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Genus *Monarda* (Lamiaceae): Chemical Composition, Biological Activity and Practical Application (a Review)

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Abstract

The review of the data on the chemical composition and biological activity of the genus *Monarda* L. represented by 20 species growing in North America is given. *M. fistulosa* L., *M. didyma* L. and *M. citriodora* Cervantes ex Lag. are successfully cultivated in various regions of Russia. The essential oils of *Monarda* are distinguished by the high content of phenols (67–89 %). Their composition depends on the species of *Monarda*, vegetation phase, site of growth, weather conditions. Under the conditions of a sharply continental Siberian climate (Novosibirsk), *M. fistulosa* has a high content (up to 4.16 %) of essential oil, more than in the Crimea and the North Caucasus. The phenolic complex contains flavonoids, anthocyanins and phenolic acids. Specific to the species of this genus is monardein, 3-O-(6-O-*trans*-*p*-coumaryl-β-*D*-glucopyranosyl)-5-O-(4,6-di-O-malonyl-β-*D*-glucopyranosyl)pelargonidine. The essential oil and extracts of *Monarda* have a high bactericidal, antiviral, antifungal activity, anti-inflammatory, analgesic, immunomodulatory, antioxidant, radioprotective, antioncological, *etc.* action. The oil extract of the aerial parts of *M. fistulosa* showed antimicrobial activity against pathogenic microorganisms: *Staphylococcus aureus*, *Enterobacter cloacae*, *Streptococcus faecalis*, *Escherichia coli*, *etc.* The aqueous-ethanolic and aqueous extracts showed high antiviral activity against influenza virus, subtype A/Aichi/2/68 (H3N2) (human) and A/chicken/Kurgan/05/2005 (H5N1) (birds). The extracts of *Monarda* also possess pronounced fungicidal properties. The activity of oil, water and water-alcohol extracts of *M. fistulosa* against the yeast-like fungus *Candida albicans* was very high. Thymol and thymoquinone from *M. fistulosa* are cytotoxic against certain lines of human tumour cells. The essential oil from *M. citriodora* and its main component, thymol, inhibit the proliferation of HL-60, MCF-7, PC-3, A-549 and MDAMB-231 cancer cells. Carvacrol also has an antioxidant and antitumour effect. Essential oil is used in the cosmetic and food industry to flavour vermouths, stabilize wines, and also as a natural flavouring agent, preservative and antioxidant instead of synthetic food additives. To expand the domestic market of raw materials, it is advisable to grow *Monarda* in culture.

Keywords: genus *Monarda* L., essential oils, non-volatile low-molecular compounds, bactericidal, antiviral, antimycotic biological activity, practical application

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GENUS *MONARDA* L.: CHEMICAL COMPOSITION, BIOLOGICAL ACTIVITY, APPLICATION

Genus *Monarda* L. (Lamiaceae family) is represented by perennial herbaceous plants growing in North America: in the USA (the southern and central parts), Canada and Mexico. There are about 20 species in the world flora [1–3]. These are plants with straight or branchy stalks up to 150 cm high, with simple, oblong lanceolate, dentate leaves. The flowers are small, fragrant, white, red, purple, yellowish or speckled, bilabiate, assembled in dense botryoidal cluster or cephalanthium up to 7 cm in diameter. One cluster blossoms for 18–20 days. The majority of monarda species grow in arid regions – in prairies and on mountain slopes, but there are also moisture-loving species preferring wet meadows and glades [4].

The genus got its name in favour of Spanish doctor and botanist Nicolas Bautista Monardes (N. B. Monardes, 1508–1588), who was the first to describe the discovered by him in the books “Joyful news from the New World” (1569) and “Medicinal history of West India” (1580). At the beginning of the XVI century, monarda was brought to Spain and other European countries, later in Russia, to the Urals and in Siberia. In the XIX century, monarda started to be used as a spice plant in Spain, France, Portugal, Great Britain under the names of Oswego tea, bergamot, mountain balm, bee or fragrant red balm, American melissa, Indian feather, lemon mint, *etc.* [5]. It was noted in ethnobotanic studies that among 200 plant species that were used by the Indian tribes of North America, settlers and doctors for medical purposes, two monarda species, *Monarda fistulosa* L. and *Acorus calamus* L., were used to treat multiple diseases [6]. Monarda species are grown in many countries of Europe and Asia as decorative, medicinal and spice aromatic plants. These plants are used for a long time in folk medicine, as kitchen herbs for food preparation and for tea aromatization [7, 8].

The goal of the present work is to review the data on the chemical composition, biological activity and practical use of the species of *Monarda* L. genus in the world flora published mainly within the two recent decades.

COMPOSITION AND CONTENT OF ESSENTIAL OILS IN MONARDA

Essential oils are multicomponent mixtures of individual substances. The most complete component composition of the oils was revealed in blossom clusters and leaves of the plants in the phase of mass blossoming [9]. They are extracted most frequently by the vapour distillation of crushed freshly mown or dried green mass of plants with leaves [10]. Essential oils of monarda, as a rule, are distinguished by the high phenolic content (67–89 %) [11]. Essential oils of 10 monarda species were studied in the Nikitskiy Botanical Garden (Yalta, Crimea), and 13 components were determined. The high content of tymol (60–84.8 %) was reported; other substances that were identified include carvacrol (4.13–9.6 %), γ-terpinene (13–16.6 %), sabinene (3.75–4.51 %), *p*-cymene (2.25–7.76 %), borneol, α-thujene, α-thujol, myrcene, linalool, cineol [12]. The composition of essential oil and the ratio of thymol to carvacrol in it depend on monarda species, phase of plant vegetation, growing site, weather conditions during the period of plant development, picking time [13]. In the Non-Black Soil Zone, the content of essential oil in the samples of *M. fistulosa* varied within the range 1.39–2.43 %, *M. didyma* – 1.66–2.13 % depending on the conditions of the year. The composition of essential oil was due both to cropping time and to sample kind. A broad range of genetic variability of monarda samples was detected [14]. It was established that the introduction of *Monarda didyma* L. and *Monarda citriodora* Cervantes ex Lag. species from southern regions into those situated in the northern direction, the component composition of essential oil remains practically unchanged, only slight deviations in the content of some terpenoids are observed [27]. In connection with the ability of monarda species to enter hybridization, it is possible to obtain winter-hardy hybrids stable

