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Flavonoids and Lignans of *Nepeta multifida* (Lamiaceae) Leaves and Their Biological Activity

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Abstract—A perennial plant *Nepeta multifida* L. (syn. *Schizonepeta multifida* (L.) Briq.) is one of the most common species of the Lamiaceae family growing in Eastern Siberia and used in traditional oriental medicine. The chemical composition of *N. multifida* has not been sufficiently studied. Chromatographic separation of phenolic compounds of *N. multifida* leaves using column chromatography and preparative HPLC made it possible to isolate 16 compounds, including a new flavonoid identified as luteolin-7-*O*-(3″,6″-di-*O*-acetyl)-β-*D*-glucopyranoside, according to UV, NMR spectroscopy and mass spectrometry. Known compounds were luteolin and apigenin *O*-glycosides, rosmarinic acid, salvianolic acids A and B, and schizotenuin A. Quantitative analysis of *N. multifida* leaves in various phases of plant development by HPLC-UV assay showed the high content of rosmarinic acid (8.36–35.71 mg/g), luteolin-7-*O*-glucuronide (2.03–14.18 mg/g) and schizotenuin A (5.29–9.56 mg/g). The highest level of phenolic compounds was found in the flowering and fruiting phases. Using Ellman's spectrophotometric method, it was found that *N. multifida* leaf extract and some compounds had antiacetylcholinesterase activity and luteolin glycosides being the most active showed the level of concentration of half-maximal enzyme inhibition (IC_{50}) 29.03–58.36 μg/mL. Thus, as a result of the present study, it was found that the leaves of *N. multifida* contain various groups of phenolic compounds capable of inhibiting the activity of acetylcholinesterase.

Keywords: *Nepeta multifida*, *Schizonepeta*, Lamiaceae, flavonoids, luteolin, rosemary acid, HPLC, acetylcholinesterase

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INTRODUCTION

Central nervous system diseases are widespread in the modern world and are among the pathological factors leading to disability of a wide range of the population. For the treatment and prevention of these diseases, both synthetic drugs and phytotherapeutic procedures are used. Among the many herbal remedies, drugs from the Lamiaceae family are particularly effective, which have a positive therapeutic effect in cerebral hypoxia and anoxia [1] and in cerebral ischemia and neuronal damage caused by excitotoxicity [2], and also demonstrate neuroprotective and anticholinesterase effects in Alzheimer's disease [3, 4]. The most commonly used include more than 50 species, among which *Nepeta* species with anticholinesterase, antinociceptive and anticonvulsant effects should be especially distinguished [5].

In Siberia, genus *Nepeta* L. is represented by six species, two of which, *N. multifida* L. and *N. annua* Pall., belong to the Schizonepeta Benth subsection, which separated into a separate genus *Schizonepeta* (Benth.) Briq. [6]. In Eastern Siberia, only *N. multifida* grows, occupying significant territories of steppified slopes and dry meadows [7]. The known literature data on the chemical composition of *N. multifida* relate primarily to essential oil, the main components of which are β-ocimene, 1,8-cineol, limonene, pulegone, phellandrene and menthone [8]. Docosanoic, tetracosanoic, succinic, deoxyoleanolic acids [9] and 3-imino-*N*-(α-iminoethyl-amino) have been identified in the composition of non-volatile compounds of *N. multifida* butyrolactam [10].

The study of phenolic compounds of *N. multifida* flowers from the Baikal region revealed the presence of luteolin and apigenin glycosides, as well as rosmarinic acid and other benzofuran lignans [10, 11]; phenolic compounds of leaves of this species have not been studied before.

In Tibetan medicine, *N. multifida* herb was used for the treatment of skin diseases as an antibacterial and wound healing agent, as well as for gastrointestinal diseases as an appetizing, antitumor and anthelmintic medicine [12]. Dried plants tincture was used for whooping cough and various types of cough. Of particular interest is the use of the *N. multifida* herb for the treatment of "planetary" diseases, the description of which resembles the clinical picture of a stroke. The studies of the biological activity of *N. multifida* extracts are few, but it is known that these extracts have neuroprotective, stress-protective, antioxidant [13] and antihypoxic effects [14]. This paper presents information on the chemical composition and quantitative content of individual compounds in the *N. multifida* leaves, as well as the results of the some phenolic components anticholinesterase activity examination.

