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Chemical composition of essential oil and antimicrobial properties of *Chrysantemum coronarium* (Asteraceae)

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Garland chrysanthemum (*Chrysanthemum coronarium* L.), or edible chrysanthemum, is a valuable food, medicinal, decorative plant, containing a considerable amount of biologically active substances. The herb is widely used as a dietary food in South-East Asia, whereas in spite of being spread throughout Ukraine, it is known there rather as a decorative than a vegetable plant. Introductory studies of *C. coronarium* were conducted on experimental plots at the Botanical Gardens of Zhytomyr National Agroecological University, which is located in Ukrainian Polesia. Chromatographic analysis of the essential oil composition was performed on the gas-liquid chromatographer Agilent Technologies 6890 with mass spectrometric detector 5973. The material for chromatographic studies was represented by *C. coronarium* inflorescences. The antimicrobial properties of the ethanolic extract from the areal parts of *C. coronarium* were studied on test-cultures, collected from the Ukrainian Collection of Microorganisms (UCM, Institute of Microbiology and Virology of NAS Ukraine), the test-cultures being: *Escherichia coli* UCM B-906 (ATCC 25922), *Staphilococcus aureus* UCM B-904 (ATCC 25923), *Pseudomonas aeruginosa* UCM B-900 (ATCC 9027), *Candida albicans* UCM Y-1918 (ATCC 885-653). The antimicrobial effect of the investigated substances was studied by the method of serial successive dilutions which determined minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). This article shows the results of chromatographic analysis of essential oil obtained from *C. coronarium* inflorescences and focuses on antimicrobial activity of the herb against the test cultures of the following microorganisms: *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans*. In the essential oil 26 compounds have been determined, 23 of which have been identified, the major components being: chrysanthemyl acetate (24.4%), chrysanthemol (21.8%), chrysanthenyl acetate (7.6%), camphor (7.3%), β-farnesene (5.9%), α-bisabolol (5.6%). *C. coronarium* ethanolic extract showed antimicrobial activity against gram-positive strains of *S. aureus*. In comparison with the solvent, the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) increased 4 and 2 times, respectively. We have observed only fungistatic activity against the fungus *C. albicans* – MBC values showed a twofold increase compared with the solvent. Inhibitory, bactericidal/fungicidal properties of the extract against gram-negative strains of *E. coli* and *P. aeruginosa* have not been detected. The experimental data prove that there is a good perspective for further study and application of *C. coronarium* in pharmacy and the food industry.

Keywords: garland chrysanthemum; chromatographic analysis; extract; microorganisms; antimicrobial activity

Introduction

Garland chrysanthemum (*Chrysantemum coronarium* L.) is a valuable food plant, medicinal and ornamental plant, which belongs to the family Asteraceae, tribe Anthemideae. Other botanical names are *Glebionis coronaria* L. Cassini ex Spach, *Glebionis coronaria* var. *discolor*, *Glebionis coronaria* var. *coronaria*, *Chrysanthemum roxburghii* Desf., *Glebionis roxburghii* (Desf.) Tzvelev, *Xanto-phtalmum coronarium* (L.) P. D. Sell., *Matricaria coronaria* (L.) Desr. It is an annual herbaceous plant with upright highly brachiferous stems. In the conditions of Zhytomyr Polesia the plants grow up to 60–143 cm height in culture. The leaves are bipinnately lobed, sessile, the inflorescence is anthodium. The corolla of the disk flowers is yellow, the ray florets are yellow of a different tone, sometimes white. The fruit is achene. Garland chrysanthemum or chrysanthemum greens is widely used as a dietary food plant in China, Japan, Korea, India, the USA; it is distributed throughout Ukraine, though it is used as an ornamental plant and is not commonly known as a vegetable plant. Garland chrysanthemum has a high nutritive value, due to its balanced content of vitamins, carotene, micro- and macro-elements, simple and complex carbohydrates, protein, flavonoids, lactones (Harborne et al., 1970; El-Masry et al., 1984; Lai et al., 2007; Cherevchenko et al., 2012; Geest et al., 2016). The plant has a high content of beta-carotene and potassium and includes essential oil (Flamini et al., 2003; Senatore et al., 2004; Sebastián et al., 2006; Basta et al., 2007; Tawaha and Hudaib, 2010; Preedy, 2016). In China, garland chrysanthemum is used for treatment of gonorrhea, syphilis, for normalization of metabolism, treatment of chronic constipation, and is given as an expectorant and stomachic medication; Japanese medicine uses it for preventing cancer, and treatment of headaches. The chrysanthemum is used for treating eye diseases, ringing in the ears, swamp fever, alcoholism, kidney stone disease, radiation sickness, cardio-vascular diseases, rheumatism, hypertension (Cherevchenko et al., 2012). Garland chrysanthemum activates the immune system (Tanaka et al., 2011), and has antioxidative (Chuda et al., 1996; Kim et al., 2011), hepatoprotective (Donia, 2014), antitumoral (Choi et al., 2007; Dokuparthi and Manikanta, 2015) insecticidal (Shonouda et al., 2008), nematocidal (Bar-Eyal et al., 2006) and antimicrobial properties (Hosni et al., 2013; Lograda et al., 2013).