TABLE 1

Essential oil content and its major components in *Monarda fistulosa*, *M. didyma* and *M. citriodora* plants from different regions

Sample No.	Growing place (*characterisation of raw material)*	Essential oil			Ref.
		Yield, % of the mass of abs. dry raw material	Number of components	Major components (%)	
		Monarda fistulosa			
1	Crimea, Yalta; Nikitskiy Botanical Garden (*freshly connected raw material)*	2.37	–	thymol (48.0) carvacrol (19.0) camphene (11.2) cyneol (7.0) terpeniol (2.6)	[12, 15]
2	the same *(air-dry raw material)*	1.67	41	*p*-cymene (28.1) thymol (21.8) thymoquinone (11.7) thymohydroquinone (6.7) carvacrol (5.23) γ-terpinene (5.10)	[16]
3	Ukraine *(air-dry raw material)*	–	38	thymol (42.0) *n*-cymene (15.4) 1,8-cineol	[17]
4	Krasnodar Territory	0.6–0.9	34	*n*-cymene (32.5) carvacrol (23.9) thymol (12.6) methyl ether of carvacrol (5.5)	[18]
5	Leningrad Region	–	more than 30	carvacrol (55.0) γ-terpinene (24.8) *n*-cymene (4.4) thymol (0)	[19]
6	Samara Region	1.67	–	carvacrol (46.3) β-cymene (30.9) thymol (1.2)	[20]
7	the same	2.84	–	thymol carvacrokl	[21]
8	Novosibirsk Region (*collected on July 20*)	3.40	около 40	thymol (56.3) linalool (20.6) γ-terpinene (6.7) carvacrol (5.4)	[22]
9	the same (*collected on July 29*)	4.16	about 40	carvacrol (50.7) thymol (14.3) γ-terpinene (12.2) *n*-cymene (4.7)	[22]
10	The same, Akademgorodok (*collection of CSBG*)	3.68	about 40	carvacrol (33.7) thymol (22.7) γ-terpinene (18.6) *n*-cymene (5.3)	[22]
		Monarda didyma			
11	Leningrad Region	–	more than 30	carvacrol (57.2) γ-terpinene (22.5) *n*-cymene (4.8) thymol (0.4)	[19]
12	Novosibirsk Region	2.16	about 40	thymol (65.9) carvacrol (10.7) γ-terpinene (6.3) 1-octene-3-ol (3.5) *n*-cymene (2.8)	[22]

Sample No.	Growing place (*characterisation of raw material)*	Essential oil			Ref.
		Yield, % of the mass of abs. dry raw material	Number of components	Major components (%)	
13	the same (*Makhogeni variety*)	2.38	около 40	carvacrol (54.4) γ-terpinene (16.5) metyl ether of carvacrol (7.3) *n*-cymene (3.8) thymol (3.4)	[22]
14	Omsk Region	1.26	more than 50	thymol (64.9) *n*-cymene (9.5) methyl ether of thymol (7.6) γ-terpinene (3.6) carvacrol (1.2)	[23]
		Monarda citriodora			
15	Leningrad Region	–	more than 30	thymol (62.4) γ-terpinene (9.8) *n*-cymene (5.0) carvacrol (3.4)	[19]
16	India	–	–	thymol (82.3) carvacrol (4.8) terpinen-4-ol (2.8) *n*-cymene (1.5)	[24]
17	Switzerland	1.46	30	thymol (44.6) 1,8-cineol (23.6) α-phellandrene (4.8) β-cymene (4.0)	[25]
18	Great Britain *(flowers, leaves)*	–	–	*flowers* thymol (61.8) γ-terpinene (13.3) *n*-cymene (4.2) *leaves* thymol (50.7)	[26]

Note: 1. Dash means that the data are absent. 2. CSBG is the Central Siberian Botanical Garden SB RAS.

against diseases and enriched in one component or another. For example, as a result of a series of open cycles of pollination and selection, five hybrids of *M. fistulosa* L. sp. *menthaefolia* with *M. didyma* were obtained. The oil of one of these hybrids contained mainly geraniol (92 %), the second hybrid contained mainly carvacrol (74 %), the third – linalool (67 %), the fourth – thymol (31 %), the fifth – 1,8-cineol (22 %), with the oil yield equal to 1.17, 1.08, 1.00, 0.62 and 0.68 g per 100 g of fresh plant mass, respectively [28].

The most popular monarda species are those with the odour of citrus and thyme: *M. fistulosa*, *M. didyma* and *M. citriodora*. Results on the content of essential oil in the plants of *M. fistulosa*, *M. didyma* and *M. citriodora* from different regions and the major components of essential oil are presented in Table 1.

Monarda fistulosa

M. fistulosa is one of the best studied species which has many varieties and clones. It is cultivated in the USA, Canada, European countries, and also in the Crimean Peninsula, in the Caucasus and in other regions of Russia. The components prevailing in the oil of phenol chemotypes of *M. fistulosa* are thymol and carvacrol, compounds differing from each other by the position of hydroxyl group in the benzene ring and readily transformed into each other (Fig. 1, *a*, *b*). Their ratio may vary depending on the stage of plant development and weather factors. In separate cases, *p*-cymene or γ-terpinene may prevail in the composition of essential oil [29].