EXPERIMENTAL

Plant raw materials. *N. multifida* plants were collected in 2020 in the Republic of Buryatia (Mukhorshibirsky district, 51°02′46.4″ N, 107°46′52.7″E, 830 m in.u.m.) at the beginning of vegetation (June 2–5; 30 samples), vegetation phases (June 28–30, 28 samples), flowering (July 15–18, 45 samples), fruiting (August 10–14, 38 samples) and dying (August 28–31, 22 samples). The sample consisted of leaves collected from one plant. The species is determined by Doctor of Pharmaceutical Sciences, N.K. Chirikova (North-Eastern Federal University, Yakutsk). A sample of plant raw materials is stored in the herbarium of the IGEB SB RAS (no. BU/LAM-0720/36-114). The leaves were separated and dried in a convection cabinet at 45°C to a humidity of 4–5%.

General experimental conditions. For column chromatography (CC) we used polyamide, normal-(SiO_2) and reversed-phase silica gel (RP–SiO_2) and Sephadex LH-20 (Sigma-Aldrich, St. Louis, MO, USA). Spectrophotometric studies were carried out on the SF-2000 spectrophotometer (OKB Spectrum, St. Petersburg, Russia). Mass spectra were recorded on the LCMS-8050 TQ mass spectrometer (Shimadzu, Columbia, MD, USA) [15], NMR spectra were recorded on the VXR 500S spectrometer (Varian, Palo Alto, CA, USA). Preparative HPLC was performed on an LC-20 Prominence liquid chromatograph (Shimadzu) equipped with a Shim-pak PREP-ODS column (20 × 250 mm, d 15 microns) and a SPD-M30A photodiode detector (Shimadzu); v 1.0 mL/min, column temperature 20°C.

Extraction and isolation of compounds 1–16 from *N. multifida* leaves. The crushed raw materials of *N. multifida* (850 g) were extracted with 70% ethanol (1: 12, 70°C, three times; ultrasonic bath), after which the alcohol extract was concentrated dry in vacuum (yield 272 g). The dry extract was treated in a Soxlet apparatus with hexane and chloroform until depleted to remove lipophilic components. The fat-free extract was separated in a two-phase ethyl lipid-water system (1 : 1) and then the aqueous phase was extracted with *n*-butanol. Organic extracts were concentrated (extract yield: ethyl acetate 66 g, *n*-butanol 98 g) and applied to polyamide for CC (1 : 20), which was eluted with water (eluate 1), 50% ethanol (eluate 2) and 0.5% NH_3 in 90% ethanol (eluate 3). Eluates 2 (E2) and 3 (E3) of the ethyl acetate fraction and eluate 2 of the *n*-butanol fraction (B2) were subjected to chromatographic separation. Eluate E2 (9 g) was separated using flash chromatography into SiO_2 (2 × 40 cm, EtOAc-Me_2CO 100 : 0 → 60 : 40), RP–SiO_2 (1 × 30 cm, H_2O–MeCN 95 : 5 → 60 : 40) and Sephadex LH-20 (1 × 60 cm, MeOH–H_2O 80 : 20 → 30 : 70), which made it possible to isolate luteolin-7-*O*-glucoside (19 mg, **2**) [16] and apigenin-7-*O*-glucoside (8 mg, **3**) [16]. To separate the E3 fraction (11 g), flash chromatography was used on SiO_2 (2 × 60 cm, EtOAc–Me_2CO 100 : 0 : 0 → 70 : 30), RP–SiO_2 (1 × 20 cm, H_2O–MeCN 100 : 0 → 20 : 80), Sephadex LH-20 (1 × 60 cm, MeOH–H_2O–AcOH 90 : 5 : 5 → 20 : 75 : 5) and preparative or prep. HPLC (eluent A—MeOH, eluent B—H_2O; gradient mode, % B: 0–30 min, 5–15%, 30–45 min, 15–38%, 45–90 min, 38–58%, 90–120 min, 58–85%). As a result of separation, **1** (33 mg), luteolin-7-*O*-glucu-

Table 1. NMR spectra ^{1}H (500 MHz, DMSO-d_6, 298 K, δ_H, ppm, *J*/Hz) and ^{13}C (125 MHz, DMSO-d_6, 298 K, δ_C, ppm) of compound **1**

