxymethylfurfural	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	36.60
5	10.416	3-hydroxy-2-methyl-4H-pyran-4-one (Maltol, Larixic acid, Larixinic acid)	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	1.14
6	12.317	Succinic acid 3-methylbutyl pentyl ester	C <sub>14</sub> H <sub>26</sub> O <sub>4</sub>	1.17
7	13.116	D-Sucrose (alpha-D-Glucopyranoside, Amerfond, Beet sugar, Cane sugar)	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	23.16
8	13.789	beta-D-Glucopyranose (1,6-anhydro- <i>α</i> -mannosan, Levoglucosan)	C <sub>6</sub> H <sub>10</sub> O <sub>5</sub>	3.27
9	15.077	1,6-Anhydro-beta-D-glucofuranose	C <sub>6</sub> H <sub>10</sub> O <sub>5</sub>	2.19
10	15.453	beta-D-Glucopyranose (beta-Lactose)	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	2.05
11	15.899	beta-D-Fructose (Levulose)	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	2.52
12	18.040	Sorbitol (D-Glucitol, Diakarmon, L-Gulitol, D-1,2,3,4,5,6-hexanehexol, Nivitin)	C <sub>6</sub> H <sub>14</sub> O <sub>6</sub>	6.68
13	23.623	Hexanedioic acid bis(2-ethylhexyl) ester (Adipic acid bis(2-ethylhexyl) ester, Adipol 2EH, Bisoflex DOA, DOA, Effomoll DOA, Flexol A26, Kodaflex DOA)	C <sub>22</sub> H <sub>42</sub> O <sub>4</sub>	0.96
14	24.601	Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester (2-mono-palmitin, Palmitic acid beta-monoglyceride)	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	5.55
15	31.913	Unknown compound	–	6.84

**Table 2**  
The antibacterial effect (radius of growth inhibition zone, mm) of extract of *P. granatum* peel on cryogenic strains microorganisms in vitro (x ± SD, n = 12)

Strains	Exposition		Reference <sup>1</sup>
	24 hours	48 hours	
<i>Escherichia coli</i> 055 K 59 No. 3912/41	11.51 ± 1.12 <sup>a</sup>	14.45 ± 1.35 <sup>a</sup>	13.62 ± 0.96 <sup>a</sup>
<i>Enterobacter aegorenes</i> 10006	6.32 ± 0.67 <sup>a</sup>	8.13 ± 0.92 <sup>ab</sup>	8.99 ± 0.93 <sup>b</sup>
<i>Enterococcus faecalis</i> ATCC 19433	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	5.47 ± 2.03 <sup>b</sup>
<i>Proteus vulgaris</i> HX 19 No. 222	19.56 ± 1.78 <sup>a</sup>	20.78 ± 2.31 <sup>a</sup>	2.34 ± 0.23 <sup>b</sup>
<i>Serratia marcescens</i> 1	8.78 ± 0.89 <sup>a</sup>	10.45 ± 1.33 <sup>a</sup>	3.32 ± 0.19 <sup>b</sup>
<i>Klebsiella pneumoniae</i> K-56 No. 3534/51	8.34 ± 0.77 <sup>a</sup>	9.67 ± 1.22 <sup>a</sup>	12.09 ± 2.78 <sup>a</sup>
<i>Salmonella typhimurium</i> 144	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>
<i>Staphylococcus aureus</i> ATCC 25923	4.31 ± 0.65 <sup>a</sup>	7.15 ± 0.94 <sup>b</sup>	22.40 ± 2.36 <sup>c</sup>
<i>Bacillus cereus</i> ATCC 10702	4.89 ± 0.76 <sup>a</sup>	7.43 ± 1.23 <sup>b</sup>	15.60 ± 1.32 <sup>c</sup>
<i>Listeria innocua</i> ATCC 33090	14.12 ± 1.88 <sup>a</sup>	17.32 ± 1.87 <sup>a</sup>	15.40 ± 1.82 <sup>a</sup>
<i>L. ivanovi</i>	14.67 ± 1.76 <sup>a</sup>	16.33 ± 2.65 <sup>ab</sup>	20.30 ± 1.64 <sup>b</sup>
<i>L. monocytogenes</i> ATCC 19112	20.87 ± 2.14 <sup>a</sup>	21.89 ± 1.76 <sup>a</sup>	14.23 ± 1.44 <sup>b</sup>
<i>Corynebacterium xerosis</i> 1911	2.34 ± 0.23 <sup>a</sup>	7.15 ± 0.94 <sup>b</sup>	25.35 ± 2.21 <sup>c</sup>
<i>Candida albicans</i>	16.67 ± 1.77 <sup>a</sup>	18.56 ± 2.11 <sup>a</sup>	2.48 ± 0.21 <sup>b</sup> – azithromycin; 18.20 ± 2.35 <sup>a</sup> – amphotericin

Notes: 1 – for bacteria, discs with 15.0 µg azithromycin were used as positive control (Valle et al., 2015), and for *C. albicans* – discs with 15.0 µg amphotericin; different letters indicate the values significantly differing one from another within a line of the Table on the results of comparison using the Tukey test (P < 0.05) with Bonferroni correction.