Thymol content is 2.5 times higher than carvacrol content in freshly collected plants (1) growing under the conditions of the Crimean Peninsula (Nikitskiy Botanical Garden) (see Table 1) [15]. In air-dry raw material (2), the quantitative ratio of the components changes: *p*-cymene becomes the major component, the amount of thymol decreases by a factor of 2.2, carvacrol – by a factor of 3.6. The yield of oil from dry raw material decreases by 0.7 % [16]. It was stressed [23] that after drying the monarda raw material, the qualitative com-

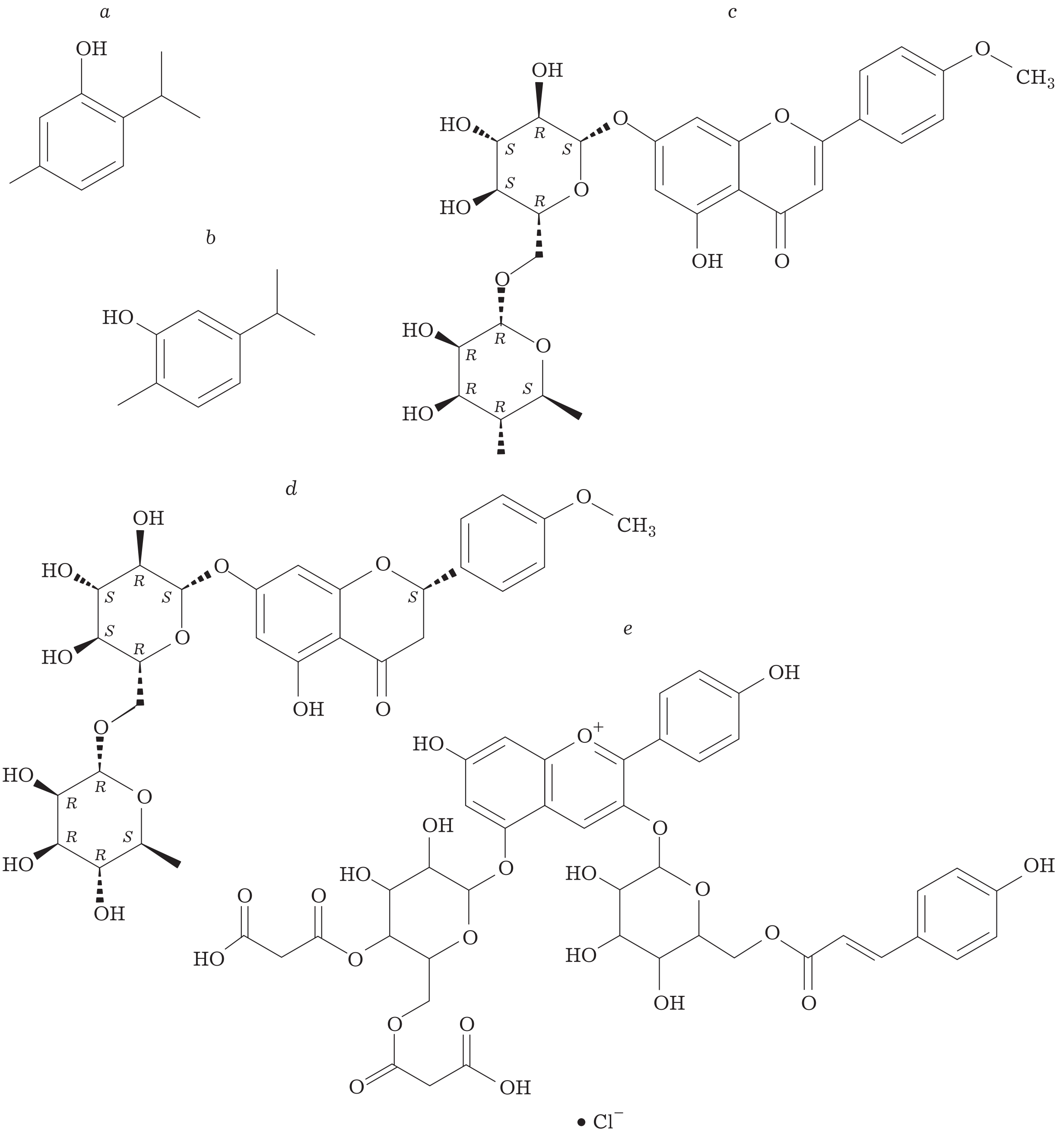

Fig. 1. Structural formulas of the major biologically active substances in the plants of *Monarda* L. family: thymol (*a*), carvacrol (*b*), linarin (*c*), didymin (*d*), monardein (*e*).

position of essential oil does not change but the content of its components varies. The air-dry raw material (3) of *M. fistulosa* cultivated under the conditions of Ukraine was determined to contain 38 volatile components, mainly aromatic terpenoids, and the major component was thymol, its content was 2.7 times higher than that of *p*-cymene [17].

It was stressed in the work by V. A. Zamureenko with co-authors [18] that the major components of the essential oil of monarda growing in the Krasnodar Territory (4) are *p*-cymene, carvacrol and thymol at a ratio of 2.5 : 1.9 : 1.0, respectively. Relying on the low yield of oil (0.6–0.9 %), one may assume that a sample of air-dry raw material was studied (these data are not reported in the work). The plants of *M. fistulosa* grown in the Leningrad Region (5) from the seeds obtained from the Krasnodar Territory are characterized by another components ratio. In this case, the first place is occupied by carvacrol; the content of γ-terpinene and *p*-cymene is lower by a factor of 2.2 and 12.5, respectively. The presence of thymol was not established. The conditions of the northern region are likely to have such a strong effect on oil composition [19]. Carvacrol is the major component also in the sample from the Samara Region (6); the content of β-cymene in it is 1.5 times lower, and thymol – almost 40 times lower [20]. However, thymol and carvacrol were mentioned as the major components of plants from the Saratov Region [21]; oil yield was relatively high – 2.84 %.