C-atom	δ_H*	δ_C*
2	–	165.5
3	6.75 (1H, s)	103.5
4	–	181.9
5	–	161.3
6	6.44 (1H, d, *J* = 2.0 Hz)	99.5
7	–	162.9
8	6.79 (1H, d, *J* = 2.0 Hz)	94.9
9	–	157.3
10	–	105.8
1′	–	121.5
2′	7.46 (1H, d, *J* = 2.1 Hz)	114.0
3′	–	146.2
4′	–	146.9
5′	6.92 (1H, d, *J* = 8.1 Hz)	116.2
6′	7.51 (1H, dd, *J* = 8.1/2.1 Hz)	119.5
1″	5.10 (1H, d, *J* = 8.1 Hz)	102.3
2″	3.69 (1H, dd, *J* = 9.0/8.1 Hz)	72.8
3″	4.98 (1H, dd, *J* = 9.5/9.0 Hz)	78.2
4″	3.40 (1H, dd, *J* = 9.5/9.6 Hz)	69.5
5″	3.72–3.73 (1H, m)	75.6
6″	4.22 (1H, dd, *J* = 12.0/5.1 Hz); 4.59 (1H, d, *J* = 12.0 Hz)	63.9
3″-CH_3CO	2.07 (3H, s)	172.5; 20.5
6″-CH_3CO	1.95 (3H, s)	170.0; 21.4

* Luteolin-7-*O*-β-*D*-glucopyranoside: signals of 7-*O*-β-*D*-glucopyranose – δ_H 5.12 (1H, d, *J* = 8.1 Hz; H-1″), 3.35–3.36 (1H, m; H-2″), 3.40–3.42 (1H, m; H-3″), 3.20–3.21 (1H, m; H-4″), 3.48–3.52 (1H, m; H-5″), 3.57 (1H, dd, *J* = 12.0/5.1 Hz; H-6_B″), 3.79 (1H, d, *J* = 12.0 Hz; H-6_A″); 102.8 (C-1″), 74.5 (C-2″), 77.0 (C-3″), 70.9 (C-4″), 78.0 (C-5″), 60.8 (C-6″).

ronide (7.5 g, **4**) [16], apigenin-7-*O*-glucuronide (10 mg, **5**) [16], luteolin-7-*O*-(6″-*O*-acetyl)-glucoside (1.4 g, **6**) [17], apigenin-7-*O*-(6″-*O*-acetyl)-glucoside (5 mg, **7**) [17], rosmarinic acid (9.1 g, **8**) [18], salvianolic acid A (24 mg, **9**) [18], salvianolic acid B (33 mg, **10**) [18], schizotenuin A (820 mg, **11**) [18] and nepetamultin A (8 mg, **12**) [11]. Eluate B2 was separated in a manner similar to that for E2, which led to the isolation of luteolin-7-*O*-neohesperidoside (7 mg, **13**) [16], luteolin-7-*O*-rutinoside (28 mg, **14**) [16], apigenin-7-*O*-neohesperidoside (6 mg, **15**) [16] and apigenin-7-*O*-rutinoside (5 mg, **16**) [16].

Luteolin-7-*O*-(3″,6″-di-*O*-acetyl)-β-*D*-glucopyranoside (1). $C_{25}H_{24}O_{13}$. UV spectrum (MeON, λ_{max}, nm): 254, 269, 347. HR-ESI-MS, *m*/*z* 531.4273 (Calculation 531.4064 for $C_{25}H_{23}O_{13}$ [*M* –H]$^-$); ESI-MS, *m*/*z* (%): 531 [*M* – H]$^-$; ESI-MS^2 [531]: 489 [(*M* – H)–42]$^-$ (9), 447 [(*M* – H)–42 × 2]$^-$ (12), 285 [(*M* – H)–42 × 2–162]$^-$ (100); NMR spectrum of ^{1}H (500 MHz, DMSO-d_6, 298 K, δ_H, ppm) (Table 1). NMR spectrum ^{13}C (125 MHz, DMSO-d_6, 298 K, δ_C, ppm) (Table 1).

