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Monarda fistulosa L. from the Forest-Steppe Zone of Western Siberia: Anti-influenza Activity of Plant Components during the Vegetation Period

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Abstract

This is a study on antiviral activity of various extracts from the plant *Monarda fistulosa* L. cultivated in the forest-steppe zone of Western Siberia during the vegetation period. The activity of aqueous and aqueous-ethanolic extracts of *M. fistulosa* during the whole vegetation period and two polyphenolic fractions from this plant at the flowering stage was investigated against human influenza virus A/Aichi/2/68 (H3N2) and avian influenza virus A/chicken/Kurgan/05/2005 (H5N1). The anti-influenza activity was compared among the extracts and the polyphenolic fractions from *M. fistulosa* at the flowering stage. It was shown that antiviral activity against each subtype of the influenza A virus differed throughout the entire vegetation period, depending on the developmental stage of *M. fistulosa* and the type of extraction. Influenza virus A/Aichi/2/68 (H3N2) infectivity was most strongly inhibited by aqueous-ethanolic extracts from *M. fistulosa* raw material collected at the growth stage and by the aqueous extract from the fruiting initiation stage. Inhibition of the infectivity of influenza virus A/chicken/Kurgan/05/2005 (H5N1) was achieved by one or another *M. fistulosa* extract almost throughout the whole vegetation period. It is found that Polyphenolic Fractions 1 and 2 are more active against influenza virus A/Aichi/2/68 (H3N2), but aqueous and aqueous-ethanolic extracts are more active against influenza virus A/chicken/Kurgan/05/2005 (H5N1). On the condition of further in-depth studies, our experimental data suggest that *M. fistulosa* plant components can be recommended as medicinal (antiviral) substances against influenza viruses A/Aichi/2/68 (H3N2) and A/chicken/Kurgan/05/2005 (H5N1).

Keywords: *Monarda fistulosa*, extract, polyphenolic fraction, antiviral activity, influenza virus A

INTRODUCTION

Currently, in Russia, as in other countries, much attention is given to the search for and development of antiviral (anti-influenza) agents, either synthetic or based on the substances of natural origin, because influenza is one of the leading infectious diseases [1, 2]. In this regard, there is a lot of research on medicinal plants in which the native sets of substances contain individual compounds and various classes of biologically active substances having anti-influenza activity [3–7]. In the literature, there is an opinion that evolutionarily late subclasses Lamiidae and Asteridae are the most prominent ones in terms of the use of plant species against viral infections in

general and against respiratory infections in particular (including influenza) [4]. Plant tissues of many species of the Lamiaceae family contain various classes of biologically active substances and can serve as a source of anti-influenza agents [8]. One of such promising plant species from this family is *Monarda fistulosa* L., also known as bee balm, which originates from North America and does not grow naturally in Russia. Nonetheless, this plant has high ecological plasticity and is cultivated in various regions of this country, namely in the European part of Russia, in Crimea, the Caucasus, the Urals, and Siberia, as a plant source of essential oils and as an aromatic, medicinal, and even nutritional plant [9, 10]. *M. fistulosa* is one of the best studied species of the genus *Monarda* and has a wide range of pharmacotherapeutic effects [11–20], which is due to the presence of diverse classes of biologically active compounds: essential oil components characterized by a high content of phenols (67–89 %), flavonoids (luteolin, naringenin, luteolin-7-glycoside, rutin, hyperoside, isorhoifolin, linarin, catechins), tannins, anthocyanins, carotenoids, polysaccharides, ascorbic, gallic and chlorogenic acids, bitterness, etc. Specific for species of the genus *Monarda* is monardein, 3-O-(6-O-trans-p-coumaryl-β-*D*-glucopyranosyl)-5-O-(4,6-di-O-malonyl-β-*D*-glucopyranosyl) pelargonidine [21–24]. At present, *M. fistulosa* is still actively investigated in various aspects, especially because the study of biological activities of extracts and essential oils increases the scope of applications of components of this nonpharmacopeial species. This state of affairs makes it possible to take advantage of the beneficial properties of *M. fistulosa* not only in medicine and cosmetics, but also in the food industry and agriculture [25–27].