A rich qualitative composition and very high content of essential oil (up to 4.16 %) were detected in the freshly cut *M. fistulosa* plants cultivated in the Central Siberian Botanical Garden SB RAS (CSBG SB RAS, Novosibirsk) (9) [22]. The authors concluded that the amount of essential oil formed in monarda under the conditions of sharply continental climate in Siberia is much higher (by 40–70 %) than in the plants growing in the southern coast of Crimea and in the Northern Caucasus. It was demonstrated that the content and composition of essential oil in monarda depend on dates of cutting. Samples (8) and (9) are blossoming *M. fistulosa* plants of the same introduced population, and the shoots were collected within a 9 days interval. The amount of essential oil in the plants cut later is higher by 22.4 %. The amount of thymol decreases by a factor of 4, the amount of carvacrol increases almost by a factor of 10, that is, thymol gives place to carvacrol as the major component. It is important to stress that sample (8) contains a valuable component linalool at a level of 20.6 %; this is higher by 100 % than the values reported in the literature for the essential oil of plants grown in the southern regions of Russia. According to the data obtained by G. Heinrich [30], 18 components (mainly hydrocarbons) were detected in the oil of the young leaves of *M. fistulosa*. The qualitative composition of the components was unchanged in ageing leaves, but thymol content increased to 68 %, and the fraction of volatile components decreased.

Under the conditions of Crimea, the accumulation of essential oil in *M. fistulosa* plants proceeds mainly in blossom clusters (up to 0.85–3.13 %) and in leaves (up to 1.23–3.51 %) during the phases of early flowering and mass flowering. Stalks do not contain essential-oil glands, oil content in them is usually very low [31]. In *M. fistulosa* grown in the Moscow Region, the maximal content of essential oil both in blossom clusters and in leaves was detected in the phase of mass flowering. By the end of vegetation, the lower leaves start to die off, and the content of essential oil in the remaining leaves decreases. The fraction of leaves in the raw material decreases, as a consequence, the content of essential oil in the raw material decreases, too. The author believes that the optimal phase for cutting off the raw material is the start of mass flowering, while for thick and oil plantations it is the stage of early flowering [9]. The shift of time of raw material gathering is also explained by the affection of monarda by *Golovinomyces biocellatus* (Ehreb.) Gel. Plant disease causes changes in the content, composition and mass fraction of the components of essential oil [32, 33].

G. Heinrich described a connection between the fine structure of glands in *M. fistulosa* with the biosynthesis of individual components of oil [34, 35]. Essential oil with high thymol content occurs mainly in peltate glandular trichomes of leaves. Non-specialized cells also produce essential oil but to a much lower extent [36]. The composition of essential oil of the glands of seed-lobes, leaves, shoots is approximately the same. The synthesis of oil components was observed in rather young plants. The component composition of 10 days old seedlings was already similar to the composition of mature leaves [37]. Vapour-distillable substances arising before and after enzymatic and acid hydrolysis of the extracts and tissues from the leaves and roots of *M. fistulosa* were analysed. Thymol and borneol, the major components of oil from leaves and stalks, did not occur in the form of glycosides, but 1-octene-3-ol bound with glycose was present [38].

In France, M. *fistulosa* L. var. *menthaefolia* is widespread. This variety has been recognised as a valuable source of geraniol. Geraniol is the major component of essential oil in this variety, in some clonal versions its content may reach 90 % of the total amount. The highest yield of oil was detected in blossom clusters and in leaves, and the highest content of geraniol was detected in the essential oil of petals and stalks. An optimal time for collecting raw material is the period of mass flowering. Mother plants of M. *fistulosa* L. var. *menthaefolia* from different climatic and geographical conditions of Normandy and Corsica were successfully reproduced *in vitro*.

The composition of essential oil from the plants grown in geographically remote territories turned out to be similar [39, 40]. It was established by means of gas-liquid chromatography that the major components of oil, in addition to geraniol, were linalool, neral and γ-terpinene. The yield of oil was affected by the extraction method and the state of the plant material: it turned out to be higher from fresh and slightly withered plants in comparison with the dry plants [41]. The essential oil of the plants grown in Quebec (Canada) was composed of geraniol by 95–98 % independently of the methods of obtaining [42].

Monarda didyma

Along with *M. fistulosa*, *M. didyma* is widely used as an essential oil plant. This species is grown in Russia on the southern coast of Crimea, in the north Caucasus, in the Moscow, Novosibirsk, Omsk and Leningrad Regions. Substantial discrepancies were revealed in the content of essential oil and its components in the samples of *M. didyma* originating from different sites [27]. The major components of essential oil from *M. didyma* (similarly to oil from *M. fistulosa*) are thymol and carvacrol (see Fig. 1, *a*, *b*). The ratio of thymol to carvacrol varies depending on the growing site and environmental conditions. For example, in the plants grown in the Leningrad Region (11) carvacrol content reaches 57.2 %, while thymol content is not high: 0.4 %. In West Siberia, in the Novosibirsk (12) and Omsk (14) Regions, vice versa, the major component is thymol – 65.9 and 64.9 %, while carvacrol content is 10.7 and 1.2 %, respectively. The samples of *M. fistulosa* often contain *p*-cymene along with thymol and carvacrol, while in *M. didyma* the compound accompanying the latter two ones is γ-terpinene. Its content also varies substantially: in the samples from the Leningrad Region the content of γ-terpinene is 22.5 %, in the Novosibirsk and Omsk Regions its content is lower by a factor of 3.5 and 6.0, respectively [19, 22, 23]. In the essential oil of *M. didyma* from the Samara Region, 11 components were revealed. Two of them, namely carvacrol and thymol, provide the manifestation of pharmacological effect [21]. The authors of [27] believe that the activity of enzymes participating in the biosynthesis of terpenoids is under the control of hormonal balance and varies through ontogenesis. Comparative analysis of the essential oil from *M. didyma* plants aged the second and the third year revealed changes in the content of major components: an increase in the amount of thymol and *p*-cymene, a decrease in the amount of carvacrol and γ-terpinene. The component composition of oil from separate organs of plants is also different: thymol content in leaves and stalks is higher than in blossom clusters, while the latter contain a higher amount of carvacrol and terpinen-4-ol [23]. The major component of *M. fistulosa* grown in France is linalool [43].