Garland chrysanthemum is not cultivated in Zhytomyr Polesia, therefore it is appropriate to conduct an introductory study of this valuable and undemanding plant, especially its biochemical and antimicrobial properties with a view to its usage in the food

industry, pharmacy, perfumery, and cosmetology. The objective of this article is to evaluate the essential oil compound and the antimicrobial properties of *C. coronarium* with a view to its introduction in Ukrainian Polesia.

Material and methods

Our introductory study of *C. coronarium* was conducted on experimental plots in the Botanical Garden of Zhytomyr National Agroecological University, which is located in Ukrainian Polesia. The output seeds were obtained from the National Botanical Garden (NBG named after M M Hryshko of the National Academy of Sciences of Ukraine. The material for chromatographic research was the inflorescences of *C. coronarium* (fresh material).

Calculation of the content of essential oils was made using the Clevenger method (Syr'e lekarstvennoe rastitel'noe…, 1988). Chromatographic analysis of essential oil components was made using a gas-liquid chromatograph Agilent Technologies 6890 with mass spectrometer 5973. The conditions of the analysis: the chromatographic column was capillary DB-5, with 0.25 mm diameter and 30 m length. The speed of the carrier-gas (helium) was 2 ml/min, the temperature of the heater when injecting the samples was 250 ºC. The programmed temperature of the thermostat ranged from 50 to 320 ºC with 4 º/min speed. For identification of components, we used the library of mass spectrums NIST05 and WILEY 2007 with total number of spectra more than 470,000 in a complex with AMDIS and NIST programmes for identification (Chernogorod and Vinogradov, 2006).

The samples for micro-biological studies were collected during their blossom stage. The extract of the above-ground part of *C. coronarium* was obtained by infusing air-dry material in 40% ethanol (1 : 5) during 7 days. The research on antimicrobial activity of the extract was conducted using microbiological cultures from the Ukrainian Collection of Microorganisms (UCM, Institute of Microbiology and Virology, National Academy of Science of Ukraine): *Escherichia coli* UCM B-906 (ATCC 25922), *Staphylococcus aureus* UCM B-904 (ATCC 25923), *Pseudomonas aeruginosa* UCM B-900 (ATCC 9027), *Candida albicans* UCM Y-1918 (ATCC 885-653). These microorganisms were test strains for assessing the antimicrobial activity of medicinal preparations (Podgorsky et al., 2007).

Assessment of the antimicrobial activity of the extract on microbiological cultures was conducted according to the following method of assessing sensitivity of microorganisms to antibacterial drugs (Viznachennya chutlivosti mikroorganizmiv do antibakterialnih preparativ [The determination of sensitivity of microorganisms to antibacterial drug]. Nakaz of MOZ of Ukraine No 167. 2007).

The antimicrobal activity of the substances was studied using the method of consequent dilution which includes defining the minimum bacteriostatic (MIC) and minimum bactericidal concentration (MBC). Daily microbiological cultures were obtained from dense growth media LB (Luria-Bertani medium, Merck, Germany) (Miller, 1976).

Results

The chromatographic analysis of the essential oil from inflorescences of *C. coronarium* discovered 26 compounds and 23 substances were identified (Table 1, Fig. 1). The main components are chrysantemyl acetate (24.4%), chrysantemol (21.8%), chrysantenyl acetate (7.6%), camphore (7.3%), β-farnesene (5.9%), α-bisabolol (5.6%). Among the identified compounds, the dominant are chrysantemol (21.8%), and its ester – chrysantemyl acetate (24.4%) (Table 1). The content of essential oil in inflorescences of *C. coronarium* was 0.5% (after conversion to oven dry mass).