In the environment of the forest-steppe zone of Western Siberia (Novosibirsk), *M. fistulosa* that is cultivated on the experimental plot of the Central Siberian Botanical Garden has been studied in various respects [2, 9, 11, 19, 21, 24, 28, 29]. Earlier, for the first time, joint studies carried out at the Central Siberian Botanical Garden SB RAS (Novosibirsk) and at the State Research Center of Virology and Biotechnology VECTOR (Novosibirsk Region) revealed a strong anti-influenza effect of the aqueous and aqueous-ethanolic extracts of *M. fistulosa* against human influenza virus A/Aichi/2/68 (H3N2) and avian influenza virus A/chicken/Kurgan/05/2005 (H5N1) [2, 29].

The aim of this work was to evaluate the antiviral properties of the extracts of *M. fistulosa* during its vegetation period against influenza viruses A/Aichi/2/68 (H3N2) and A/chicken/Kurgan/05/2005 (H5N1).

EXPERIMENTAL

Plant material for the assays

The samples of the aerial parts of *M. fistulosa* collected on the experimental plot of the Central Siberian Botanical Garden during the vegetation period in 2018 were used for the analysis of anti-influenza activity at various developmental stages: growth stage 1 (shoots up to 10 cm long), growth stage 2 (shoots up to 15 cm long), flowering, and the start and end of fruiting. The plant samples were air-dried completely and stored in paper bags.

Methods for obtaining dry extracts

The aqueous-ethanolic extraction method: Each sample of plant material (2 g) was placed in a 100 mL round-bottom reflux flask, and 25 mL of 70 % ethanol was added. Extraction was carried out in a water bath at 60 °C for 1 h. After that, the extract was separated from the solids by passing it through a glass filter (pore size 10–16 μm). The extraction procedure with 70 % ethanol was repeated two more times. These extracts were combined, dried in a rotary evaporator, and finally dried in an oven at 60 °C.

The aqueous extraction method: A sample (2 g) of each plant material was placed in a 100 mL round-bottom flask with a reflux condenser, 60 mL of purified water was added, and the mixture was heated at 95 °C for 1 h. The extract was separated from the solids by passing it through a glass filter (pore size 10–16 μm). The extraction procedure was repeated with 40 mL of water. The extracts were combined, cooled, evaporated and finally dried at 60 °C [30].

Isolation of polyphenolic fractions

Polyphenolic Fraction 1. A sample (50 g) of plant material from the flowering stage was extracted with ethyl acetate, and the solids were dried. Next, extraction with ethanol (sample/ethanol at 1 : 50) was performed three times: at concentrations 96, 70, and 50 % in that order. The extracts (1000, 750, and 750 mL) were combined and evaporated until the ethanol smell disappeared. The resulting aqueous extract was

shaken with chloroform (to remove chlorophyll and resins) until the green color in chloroform disappeared. Next, the purified extract was shaken with ethyl acetate. Anhydrous sodium sulphate was added to remove water from the ethyl acetate extract, and the latter was filtered. After that, ethyl acetate was distilled off until a precipitate began to form, and the resultant extract was poured as a thin trickle into a 6-fold volume of chloroform with stirring. The yellow precipitate that emerged was filtered off through a glass filter. The precipitate was then dried in vacuum [31].

Polyphenolic Fraction 2 was prepared in the same way as Polyphenolic Fraction 1 was, except the ethyl acetate extraction step was omitted.