Monarda citriodora

M. citriodora is also one of the promising natural sources of essential oil, which is used for medical purposes, to aromatise food and perfumes. The major components of essential oil from the plants grown in India (16) are thymol (82.3 %), carvacrol (4.8 %), terpinen-4-ol (2.8 %), *p*-cymene (1.5 %) [24]. The essential oil of *M. citriodora* from Switzerland (17) was determined to contain 30 components, among which 26 were identified; these are mainly mono- and sesquiterpenes. The major components are thymol (44.6 %), 1,8-cyneol (23.6 %), α-phellandrene (4.8 %) and β-cymene (4.0 %). The oil yield is 1.46 % [25]. Thymol is also the dominating component of essential oil in *M. citriodora* var. *citriodora* plants grown in the Leningrad Region (15) and in Great Britain (18). Thymol content in the flowers of plants from Great Britain is 61.8 %, and in the leaves it is 50.7 %. The content of *p*-cymene and γ-terpinene in the flowers and leaves is not equal: flowers contain a smaller amount of the former (4.2 %) but larger amount of the latter (13.3 %) [26].

Monarda punctata

Above-considered species *M. fistulosa*, *M. didyma* and *M. citriodora* were most ethoroughly studied from the viewpoint of content and qualitative composition of essential oil., *Monarda punctata* is studied insufficiently. By means of gas chromatography, R. Scora [44] discovered the following components in the oil of *M. punctata* var. *maritima*: thymol and carvacrol 20.2 % (a sum), γ-terpenes 18.8 %, α-pinene 5.4 %, cineol 5.5 %, heptanol 4.8 %, β-pinene 3.3 %, *D*-limonene 1.9 %, *etc*. The author concluded that oil from different species of *Monarda* genus contains the same components, and their amounts vary. It was demonstrated that the enzymatic systems of *M. punctata* are very active, and their components are readily transformed into each other. The high level of biological activity was detected for camphene, linalool, neral, isomenthone and bornyl acetate, the low level was detected for α-terpineol, α-pinene, cineol and sabinene. The level of activity of the components determines their positions on the route of biosynthesis [45].

FLAVONOIDS, PHENOLIC ACIDS AND OTHER NON-VOLATILE COMPOUNDS

The phenolic complex of the plants of *Monarda* genus was studied not so thoroughly as an essential oil. Phenolic compounds include flavonoids, anthocyanins and phenolic acids. The presence of vitamins, allyl mustard oils, resinol was also detected in the aerial organs of monarda [5, 8, 17].

The studies of methanol extracts from the aerial parts of blossoming *M. fistulosa*, *M. fistulosa* var. *rubra*, *M. didyma* var. *rosea*, *M. didyma* var. *Cambridge Scarlet*, *M. citriodora*, *Monarda pectinata* and *Monarda clinopodia* showed a similarity in the composition of their flavonoid compounds. Six flavonoid glycosides were extracted from *M. pectinata*, in particular linarine (acacetin 7-rutinoside) (see Fig. 1, *c*), didymin (isosacuranetin 7-rutinoside) (see Fig. 1, *d*) and luteolin 7-glucoside [46]. Didymin was extracted also from a 90 % ethanol extract of *M. didyma* [47]. The isolation of linarin and didymin and determination of their structure was reported by German scientists L. Hörhammer, H. Wagner et al. [48–50] as long ago as in the 60-es of the past century. Chromatographic methods were used to examine *M. fistulosa*, *M. didyma*, *M. hybrida*, *M. citriodora* and *M. russeliana* introduced in the Republic of Bashkortostan; the list of detected compounds included luteolin, naringenin, luteolin 7-glucoside, rutin, hyperoside, catechine, gallic and chlorogenic acids. Luteolin content in these species was found to be 1.57, 1.63, 1.52, 1.61 and 0.91 %, the content of tannins was 9.70, 12.11, 9.90, 6.72 and 7.29 %, respectively [51, 52]. Not less than 8 flavonoid glycosides with the total content 3.2 % were detected by us in the leaves of *M. fistulosa* [53]. Flavon glucuronide keshonin was identified in the aerial parts of *M. punctata* [54].

Bright coloured flowers of monarda contain anthocyanins. Five anthocyanin pigments were detected in the fresh petals of *M. fistulosa* with the help of high-performance liquid chromatography. The amount of anthocyanins was 214.8 mg per 100 g of fresh plant mass. The major anthocyanin was 3,5-diglucoside of pelargonidine acylated with cumaric and malonic acids. It accounts for 81 % of the total anthocyanin content and 17 % of the total amount of flavonoids. The compounds detected among flavonoids are: flavone, apigenin 7-O-glucoside, 5-hydroxyflavone and 8-C-glucoside of dihydroxyflavone [55]. It is likely that monardein is specific for the species of *Monarda* genus. The structure of this compound was established as 3-O-(6-O-*trans*-*p*-coumaroyl-β-*D*-glucopyranosyl)-5-O-(4,6-di-O-malonyl-β-*D*-glucopyranosyl) pelargonidin (see Fig. 1, *e*) [56].

Six monoterpene glycosides were extracted from the aerial parts of *M. punctata* plants and identified using spectroscopic methods [54]; β-sitosterol was detected in *M. citriodora* [57]. In the aerial parts of three varieties of *M. punctata*, *n*-alkanes were discovered: 9 compounds in *M. punctata* var *maritima*, 7 in *M. punctata* var *fruticulosa*, 5 in *M. fistulosa* var *mollois*. The amount of odd alkanes exceeded the number of even ones in all three axons. The composition varied from $C_{27}H_{56}$ to $C_{35}H_{72}$ [58]. The composition of fatty acids and triglycerides in the oil from the seeds of seven monarda species was investigated. The acids that were detected in relatively large amounts included palmitic, stearic, oleic, linoleic and linolenic; among triglycerides, the detected compounds are trilinolenine, linoleodilinolenin, dilinoleolinolenin, oleodilinolenin, palmitodilinolenin, trilinolein, oleolinoleolinoolenin, *etc*. [59, 60]. Monardic acids A and B were extracted from the aerial organs of *M. fistulosa*. These compounds are diastereomers of lithospermic acid A and lithospermic acid B [61].