Micro-column HPLC-UV. Quantitative analysis of phenolic compounds was carried out using a micro-column liquid chromatograph Milichrome A-02 (Econova, Novosibirsk, Russia) on a ProntoSIL column-120-5-C18 AQ (2 × 75 mm, Ø5 microns; MetrohmAG, Herisau, Switzerland); mobile phase: 0.2 M $LiClO_4$ in 0.006 M $HClO_4$ (A), MeCN (B). Gradient conditions (%B): 0–15 min 5–100%; in 150 μL/min; column temperature 35°C; UV detector, λ 330 nm. The content of phenolic compounds was calculated reference standards ChemFaces (Wuhan, Hubei, PRC)—luteolin-7-*O*-glucuronide (cat. no. CFN98512, ≥98%), luteolin-7-*O*-rutinoside (cat. no. CFN93556, ≥98%), apigenin-7-*O*-glucu-

ronide (cat. no. CFN98500, ≥98%); and Sigma-Aldrich (St. Louis, MO, USA) —luteolin-7-*O*-glucoside (cat. no. 74284, ≥98%), apigenin-7-*O*-glucoside (cat. no. 44692, ≥97%), rosmarinic acid (cat. no. R4033, ≥98%), salvianolic acid A (cat. no. 97599, ≥95%), salvianolic acid B (cat. no. PHL89783, ≥90%). For the quantitative analysis of some compounds, external comparison substances were used: luteolin-7-*O*-glucoside for luteolin-7-*O*-(6″-*O*-acetyl)-glucoside and luteolin-7-*O*-(3″,6″-di-*O*-acetyl)-β-*D*-glucopyranoside, apigenin-7-*O*-glucoside for apigenin-7-*O*-(6″-*O*-acetyl)-glucoside, rosemary acid for schizotenuin A. To prepare a solution of a standard substance, 5 mg of the compound was dissolved in a measuring flask (5 mL) in 70% acetonitrile and the volume of the solution was brought to the mark with the same solvent. Next, a series of dilutions with a concentration of 5–500 μg/mL was prepared and analyzed by HPLC-UV. The results were used to construct calibration graphs in the coordinates "concentration, μg/mL—chromatographic peak area," which were used for further calculation. The data are presented as an average of three parallel definitions (± standard deviation, S.D.).

Sample preparation of plant samples. To carry out quantitative analysis of phenolic compounds in *N. multifida* leaves, an exact sample of crushed plant raw materials (200 mg) was placed in an extraction container (5 mL) with a screw cap, 2 mL of 70% ethanol was poured and extracted in an ultrasonic bath (100 W, 35 kHz) at 50°C for 20 min. The resulting sample was centrifuged (3000 g, 15 min) and the supernatant was transferred to a measuring flask with a capacity of 5 mL. The extraction was repeated under the same conditions again. The volume of the combined extract was adjusted to the label with 70% ethanol. Before the HPLC procedure, the test solution was filtered through a PTFE filter (0.22 microns) and used for analysis without prior dilution. The extract for the study of biological activity was obtained in a similar way, after which the extractant was removed in vacuum and the dry residue was crushed.

Biological activity. Inhibitory effect of *N. multifida* extract and pure compounds on acetylcholinesterase from *Electrophorus electricus* (Sigma-Aldrich, cat. no. C3389, type VI-S, 1000 units/mg) was studied using the Ellman method, in which thiocholine formed from an enzyme reacts with 5.5′-dithiobis(2-nitrobenzoic acid) to form a colored complex recorded by spectrophotometric method at a wavelength of 412 nm. The Acetylcholinesterase Activity Assay Kit (Sigma-Aldrich, cat. no. MAK119). The composition of *N. multifida* extract metabolites was studied before and after incubation with acetylcholinesterase under the HPLC-UV conditions described above. The extract sample (10 mg) was dissolved in a 20 mM tris-HCl buffer (pH 7.5; 5 mL), centrifuged (6000 *g*, 10 min), the supernatant (50 mL) was mixed with 50 mL of acetylcholinesterase solution in a 20 mM tris-HCl buffer (1 mg/mL) and incubated at 37° from (30 min). After that, 100 mL of acetonitrile was poured into the sample, centrifuged (6000 *g*, 5 min) and the supernatant was analyzed by HPLC-UV.

Statistical analysis was performed using single-factor analysis of variance (ANOVA). The significance of the mean differences was determined using the Duncan multi-rank test. Differences at $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

As a result of chromatographic separation of methanol extract from *N. multifida* leaves using CC on polyamide, normal- and reversed-phase silica gel, Sephadex LH-20 and preparative HPLC, known compounds **2–16** and a new flavonoid **1** were isolated.

Compound **1** was determined by the formula $C_{25}H_{24}O_{13}$ according to MS (HR-ESI-MS, *m*/*z* 531.4273; calculation 531.4064 for the ion $C_{25}H_{23}O_{13}$ [$M - H]^-$) and NMR spectroscopy ^{13}C. UV spectroscopy data indicated that **1** is a flavonoid of the luteolin group (Fig. 1a), and in the mass spectrum of MS^2, ions were detected due to the removal of two acetyl groups (*m*/*z* 531 → 489, 447) and a hexose fragment (*m*/*z* 447 → 285) (Fig. 1b) [19].