Determination of antiviral properties of the extracts and polyphenolic fractions

These experiments involved avian influenza virus A/chicken/Kurgan/05/2005 (H5N1) and a strain [A/Aichi/2/68 (H3N2)] of human influenza virus adapted to laboratory mice; the viruses were obtained from the State Collection of Viral and Rickettsial Disease Agents at the State Research Center of Virology and Biotechnology VECTOR (Koltsovo, Novosibirsk Region, Russia) affiliated with Rospotrebnadzor. Influenza virus production and titration were performed on cultured immortalized cells (MDCK cell line) obtained from the Cell Culture Collection at the State Research Center of Virology and Biotechnology VECTOR and grown in the DMEM medium (Biolot Ltd., St. Petersburg, Russia), supplemented with 10 % inactivated fetal bovine serum (Biolot Ltd., St. Petersburg, Russia) and antibiotics (11 IU/mL penicillin and 100 μg/mL streptomycin (Biolot Ltd., St. Petersburg, Russia)). The study of the antiviral activity of the samples was conducted using its maximum tolerated concentrations (MTC). The virus titer in the culture supernatant was calculated by the Spearman–Kärber method, expressed as the decimal logarithm of 50 % tissue culture infectious dose per mL (lgTCID50/mL), and is presented as the mean with standard deviation (M ± SD) [32]. The effect of the plant extracts and polyphenolic fractions on the influenza virus of each subtype was evaluated by means of a logarithmic metric called a neutralization index (NI): NI = lg(50 % infectious virus titer in control [no treatment]) − lg(50 % infectious virus titer in experiment) [33].

Testing of the cytotoxicity of the extracts and polyphenolic fractions

To determine cytotoxicity (maximum tolerated concentration; MTC) of the analysed extracts and polyphenolic fractions, these samples were serially diluted, and the presence of toxic effects in the MDCK cell culture monolayer was assessed under an inverted microscope. After that, MTC was determined as the concentration at which 100 % of cells were retained in the monolayer. For determining the antiviral activity of the extracts and polyphenolic fractions, the following cells served as controls: MDCK cells cultivated in the DMEM medium containing 2 μg/mL trypsin (no virus and no treatment) and MDCK cells infected with either influenza virus A/Aichi/2/68 or A/chicken/Kurgan/05/2005 without the *M. fistulosa*-derived substances [33].

RESULT AND DISCUSSION

Anti-influenza activity of the M. fistulosa extracts during the vegetation period

The anti-influenza properties of water and water-ethanol extracts obtained from raw materials of the above-ground part of *M. fistulosa* during the growing season were studied. The present study was carried out in continuation of earlier works, where the anti-influenza activity of *M. fistulosa* extracts against human and avian influenza viruses was shown for the first time [29]. The results on changes in the anti-influenza activity of extracts from *Monarda* raw materials collected according to the phases of plant development were obtained for the first time, and we are not aware of any publications in the scientific literature on the antiviral (anti-influenza) properties of *M. fistulosa*. Table 1 presents the data on the antiviral activity of aqueous and aqueous-ethanolic extracts from above-ground parts of *M. fistulosa* during the vegetation period. The anti-influenza activity was quantified with the help of the NI (a logarithmic metric). According to guidelines for the experimental (preclinical) study of new pharmacological substances [34], drugs that reduce the infectivity of a virus in cell culture by a factor of at least 100 (lg ≥ 2) are promising for further *in vivo* studies. From the data in Table 1, it follows that antiviral activity against influenza A of both subtypes was found in at least one of the two extracts throughout the vegetation period, but the magnitude of inhibi-

TABLE 1

Antiviral activity of extracts from *M. fistulosa* toward influenza viruses A/Aichi/2/68 (H3N2) and A/chicken/Kurgan/05/2005 (H5N1)

A/Aichi/2/68 (H3N2)				*M. fistulosa* developmental stages	A/chicken/Kurgan/05/2005 (H5N1)			
Extracts					Extracts			
Aqueous		Aqueous-ethanolic			Aqueous		Aqueous-ethanolic	
Virus titer, lgTCID50/mL, (M ± SD, n = 4)	NI	Virus titer, lgTCID50/mL (M ± SD, n = 4)	NI		Virus titer, lgTCID50/mL (M ± SD, n = 4)	NI	Virus titer, lgTCID50/mL, (M ± SD, n=4)	NI
5.00±0.29*	1.25	1.50±0.00*	4.75	Growth stage 1	2.50±0.00*	3.75	3.00±0.35*	3.25
4.50±0.00*	1.75	2.50±0.00*	3.75	Growth stage 2	4.50±0.00*	1.75	3.00±0.29*	3.25
4.50±0.00*	1.75	4.50±0.00*	1.75	Flowering	4.00±0.29*	2.25	4.00±0.29*	2.25
2.50±0.00*	3.75	5.00±0.29*	1.25	Fruiting initiation	4.50±0.00*	1.75	4.50±0.00*	1.75
5.00±0.29*	1.25	5.00±0.29*	1.25	Fruiting	3.50±0.41*	2.75	3.50±0.00*	2.75
6.25±0.25				Control (extract not added)	6.25±0.38			