Table 1
Chemical compound of essential oil from *C. coronarium* (blossom phase)

No	Duration of exposure, min	Component	Numeric content within the essential oil, %
1	7.46	yomogi alcohol	0.82 ± 0.011
2	11.18	linalool	2.89 ± 0.068
3	13.73	chrysantemol	21.83 ± 0.048
4	14.10	–	1.15 ± 0.035
5	14.63	terpinen-4-ol	2.53 ± 0.024
6	14.77	camphore	7.34 ± 0.037
7	15.46	α-terpineol	1.03 ± 0.035
8	16.26	linalyl acetate	0.86 ± 0.049
9	17.12	chrysantenyl acetate	7.55 ± 0.072
10	17.65	chrysantemyl acetate	24.40 ± 0.196
11	17.78	lavandulyl acetate	0.66 ± 0.042
12	18.11	bornyl acetate	0.97 ± 0.066
13	19.40	2-hexenyl caproate	0.48 ± 0.018
14	19.87	β-elemen	0.57 ± 0.073
15	20.69	neryl acetate	0.23 ± 0.034
16	21.06	β-caryophyllene	0.31 ± 0.029
17	21.49	geranyl acetate	0.61 ± 0.041
18	21.90	β-farnesene	5.88 ± 0.091
19	23.02	–	0.82 ± 0.046
20	23.10	–	1.15 ± 0.057
21	23.50	germacrene D	4.50 ± 0.493
22	23.84	α-farnesene	3.45 ± 0.453
23	24.23	biciklogermakren	0.62 ± 0.049
24	24.56	β-seskvifelandren	2.57 ± 0.082
25	28.91	epi-α-cadinol	1.21 ± 0.052
26	29.39	α-bisabolol	5.58 ± 0.069

Note: "–" – unidentified components.

At the first stage of studying the antimicrobial effect of *C. coronarium* alcohol extract, we studied the bacteriostatic and bactericidal activity of the solvent 40% ethanol. Bacteriostatic activity of the solvent against all used microbiological cultures was observed only after at a concentration of 1 : 2. Bactericidal/fungicidal concentration of alcohol in the solvent of *P. aeruginosa* and *C. albicans* was the same as for the bacteriostatic concentration. None of the alcohol solutions had any bacteriostatic effect on *E. coli* and *S. aureus*.

We observed moderate antimicrobial activity of the *C. coronarium* extract against gram-positive strains of *S. aureus* bacteria. In the liquid culture, the extracted substances stopped the growth and reproduction of bacteria in solutions of 1 : 8 and below (Table 2, Fig. 2). We observed that microorganisms inoculated in solid growth media ceased to grow after use of 1 : 2 solution (Table 3, Fig. 3). Thus, the indicators of minimum bacteriostatic (MIC) and minimum bactericidal concentration (MBC) of the sample against *S. aureus* increased by 4 and 2 times respectively in comparison with the solvent.

Table 2
Identifying minimum bacteriostatic concentration (MIC) of *C. coronarium* ethanol extract against microbiological cultures

Microbiological culture	Growth among the bacteria of the culture in tested variants at a particular solution of sample							Growth of microorganisms in culture in control variants			
	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	+K	–K	K_c	K_s
Escherichia coli UCM B-906	–	+	+	+	+	+	+	+	–	–	–
Staphylococcus aureus UCM B-904	–	–	–	+	+	+	+	+	–	–	–
Pseudomonas aeruginosa UCM B-900	–	+	+	+	+	+	+	+	–	–	–
Candida albicans UCM Y-1918	–	–	+	+	+	+	+	+	–	–	–

Note: "+" – growth of microorganisms in culture; "–" – absence of growth of microorganisms in culture; +K – positive control of growth of microorganisms in culture; –K – negative control of growth of microorganisms in culture; K_m – control of media cleanliness; K_s –control of the sample cleanliness (in 1 : 2 solution).