Moderate resistance of *C. xerosis* to extracts from peel of *P. granatum*: growth inhibition zone ranged 2.34 mm (24 h) to 4.81 mm (48 h of exposition). Susceptibility of *C. albicans* to extracts from peel of *P. granatum* was observed: after 48 h of incubation this parameter was higher compared to azithromycin and amphotericin by 16.08 and 0.36 mm.

**Table 2**  
Mortality of larvae of (%) *Strongyloides papillosus* Wedl, 1956 in extracts of *P. granatum* peel in different concentrations

Concentration, %	Development stages of <i>S. papillosus</i> larvae	
	L <sub>1</sub> –L <sub>2</sub>	L <sub>3</sub>
0 (control)	1.6 ± 0.3	0.0
1	1.7 ± 0.4	0.0
5	4.1 ± 0.9	0.0
10	5.9 ± 1.4	0.0
15	82.1 ± 16.2	75.8 ± 9.2
20	100.0	100.0
25	100.0	100.0

Right after the contact with the solutions of 0.5% and 1.0% concentrations, the ciliates began to move rapidly, moving with the front end, and then died, obtaining spherical shape. In concentration of 0.2%, *P. caudatum* slowed, maintained shape without any changes, but a large proportion of them died. Further dilution had no effect on the ciliates. For pomegranate extract, LC<sub>50</sub> equaled 2.97 g/L, or 0.3% concentration. In this product, LC<sub>100</sub> was determined at the level of 4.51 g/L or around 0.45%.

According to the results of the studies on the impact of *P. granatum* extract on the level of mortality of nematode larvae of *S. papillosus*, parasites of gastrointestinal tract of ruminants, the number of dead specimens in 1% solution on average did not exceed the control. No positive reaction was observed either during use of 5% and 10% extract. Notable effect was recorded after affecting the larvae with exposures equaling

24 h with 15% extract of the studied substance. During use of extract in this concentration, over 80% of the dead individuals were of the first and second stage of the development. Among third stage larvae, mortality of 75% of specimens was observed. Best results were obtained after using 20–25% extract of *P. granatum* (Table 2).

## Discussion

Aqueous extracts of henna and pomegranate showed higher anti-fungal activity as compared to their corresponding ethanolic extracts (Singla et al., 2013). The increase in total aerobic plate counts, pH and total volatile basic nitrogen values were significantly inhibited in shrimps treated with chitosan coating combined with pomegranate peel extract (Yuan et al., 2015). Ari et al. (2018) report that pomegranate juice had a synergistic effect when used in combination with antibiotics. *In vitro*, methanolic extract of pomegranate peel caused inhibitory effect to the linear growth of six economically important fungal phytopathogens isolated from different hosts including: *Botrytis cinerea*, *Colletotrichum dematium*, *Fusarium oxysporum*, *F. solani*, *Phoma* spp., and *Rhizoctonia solani* (Mohamad et al., 2015). Potent inhibitory effects of PoP extracts were observed against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium* and *Aspergillus niger* and minimum inhibitory concentration (MIC) was recorded between 0.25–0.89 mg/mL (Ismail et al., 2016).

After analyzing the results of our research, it was found that the extract of *P. granatum* can compete with azithromycin, a broad-spectrum antibiotic from the macrolide group, whose spectrum of action includes *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus agalactiae*, *S. pneumoniae*, and *S. acupunctura*, *Chlamydia trachomatis*, *Ch. pneumoniae*, *Mycobacterium avium* complex, *Mycoplasma pneumoniae*, *Ureaplasma*

*urealyticum*, *Treponema pallidum*, *Borrelia burgdorferi* (Rodriguez-Cavallini, 2004). Extract of *P. granatum* can compete with azithromycin by acting on strains of *Escherichia coli*, *Enterobacter aegorenes*, *Proteus vulgaris*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Listeria ivanovi*, *L. innocua*, *L. monocytogenes* and *Candida albicans*.