Note. Growth stage 1 – shoots up to 10 cm long; growth stage 2 – shoots up to 15 cm long; lgTCID50/mL – the virus titer in the culture supernatant, *i. e.*, $\log_{10}$ of 50 % tissue culture infectious dose (these titers were calculated and compared by the Spearman–Kärber method) per mL; M ± SD mean value ± standard deviation; n – the number of wells with a cell monolayer infected with various dilutions of a virus suspension; NI – neutralization index: NI = lg(50 % infectious virus titer in control) – lg(50 % infectious virus titer in experiment).

* A significant difference from the untreated-infection control ($p \leq 0.05$).

tion depended on the type of extract and developmental stage. For instance, aqueous-ethanolic extracts showed antiviral activity against the human influenza virus with the highest NI (4.75–3.75) at growth stages 1 and 2. By contrast, in aqueous extracts, the highest anti-influenza activity (NI = 3.75) was registered at the fruiting initiation stage. As for the avian influenza virus, strong activity (NI = 2.25 to 3.75) was detectable almost throughout the entire vegetation period: at growth stages 1 and 2, the NI was 3.75 in aqueous extracts and 3.25 in aqueous-ethanolic extracts; at the flowering and fruiting stages, the anti-influenza activities of the aqueous and aqueous-ethanolic extracts (NI = 2.25–2.75) were similar toward this virus subtype. Overall, the extracts from the aerial parts of *M. fistulosa* significantly reduced the infectivity of the influenza virus of both subtypes, depending on the developmental stage of this plant and on the type of extraction.

Cytotoxicity of the M. fistulosa extracts throughout the vegetation period

Cytotoxicity of the two extracts from *M. fistulosa* was measured next, and MTCs were determined in the range of concentrations 0.005 to 2.500 mg/mL (see Table 2).

The aqueous and aqueous-ethanol extracts were found to have low toxicity to MDCK cells, but differed in MTC, as was the case for the magnitude of inhibition of human and avian influenza viruses throughout the vegetation period (see above). Aqueous and aqueous-ethanolic extracts at growth stage 1 were the least toxic, as was the aqueous-ethanolic extract at the flowering stage.

Anti-influenza activity and cytotoxicity of the polyphenolic fractions

In this part of the project, two types of polyphenolic compounds (Polyphenolic Fractions 1

TABLE 2

Toxicity of plant extracts from *M. fistulosa* during the vegetation period

M. fistulosa developmental stages	MTCs (mg/mL) of extracts	
	Aqueous	Aqueous-ethanolic
Growth stage 1: shoots up to 10 cm long	2.5	2.5
Growth stage 2: shoots up to 15 cm long	1.43	1.43
Flowering	1	2.5
Fruiting initiation	1	1
Fruiting	1	1

TABLE 3

Toxicity and antiviral activity of polyphenolic fractions from *M. fistulosa* (flowering stage) toward influenza viruses A/Aichi/2/68 (H3N2) and A/chicken/Kurgan/05/2005 (H5N1)

Sample type	MTC, mg/mL	A/Aichi/2/68 (H3N2)		A/chicken/Kurgan/05/2005 (H5N1)	
		Virus titer, lgTCID50/mL (M ± SD, n = 4)	NI	Virus titer, lgTCID50/mL (M ± SD, n = 4)	NI
Polyphenolic Fraction 1	0.2	4.00±0.29*	2.00	4.50±0.00*	1.25
Polyphenolic Fraction 2	0.5	3.50±0.00*	2.50	4.00±0.29*	1.75
Control		6.00±0.29		5.75±0.25	

Note. Virus titers in the culture supernatants were calculated and compared by the Spearman–Kärber method; lgTCID50/mL – $\log_{10}$ of 50 % tissue culture infectious dose per mL; M ± SD mean value ± standard deviation; n – the number of wells with a cell monolayer infected with various dilutions of a virus suspension; NI – neutralization index: NI = lg(50 % infectious virus titer in control) – lg(50 % infectious virus titer in experiment).