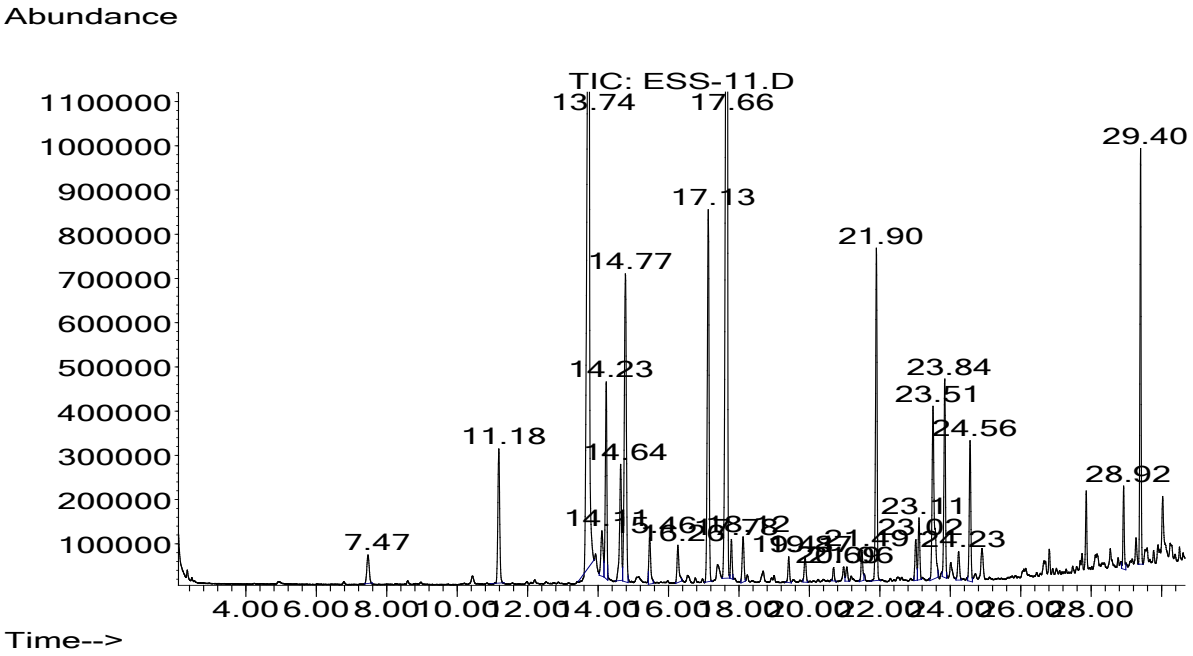

Fig. 1. Chromatogram of essential oil of *C. coronarium*

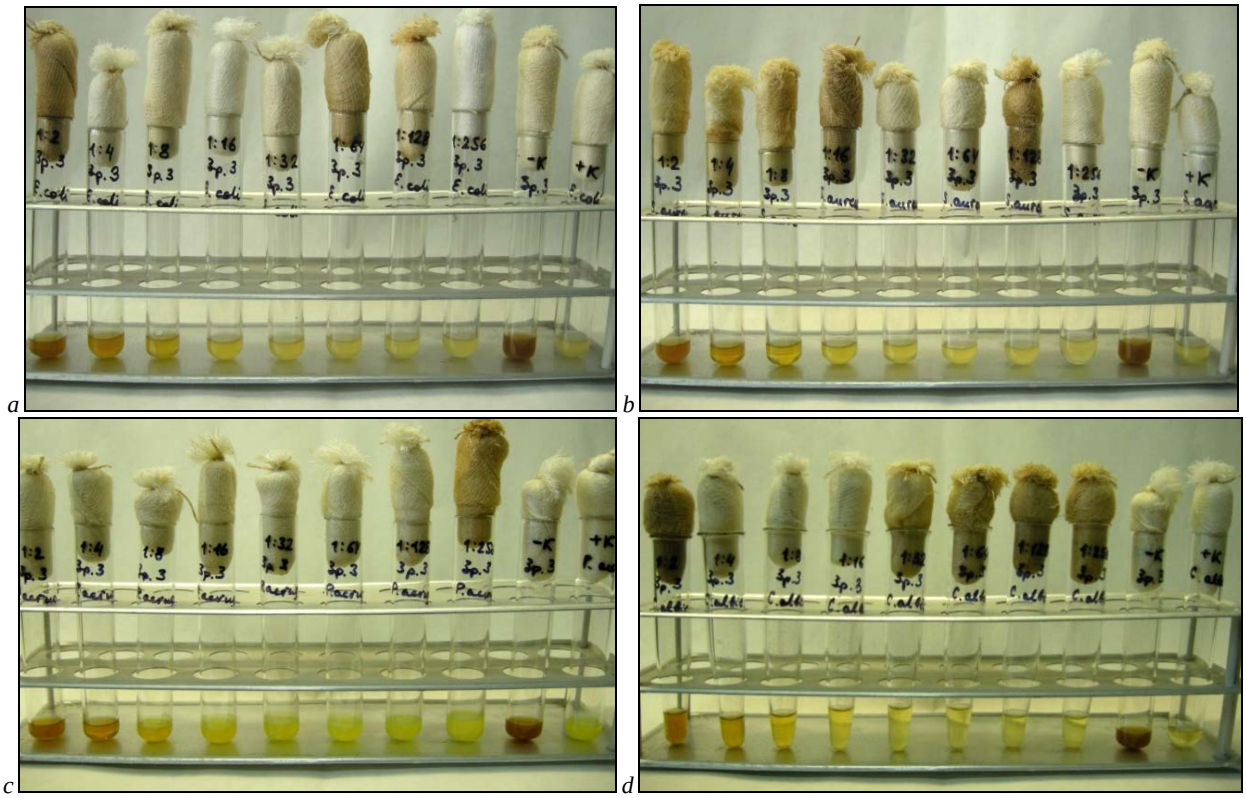

Fig. 2. Identifying minimum inhibitory concentration (MIC) of ethanol extract of *C. coronarium* against microbiological cultures: *a* – *Escherichia coli* UCM B-906; *b* – *Staphylococcus aureus* UCM B-904; *c* – *Pseudomonas aeruginosa* UCM B-900; *d* – *Candida albicans* UCM Y-1918

Table 3
Identifying minimum bactericidal/fungicidal concentration (MBC/MFC) of ethanol extract of *C. coronarium* against microbiological cultures

Tested microbiological cultures	Growth among bacteria of the culture in solid media after using the corresponding diluted sample						