The content of inorganic chemical elements was determined by means of X-ray fluorescence analysis using the synchrotron radiation. The elements that were discovered in the leaves and blossom clusters of *M. fistulosa* include (mg/kg of dry mass, respectively): K (6207, 10917), Ca (33208, 13503), Mn (66.19, 50.86), Fe (454.87, 194.30), Cu (6.33, 17.74), Zn (17.11, 46.01), Br (11.22, 2.07), Rb (18.96, 79.03), Sr (113.56, 53.93), Zr (2.14, 0.45), Mo (4.23, 2.08), W (2.76, 3.63), Pb (2.01, 2.45), Th (0.53, 0.62). Some elements are present only in the leaves: Y (0.65) and Bi (0.53), while others are present only in blossom clusters: Co (4.38), Ni (7.28), Ga (0.20), Hg (1.18) [53]. Selenium content was 169.0 μg/kg of dry mass [62].

BIOLOGICAL ACTIVITY OF THE PLANTS OF *MONARDA* GENUS

Essential oil and extracts of monarda exhibit high antiviral, antifungal, antimycoplasmic and antihelminthic activity, immunomodulatory action, antioxidant, radioprotective, antisclerotic, desensibilizing, anticancer, antiinflammatory and analgesic effect. They recover disturbed oxidation-reduction processes in the organism, cause sedative action on the central nervous system, stimulate regeneration of damaged skin, bring the increased parameters of lipid exchange down to the normal level [7, 29]. The presence of flavonoids is believed to be the reason for the efficiency against various pathogens (bacteria, fungi, protozoa, *etc.*) [5].

Bactericidal and antiviral action

The essential oil of monarda is distinguished by bactericidal and antibiotic activity, as a consequence of the high phenolic content (67–89 %). The studies showed that terpene compounds of phenolic nature possess a broad range of antimicrobial action against different microorganisms. The formation of the resistance of bacteria to the essential oil of monarda proceeds much slower than to antibiotics; some staphylococcal strains do not develop stability to monarda oil [11, 63]. Oil from the blossom clusters of *M. citriodora*, containing mostly monoterpenes and sesquiterpenes, caused higher antibacterial activity than penicillin against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus albus* [25]. Antibacterial activity of the oil of *M. punctata*, in which thymol (75.2 %), *p*-cymene (6.7 %), limonene (5.4 %) and carvacrol (3.5 %) (oxygenated monoterpenes) are prevailing compounds, was evaluated with respect to the pathogens of frequent respiratory infections – *Streptococcus pyogenes*, *E. coli*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Haemophilus influenzae*. The first three strains turned out to be the most susceptible to this action [64]. Tests for antimicrobial activity of essential oil from *M. didyma* against nine bacterial strains showed that gram-positive bacteria are more sensitive in comparison with gram-negative ones; in general, the oil possesses inhibiting action against all test cultures and is especially efficient against *Bacillus cereus*. It is assumed that the high content of a strong antimicrobial agent thymol in the oil of *M. didyma* is the major factor of its pronounced antimicrobial activity [65, 66]. Antibiotic effects of oil samples were tested with *E. coli*, which is most stable against essential oil. A strong action was exhibited by oil from *Monarda ramoleyi*, *M. citriodor*a and *Monarda violacea*; tripanosomes in contact with essential oil lost their mobility and died soon [67]. According to the data obtained by B. V. Bogutskiy with co-authors [68], the addition of essential oil from *M. fistulosa* to the bacterial suspensions of *S. aureus* and *E. coli* caused cell lysis and a substantial decrease in the optical density of the culture.

Antibiotic activity is exhibited not only by essential oil from monarda species but also by the extracts from the aerial parts of the plants. Oil extract from the raw *M. fistulosa* (collected in July) demonstrates antimicrobial activity against both gram-negative and gram-positive microorganisms. In the concentration of 30 mg/mL, it causes complete suppression of the growth of *Enterobacter cloacae*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *S. aureus*, *Streptococcus faecalis*. In the concentration of 50 mg/mL, complete suppression of all tested pathogenic bacteria was detected, in addition to the listed species, also *E. coli*, *Citrobacter freundii*, *Pseudomonas aeruginosa* were suppressed [69]. The possibility to use the oil extract of monarda as an antimicrobial agent is patented in the RF [70]. Water-ethanol extracts from *M. fistulosa* possess bactericidal action against gram-positive bacteria *S. aureus*, *Enterococcus faecium* and *B. subtilis*; the effect against the first two species is extremely strong. Water extract exhibits lower activity and only against one test strain: *S. aureus* [71].

Water-ethanol and water extracts of the aerial parts of *M. fistulosa* plants exhibit high antiviral activity with respect to the influenza virus of A/Aichi/2/68 (H3N2) (human) and A/chicken/Kurgan/05/2005 (H5N1) (bird) subtypes [72].

Antimycotic action

Antimycotic activity of the species of *Monarda* genus is investigated in connection with the possible application in plant cultivation. Search for alternatives to synthetic fungicides is an extremely important problem connected with the safety of food products and environmental protection. It was established that essential oil from the aerial parts of *M. didyma*, *M. didyma* var. 80-1A and *M. fistulosa* are promising means against fungal pathogens causing grey mold of strawberry – *Botrytis cinerea*, and tomatoes – *Rhizoctonia solani* [73–76]. Japanese scientists [77] studied essential oil of monarda and aerial parts in combination with other herbs as part of hydrosols and herbal preparations against *Candida albicans*, *Aspergillus fumigatus* and *Trichophyton mentagrophytes*. The tested herb compositions demonstrated suppression of *C. albicans* in different extents, however, monarda exhibited the high degree of inhibition of both the thread and yeast-like forms of candida. In experiments with volatile emissions of 11 plant species, essential oil of monarda proved to be the most efficient agent suppressing *T. mentagrophytes* and (to a lower extent) *A. fumigatus*. This effect is explained by the presence of large amounts of thymoquinone [78]. Some plants exhibit the high activity of their volatile emissions, but the major part demonstrates activity in the contact action. Antifungal activity of *M. citriodora* var. *citriodora* turned out to be high in both cases in the tests with 15 pathogens [79–81].