* A significant difference from the untreated-infection control ($p \leq 0.05$).

and 2) were isolated from the aerial parts of *M. fistulosa* in the flowering stage, and their anti-influenza activity was investigated (see Table 3). The Table shows the NIs of the *M. fistulosa* polyphenolic fractions toward the influenza viruses. The NIs of Polyphenolic Fractions 1 and 2 toward the human influenza virus were 2.0 and 2.5, respectively. By contrast, these substances did not exert such a strong effect on the avian influenza virus. Nevertheless, there was statistically significant suppression of the replication of influenza viruses A/Aichi/2/68 (H3N2) and A/chicken/Kurgan/05/2005 (H5N1) in comparison with the control (infection without treatment). This experiment has also revealed that these polyphenolic fractions from the aerial part of bee balm have low toxicity to cultured MDCK cells, and that Polyphenolic Fraction 2 is less toxic than Polyphenolic Fraction 1 (see Table 3).

Overall, in the comparison of the anti-influenza effects among the four types of substances at the flowering stage, Polyphenolic Fractions 1 and 2 turned out to be more active against the human influenza virus, whereas the aqueous and aqueous-ethanolic extracts were more active against the avian influenza virus.

CONCLUSION

This paper presents the results from assays of antiviral activity of the substances isolated from the plant *M. fistulosa* collected during the vegetation period (several stages of development) against influenza viruses A/Aichi/2/68 (H3N2) and A/chicken/Kurgan/05/2005 (H5N1). Anti-influenza activity against both influenza virus subtypes was found in aqueous and aqueous-ethanolic extracts throughout the vegetation period. The magnitude of inhibition depended on the influenza virus subtype, the developmental stage of *M. fistulosa*, and on the type of extraction. Regarding influenza virus A/Aichi/2/68 (H3N2), the strongest inhibition of its infectivity was caused by aqueous-ethanolic extracts from *M. fistulosa* collected at the growth stages with a shoot length of ≤10 and ≤15 cm (NIs of 4.75 and 3.75, respectively) and by the aqueous extract at the initiation of fruiting (NI = 3.75). As for the influenza A/chicken/Kurgan/05/2005 (H5N1) virus, strong inhibition of its infectivity was induced by the extracts of both types at the growth stage with shoots up to 10 cm long (NI = 3.25 to 3.75), at the flowering stage (NI = 2.25), and at the fruiting stage (NI = 2.75) as well as by the aqueous-ethanolic extract at the growth stage with shoots up to 15 cm long (NI = 3.25). Comparison of the anti-influenza effects among the four types of substances at the flowering stage revealed that Polyphenolic Fractions 1 and 2 proved to be more active against the human influenza virus [A/Aichi/2/68 (H3N2)], whereas the aqueous and aqueous-ethanolic extracts were more active against the avian influenza virus [A/chicken/Kurgan/05/2005 (H5N1)].

The findings indicate that, because of the anti-influenza properties, *M. fistulosa* plant components hold promise as antiviral therapeutics for influenza viruses A/Aichi/2/68 (H3N2) and A/chicken/Kurgan/05/2005 (H5N1).