	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128
Escherichia coli UCM B-906	+	+	+	+	+	+	+
Staphylococcus aureus UCM B-904	–	+	+	+	+	+	+
Pseudomonas aeruginosa UCM B-900	–	+	+	+	+	+	+
Candida albicans UCM Y-1918	–	+	+	+	+	+	+

Note: "+" – growth among the bacteria, "–" – absence of growth among the bacteria.

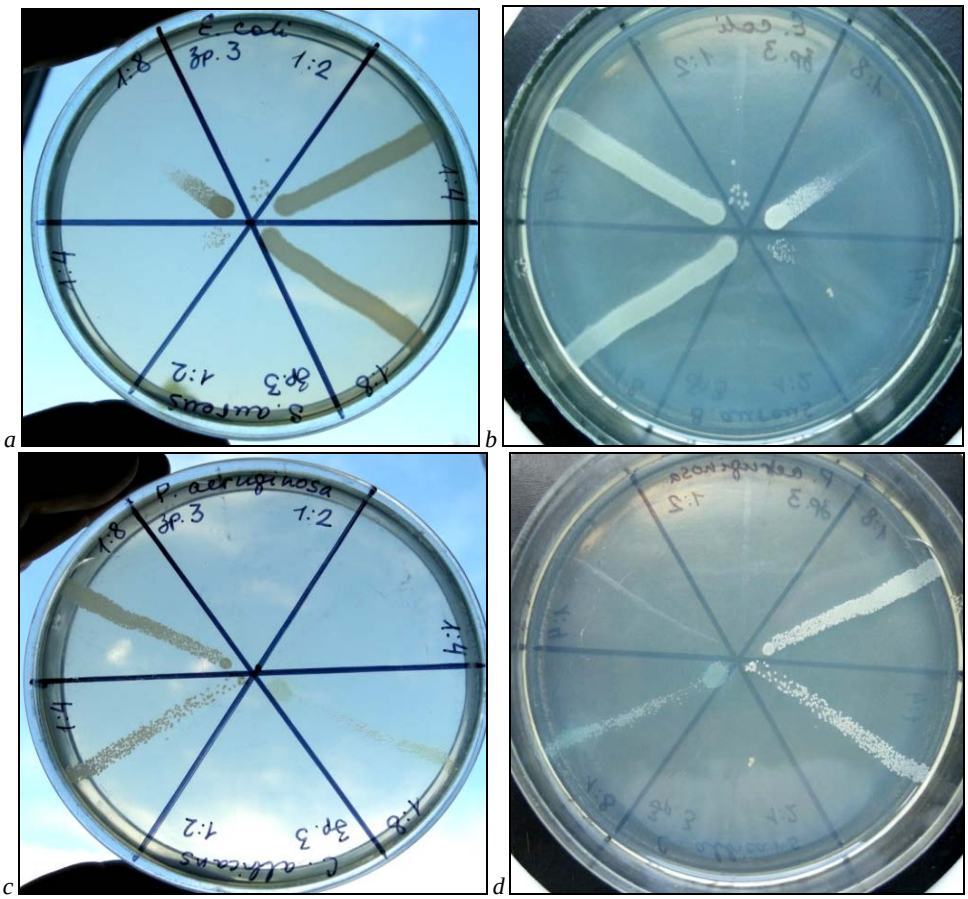

Fig. 3. Identifying the minimum bactericidal/fungicidal concentration (MBC/MFC) of ethanol extract of *C. coronarium* against tested microbiological cultures: *Escherichia coli* UCM B-906 and *Staphylococcus aureus* UCM B-904 (*a* – obverse, *b* – internal view); *Pseudomonas aeruginosa* UCM B-900 and *Candida albicans* UCM Y-1918 (*c* – obverse, *d* – internal view)