A mathematical model was developed for the first time to describe the effect of essential oil of monarda and its component cymene on the germination and growth of the spores of *Beauveria bassiana*, entomopathogenic fungus able to colonise a large number of plant species. It was established that it is convenient to use spore germination as a parameter in monitoring the effect of essential oils on the growth of *B. bassiana* because this period is the weakest spot in the vital cycle of the fungus [66]. The extracts of monarda also possess clearly pronounced fungicidal properties. For instance, the activity of oily, aqueous and water-alcohol extracts of *M. fistulosa* against the yeast-like fungus *C. albicans* was found to be very high [53, 69, 71, 82].

Antioxidant activity

Biologically active substances from monarda possess antioxidant and antiradical properties. The studies carried out by A. G. Shutova [83, 84] confirmed that the highest antiradical activity is exhibited by phenolic compounds – thymol and its isomer carvacrol. The essential oil from the aerial parts of *M. fistulosa* containing thymol and carvacrol as the dominating components has the strongest antioxidant effect. In this connection, broad possibilities to use the essential oil of monarda as a natural source of antioxidants for the pharmaceutical industry are opened [75, 85, 86]. According to the data of the authors of [87], the aerial parts of *M. fistulosa* contain phenolic antioxidants in the amount of up to 5.52 mg/g. This may be a good basis for the development of innovative functional food products and the products for curative and prophylactic purposes. According to other data [88, 89], during the mass blossoming of *M. fistulosa* plants, the total content of water-soluble antioxidants reaches 16.1–17.3 μg-equiv. of gallic acid per 1 g of the raw mass, which is 10 times higher than the values for thyme and lavender. The essential oil of *M. citriodora* var. *citriodora* was screened for antioxidant properties in a matrix enriched with lipids. It was concluded that this oil is highly effective both in antioxidant and in antibacterial test systems during important life periods – fetus, neonate, and aging [90, 91].

Cytotoxic action

Flavonoid fractions isolated from plants have weak antomycotic and clear antomitotic activity [46]. Few data concerning the antitumour action of essential oil from monarda species and their components give some hope for the extensification of these studies. In the tests of biologically active monoterpenes from *M. fistulosa* against 60 lines of human tumour cells, thymol and thymoquinone exhibited selective cytotoxicity against definite cell lines (SF-539 (CNS), PC-3, M-14, OVCAR-5, MCF-7) [92]. Scientists from India showed that essential oil from *M. citriodora* and its major component thymol inhibit the proliferation of cancer cells of HL-60, MCF-7, PC-3, A-549 and MDAMB-231 lines. The essential oil was twice as cytotoxic as thymol in the cells with promyelocytary leucemia HL-60. In comparison with thymol the degree of apoptosis induction and violation of the signalling cascade turned out to be substantially higher [93]. It was demonstrated that carvacrol, similarly to its isomer thymol, possesses antioxidant and antitumour (cytotoxic) properties [94].

PHARMACOLOGICAL STUDIES

Pharmacological studies of the species of *Monarda* genus are few in number. The available data refer to the tests of essential oil and its components. For instance, V. V. Nikolaevskiy with coworkers [95] revealed that essential oil of monarda causes a decrease in the amount of cholesterol in the aorta and in the size of atherosclerotic plaques, thus exhibiting angioprotective action. Fractions of essential oil introduced into the atmosphere normalize some enzymatic reactions in the blood and liver of rats [96]. The essential oil of *M. didyma* and its components exhibit high allelopathic activity [97]. The possibility to stimulate or suppress the secondary antibody immune response, depending on the scheme of essential oil administration, was established in experiments with mice [98]. Acetone extract from the aerial parts of *M. punctata* plants exhibited the inhibiting action on lipase activity in isolated murine plasma *in vitro*; the active component of essential oil of this species is carvacrol [54].

PRACTICAL SIGNIFICANCE AND OUTLOOKS FOR THE USE OF THE SPECIES OF *MONARDA* GENUS

The species of *Monarda* genus are known long ago as a source of thymol possessing strong antibacterial and fungistatic activity. The essential oil of monarda was manufactured mainly to isolate this monoterpenoid, which was of great value for medicine and thus it was exported into India and

European countries. Cultivation of monarda for obtaining thymol to be exported to India and European countries was reported as long ago as in 1916 [99, 100]. To extract thymol, it is necessary to select thymol-containing varieties and clones with the high content of this compound in essential oil. It was stressed in the review by S. V. Fedotov [29] dealing with the chemotypes of essential oil of monarda that the production of individual compounds from the essential oil should proceed under strict control because "the component composition of commercial essential oil often differs substantially from those studied and described for their biological activity". The author states that thymol, carvacrol, geraniol and other chemotypes of natural essential oil are substantially different from each other in their properties and biological activity, so they cannot be used in the same manner. This limitation relates to all aspects of the use of monarda species in connection with its biological activity. Due to the undesirable presence of one component or another, it is necessary to possess complete information on the composition of the oil sample to be used. Separation of essential oil into fractions is used in the perfumery industry to improve the odour of products, some components are removed, others are added, for example, linalool which renders a pleasant floral odour, limonene – the citrus odour, borneol and camphene – camphor odour. The approach of this kind is necessary also for the use of essential oil in medicine. Oil with high thymol content possesses a strong bactericidal action, high content of carvacrol provides mycostatic activity against various strains of *Candida* [101]. Geraniol renders antihelminthic and toxic action to the essential oil [102, 103].