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REFERENCES

1 Ershov F. I. Antiviral Preparations, Moscow: Meditsina, 1998. (In Russ.).
2 Mazurkova N. A., Filippova E. I., Makarevich E. V., Lobanova I. E., Vysochina G. I. Higher plants as a basis for the development of anti-influenza preparations // Voprosy Biol., Med. and Farmatsevt. Khimii. 2014. No. 4. P. 55–56. (In Russ.).
3 Balunas M. J., Kinghorn A. D. Drug discovery from medicinal plants. // Life Sci. 2005. Vol. 78, No. 5. P. 431–441.
4 Popov P. L. Plant species, using against virous infections of man and animals: Regularities of the distribution in the phylogenetic classification system // J. Stress Physiol. & Biochem. 2008. Vol. 4, No. 3. P. 17–64.
5 Preziosi P. Influenza pharmacotherapy: Present situation, strategies and hopes // Expert Opin. Pharmacother. 2011. Vol. 12, No. 10. P. 1523–1549.
6 Martinez J. P., Sasse F., Bronstrup M., Diez J., Meyerhans A. Antiviral drug discovery: Broad-spectrum drugs from nature // Nat. Prod. Rep. 2015. Vol. 32, No. 1. P. 29–48.
7 Lobanova I. E., Filippova E. I., Kukushkina T. A., Protsenko M. A., Khramova E. P., Mazurkova N. A., Vysochina G. I. Comparative evaluation of the antiviral activity of extracts of some higher plants against influenza A virus *in vitro* // Chem. Sustain. Dev. 2021. Vol. 29, No. 6. P. 675–682.
8 Protsenko M. A., Mazurkova N. A., Filippova E. I., Kukushkina T. A., Lobanova I. E., Pshenichkina Yu. A. Anti-influenza activity of extracts of plants of the Lamiaceae family // Khimiya Rastitelnogo Syrya. 2021. No. 2. P. 181–190. (In Russ.).
9 Pat. RU 2250596 C2, 2005.
10 Kharchenko V. A., Bespalko L. V., Gins V. K., Gins M. S., Baikov A. A. Monarda – A valuable source of biologically active compounds // Vegetables Crops of Russia. 2015. No. 1. P. 31–35. (In Russ.).
11 Kislenko V. N., Reimer V. A., Cheremushkina V. A., Vysochina G. I., Kolesnikova O. P., Koptev V. Yu., Alekseeva Z. N., Lyakhovskaya N. V., Tarabanova E. V., Lysenko N. A., Sviridova O. A., Seliverstova M. N. Some pharmacological features of *Monarda fistulosa* L. and *Solsola collina* Pall. // Bulletin of the Novosibirsk State Agrarian University. 2011. No. 2 (18). P. 87–91. (In Russ.).
12 Naumenko E. N., Zhilyakova E. T., Novikov O. O., Krichkovskaya L. V., Vankhin O. A. Research of anti-inflammatory activity of medicine "Monavitol" *in vivo* // Scientific Bulletin of the Belgorod State University. Series: Medicine. Pharmacy. 2012. No. 22-1 (141). P. 195–198. (In Russ.).
13 Bedulenko M. A. Introduction, environmental aspect and modern trends in the study and application of medicinal, aromatic and essential oils *Monarda fistulosa* L. (review) // Proceedings of the Belarusian State University. Series: Physiological, Biochemical and Molecular Biology Sciences. 2013. Vol. 8, No. 2. P. 52–60. (In Russ.).
14 Zhilyakova E. T., Novikov O. O., Naumenko E. N., Kuzmichyova O. A., Bocharova K. A., Titareva L. V. Antimicrobial and anti-inflammatory activity of new formulation with Monarda oil // Scientific Bulletin of the Belgorod State University. Series: Medicine. Pharmacy. 2013. No. 25-1 (168). P. 198–201. (In Russ.).
15 Krasyuk E. V., Makarova N. N., Petrova I. V., Pupykina K. A., Valeeva L. A. Pharmacological activity of Monarda types, introduced in the Republic of Bashkortostan // Med. Vestn. Bashkortostan. 2015. Vol. 10, No. 5 (59). P. 67–70. (In Russ.).
16 Krasyuk E. V., Pupykina K. A., Krasyuk A. D. The study of the antimicrobial activity of Monarda plants introduced in the Republic of Bashkortostan // Bulletin of the Bashkir State Medical University. 2019. No. 6. P. 120–123. (In Russ.).
17 Marchenko M. A. Development of an anti-inflammatory dental gel based on an extract from the herb *Monarda fistulosa* // Materials of the Scientific and Practical Conference "Young Science". Pyatigorsk, October 21–22, 2016. P. 36–38. (In Russ.).
18 Pat. RU 2580304 C1, 2016. (In Russ.).
19 Lobanova I. E., Andreeva I. S., Vysochina G. I., Solovyanova N. A. Screening of wild and cultivated plants of the Novosibirsk region for the presence of antibiotic activity // Rastitelniy Mir Aziatskoy Rossii. 2017. No. 2 (26). P. 85–91. (In Russ.).
20 Lapina A. S., Varina N. R., Kurkin V. A., Avdeeva E. V., Ryazanova T. K., Ryzhov V. M., Ruzaeva I. V. *Monarda fistulosa* as a promising source of medicinal products preparations // Collection of Scientific Works of the State Nikitsky Botanical Garden. 2018. No. 146. P. 175–178. (In Russ.).
21 Oparin R. V., Pokrovsky L. M., Vysochina G. I., Tkachev A. V. Studies of the chemical composition of the essential oils of *Monarda fistulosa* L. and Monarda *didyma* L. cultivated in Western Siberia // Khimiya Rastitelnogo Syrya. 2000. No. 3. P. 19–24. (In Russ.).
22 Fedotov S. V. Essential oils of *Monarda fistulosa* L., *Monarda didyma* L., *Monarda citriodora* Cervantes ex Lag., their chemotypes and biological activity // Collection of Scientific Works of the State Nikitsky Botanical Garden. 2015. No. 141. P. 131–147. (In Russ.).
23 Krasyuk E. V., Pupykina K. A. Qualitative analysis and development of methods of quantification of flavonoids in Monarda species introduced in the Republic of Bashkortostan // Medical Bulletin of Bashkortostan. 2016. Vol. 11, No. 5 (65), P. 73–77. (In Russ.).
24 Kukushkina T. A., Lobanova I. E., Vysochina G. I. Nonvolatile biologically active substances of *Monarda fistulosa* during the growing season // Rastitelniy Mir Aziatskoy Rossii. 2019. No. 3. P. 57–61. (In Russ.).
25 Tabanca N., Bernier U. R., Ali A., Wang M., Demirci B., Blythe E. K., Khan Sh. I., Baser K. H. C., Khan I. A. Bioassay-guided investigation of two Monarda essential oils as repellents of yellow fever mosquito Aedes aegypti // Agricult. Food Chem. 2013. Vol. 61, No. 36. P. 8573–8580.
26 Lapina A. S., Kurkin V. A. Elaboration of approaches to standardizing wild bergamot (*Monarda fistulosa* L.) herb // J. Pharmacy. 2019. Vol. 68, No. 4. P. 11–16. (In Russ.).