Low activity of the extracted substances was also observed against the fungus *C. albicans*. The absence of growth among the microorganisms of the culture was observed in solutions 1 : 4 and lower (Table 2, Fig.2). So, the extracted substances doubled the fungicidal activity of alcohol. At the same time, the indicator of MBC sample did not differ from the similar indicator at 40% ethanol. Both cases showed absence of growth among the bacteria inoculated in solid media at the solution 1:2. The extracted substances did not increase the fungicidal activity of 40% ethanol. The increase in fungicidal activity of the solvent against the fungus *C. albicans* was not observed (Table 2, 3). Gram-negative bacteria *P. aeruginosa* and *E. coli* appeared to be insensitive to the substances of extract (Table 2, 3, Fig. 2, 3).

Discussion

The results of our studies concur with the data of Hosni et al. (2013), who identified 40 components of *C. coronarium* essential oil with the following dominants: cis-chrysanthenyl acetate (21.8%), trans-chrysanthenyl acetate (12.8), (E)-β-farnesene (9.0%), germacrene D (8.9%) and camphor (6.0%). We also found these compounds, but at different percentages. In essential oil extracted from samples of Garland chrysanthemum, grown in Greece, the dominant components were as follows: trans-chrysanthenyl acetate (7.8–13.2%), cis-chrysanthenyl acetate (9.1–9.9%), camphor (9.1–15.7%), β-pinene oxide (7.7–8.8%), trans-chrysanthenyl isovalerate (5.8–10.2%) and myrcene (6.2–7.0%) (Basta et al., 2007). Our samples contained no β-pinene oxide, trans-chrysanthenyl isovalerate and myrcene. Samples from Algeria contained no trans-chrysanthenyl acetate, and β-pinene oxide (Dokuparthi and Manikanta, 2015).

According to research by Spanish scientists, the dominant components of *C. coronarium* essential oil were: camphor (29.2%), α-pinene (14.8%), β-pinene (9.5%), liratyl acetate (9.8%) (Alvares-Castellanos and Pascual-Vilalolos, 2003; Alvares-Castellanos et al., 2001). So, our results differ from the Spanish research data in the absence of α-pinene, β-pinene, liratyl acetate. According to literature sources, the chemical compound of Garland chrysanthemum is variable and depends upon genetic, climatic and geographical factors (Flamini et al., 2003; Senatore et al., 2004; Sebastian et al., 2006; Hosni et al., 2013; Dokuparthi and Manikanta, 2015; Preedy, 2016).

The antimicrobial activity of extract of *C. coronarium* against *S. aureus* and *C. albicans*, which we observed, is probably related to the plant's content of essential oil and phenol compound. Our study concurs with the data obtained by Hosni et al. (2013), who established that essential oil of Garland chrysanthemum had antimicrobial properties against *S. aureus*, *C. albicans*, *Salmonella typhymurium* and *Bacillus cereus*.

Conclusions

Chromatographic analysis of essential oil extracted from inflorescences of *C. coronarium* found 26 compounds, 23 of which were identified. The dominant components were chrysantemyl acetate (24.4%), chrysantemol (21.8%), Chrysantenyl acetate (7.6%), camphora (7.3%), β-farnesene (5.9%), α-bisabolol (5.6%).

We observed antimicrobial activity of alcohol extract of *C. coronarium* against gram-positive strains of *S. aureus* bacteria. Compared to the solvent, the indicators of minimum bacteriostatic (MIC) and minimum bactericidal concentration (MBC) increased by 4 and 2 times, respectively. Fungicidal activity was observed only against the fungus *C. albicans* – the indicators doubled compared to the solvent. We observed no bacteriostatic or bactericidal properties of the extract against gram-negative strains of *E. coli* and *P. aeruginosa* bacteria. The obtained experimental data indicate prospects of further study and usage of *C. coronarium* in pharmacy and the food industry.

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