In 1996, the International Association of Medicinal Herbs announced *Monarda* to be the medicinal herb of the year [104]. The essential oil of monarda may be used for the prophylactics of bronchitis and acute respiratory diseases, for the correction of secondary immunodeficiency (mainly for the T system), for enhancement of stability against various infectious diseases [105]. Due to a broad pharmacological range of action, the aerial parts of all monarda species are used along with the essential oil for inhalations to treat infectious diseases of the upper airways and lungs (mycoplasmal pneumonia, chronic bronchitis, tuberculosis, bronchiectatic disease, influenza, acute respiratory disease). Water-alcohol or water extracts are applied in the form of rinsing to treat inflammations of mucous membranes of oral cavity and nasopharynx (angine, sinusitis, stomatitis, gingivitis, highmoritis, rhinitis), against atherosclerosis, oncological risk, anemia, distress, hypoxia, low radiation exposure, *etc*. [7, 8]. Thymol is a tracheal relaxant causing relaxation of the trachea, it is used in stomatology as analgesic means for dentin anesthesia, as a component of antiseptic liquids for rinsing and treating ulceration in the oral cavity, it possesses antiseptic, bactericidal, disinfecting, deodorising, antitumour (cytotoxic), spasmolytic, fungicidal activity [106–108]. Carvacrol is close in its biological activity to thymol, however, it is not so widely used in medicine [29]. Carvacrol is used to suppress the development of various strains of *Candida* genus [109].

It is recommended to apply the essential oil of monarda to treat pneumonia, chronic bronchitis, tuberculosis, loss of immunity, ageing, atherosclerosis, hypoxia, stress, anemia, candidosis, gingivitis, periodontitis, stomatitis, low radiation exposure, and also for the prophylactics of acute respiratory diseases and influenza, for optimization of adaptation to new climatic conditions [15, 104, 110]. Investigations aimed at the development of new medicinal agents based on *M. fistulosa* are carried out for medicinal and prophylactic applications in elderly patients [21, 111]. The essential oil of *M. fistulosa* is proposed as the means to treat seborrhea; it inhibits the growth of microorganisms, and its antiinflammatory effect is stronger than that of hydrocortison in combination with vitamin B [112]. Experiments on the use of phytoextracts from *M. fistulosa* and other species for sanitation of medical rooms (dentist's) showed a decrease in the level of microbial contamination, with a 2–3 times decrease in microbial number as average. Air saturation with the volatile molecules of essential oil causes a positive effect on the emotional sphere of medical personnel and patients, invigorative, antidepressant, adaptogenic action [113, 114]. It is recommended to plant monarda species indoors, as well as in the parks and gardens of sanatoria for therapeutic (aerostimulating) effect, which is due to the emission of volatile compounds [115, 116].

The essential oil of monarda is used in perfumery and cosmetic industry, in the food industry to aromatise vermouth, to provide biological stabilization of table dry, semi-dry and semi-sweet wine, and as an antiseptic component in nonalcoholic drinks [7, 8, 117]. Monarda is used as a natural aromatising agent, conservant and antioxidant, as a natural substituent of synthetic food additives. For example, essential oil of *M. didyma* exhibited high antioxidant activity in the dose of 0.2 %, exceeding the activity of synthetic preparation BHT (butylhydroxytoluene). Bacteriostatic and bactericidal properties of carvacrol against a number of bacteria, for example *E. coli, B. cereus* and others, in combination with a rather pleasant odour and specific poignant taste, allowed recommending it as a food additive with conserving effect [107, 109]. The aerial parts collected during an interval after budding but before flowering are used for the preservation of vegetables and for the production of marinades as an alternative for black pepper [118, 119]. Fragrant leaves of monarda may be used almost all year round because it starts to grow very early in spring and remains green in November under frost of −5... −7 °C; monarda is a source of vitamins C, B_1, B_2 [89].

In connection with the use of the components of essential oil in the food industry as substituents of synthetic food additives, evaluation of their prooxidant and toxic properties was carried out; the studies of cytotoxic, genotoxic and DNA-protective effects were carried out with a long-term (24 h) incubation of the cells of mammals with two major components of essential oil of monarda (carvacrol and thymol) *in vitro*. Carvacrol was determined to be somewhat more cytotoxic than thymol, and the cells of Caco-2 were more stable to carvacrol and thymol than HepG2 and V79 cells [120].

Biochemical evaluation of different species and varieties of monarda was carried out during 1990–1991 at the All-Union Research Institute of Vegetable Breeding and Seed Production (VNIISSOK) in the Moscow Region and demonstrated that this plant is promising to be introduced as a new spice plant. The researcher collected and studied more than 43 samples belonging to 12 species of the genus. In the opinion of the researchers, it is an important task to make new home varieties of monarda with the high content of biologically active substances possessing curative and prophylactic effects on human organism [89, 121]. In 1997, the first vegetable variety of *M. fistulosa* was obtained and recorded into the State Registry; this was Viktyulia variety [122].

As a result of the introduction under different climatic and soil conditions of Russia, some species of *Monarda* genus were recognized as promising for industrial planting [4, 9, 14, 19, 22, 123]. *M. fistulosa* and *M. didyma* may be grown as perennial cultures in Siberia. Other species freeze out in winter, so they are sown every year [53]. An RF Patent was obtained at the CSBG SB RAS on the method of growing *Monarda fistulosa* in West Siberia (the Novosibirsk Region) [124].

The published information provides evidence that attention to the species of the *Monarda* genus increases with time. Different versions of the practical application of monarda are under development on the basis of biocide properties of these plants: in agriculture, this direction includes inhibition of seed germination and toxic effect on the seedlings of weeds [125], and the application against plasmodia [126], larvae of tortoise bugs [127], Mexican bean billbugs [128], as a domestic application – treatment of footwear against fungi [129], use as repellents against mosquitoes [130] and so on. Numerous results of the studies proved the economical value and practical significance of monarda species. To broaden the home market of raw materials, it is reasonable to grow monarda species in culture, which is possible in various regions of Russia.

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