27 Vysochina G. I. Genus *Monarda* L. (Lamiaceae): Chemical composition, biological activity and practical application (a review) // Chem. Sustain. Dev. 2020. Vol. 28, No. 2. P. 105–120.
28 Pat. RU 2244552 C2. 2003. (In Russ.).
29 Lobanova I. E., Filippova E. I., Vysochina G. I., Mazurkova N. A. Antiviral properties of the wild-growing and cultivated plants of Southwest Siberia // Rastitelniy Mir Aziatskoy Rossii. 2016. No. 2 (22), P. 64–72. (In Russ.).
30 Kostina N. E., Ibragimova Zh. B., Protsenko M. A., Makarevich E. V., Skarnovich M. A., Filippova E. I., Gorbunova I. A., Vlasenko V. A., Troshkova G. P., Mazurkova N. A., Shishkina L. N. Isolation, characterization and antiviral properties of biologically active agents of the highest mushrooms of Western Siberia // Modern Problems of Science and Education. 2013. No. 3. Art. 336. (In Russ.).
31 Pat. RU 2128516 C1, 1999 (In Russ.).
32 Zaks L. Statistical Evaluation. Moscow: Statistica, 1976. (In Russ.).
33 Filippova E. I., Mazurkova N. A., Kabanov A. S., Teplyakova T. V., Ibragimova Zh. B., Makarevich E. V., Mazurkov O. Yu., Shishkina L. N. Antiviral properties of aqueous extracts isolated from higher basidiomycetes as respect to pandemic influenza virus A(H1N1)2009 // Modern Problems of Science and Education. 2013. No. 2. Art. 413. (In Russ.).
34 Guidelines for Conducting Preclinical Study of Medicines / Mironov A. N. (Ed.). Part one. M.: Grif and K, 2012. 944 p. (In Russ.).