During biotesting with *Paramecium caudatum* in our experiment, LC<sub>100</sub> equaling within 0.1–1.0% allows the extract of pomegranate to be classified to the third class of toxicity (according to GOST 12.1.007-76 System of standards of labour safety. Toxic substances. Classification and general requirements for safety). Also the obtained results were compared to the calculations of toxicity according to method of Pershyn and Bliss-Prozorovskii (Prozorovskii, 2007): according to Pershyn method LC<sub>50</sub> = 2.81 g/L, and Bliss-Prozorovskii LC<sub>16</sub> = 2.32 g/L, LC<sub>50</sub> = 3.15 g/L, LC<sub>84</sub> = 3.99 g/L, i.e. the curve of dependence of lethality corresponds to the graph  $y = 1.25 + 1.19 \cdot x$ . Therefore, the results on activity of extract of pomegranate towards ciliates allow it to be classified as a moderately toxic substance, LC<sub>50</sub> ranging 151–5000 mg/kg (Kotsumbas, 2006). These data coincide with the data obtained for laboratory animals with similar ethanol extracts of pomegranate, differences can be caused by different conditions of obtaining the product from local raw material. Therefore, Satheesh Kumar Bhandary et al. (2013) indicate that LD<sub>50</sub> of ethanol extract from fruits and seeds of *P. granatum* in white rats exceeded 2 g/kg, and Patel (2008) determined that LD<sub>50</sub> in extracts of pomegranate fruits for rats and mice exceeds 5 g/kg of body weight, level of no-observed-adverse-effect level (NOAEL) was determined as 600 mg/kg of body weight a day.

The impact of plants and plant-based preparations on the development of helminths *in vitro* and *in vivo* was studied by several authors. The experiments were conducted on the impact of alcohol extract of *P. granatum* on the development of cestodes and nematodes. Akhtar & Riffat (1985) have demonstrated the impact of peel of *P. granatum* on different species of sheep cestodes. During use of *P. granatum*, the intensity of infection of sheep with different species of cestodes decreased. Kalesaraj (1974) proved anthelmintic activity *in vitro* of alcohol extract from peel of *P. granatum* against *Ascaris lumbricoides* (Linnaeus, 1758). A number of authors conducted experiments on impact of alcohol extract of *P. granatum* on the development of nematodes of the Strongyloida order. Pradhan et al. (1992) observed therapeutic efficiency of *P. granatum* in sheep with *Nematodirus*. Positive anthelmintic effect was observed *in vitro*. Prakash et al. (1980) determined the effect of alcohol extract of *P. granatum* on inhibition of the development of eggs of *Haemonchus contortus* (Rudolphi, 1803).

Thus, internal parts (interior walls or mesocarp) of fruits of *P. granatum* can have a significant impact on microbiocenosis of soil, fauna of soil protists and invertebrates. According to the results of previous experiments mass species of insects can die after consuming plant remains not characteristic of their habitat. For example, *Opatrum sabulosum* (Linnaeus, 1761), a dangerous pest in Steppe and Forest-Steppe zone of Eurasia, cannot consume plants of certain species (Brygadyrenko & Nazimov, 2014, 2015). Volatile compounds present in some species of plants can scare off or lure insects (Martynov et al., 2019). Plant remains on the soil surface change horizontal structure of communities of invertebrates (Faly & Brygadyrenko, 2014), which can occur directly during consumption of food, as well as indirectly by changes in species composition of biocenosis in certain parts. A complex study of chemical composition and effect on different groups of living organisms would allow determination of what are still hidden interrelations in natural ecosystems, and for the researched substances to be used with minimum damage to biodiversity.

## Conclusion

The experiment *in vitro* revealed a positive antibacterial effect from the use of extracts of *P. granatum* on strains of Enterobacteriaceae microorganisms: *E. coli*, *E. aegorenes*, *P. vulgaris*, *S. marcescens*, *K. pneumoniae*; Listeriaceae: *L. ivanovi*, *L. innocua*, *L. monocytogenes*; and Saccharomycetaceae: *C. albicans*. We consider it possible to recommend the investigated extracts of *P. granatum* for further research in the fight against polyresistant strains of the above-mentioned microorgan-

isms. Ethanol extract of pomegranate belongs to moderately toxic substances during biotesting on *P. caudatum*, LC<sub>50</sub> corresponds to 0.3% concentration of the preparation. Nematode larvae of *S. papillosus* die during processing with 20% extracts of *P. granatum* peel.

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