Luteolin and *D*-glucose were found in the products of acid hydrolysis of compound **1** with TFA. The signal of the anomeric proton of the carbohydrate fragment was

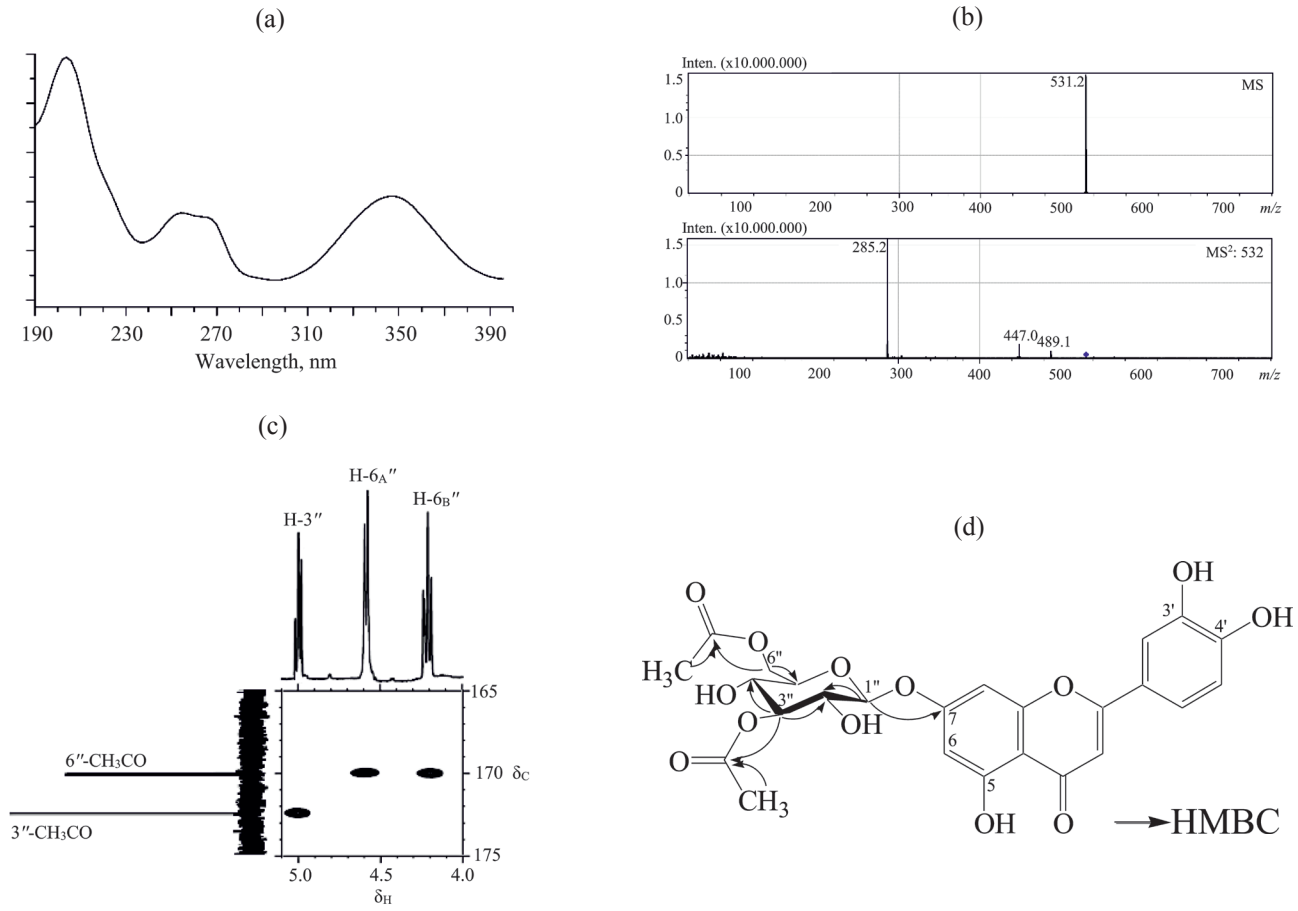

Fig. 1. Absorption spectrum (a), mass spectra (MS and MS^2, negative ionization; (b)), fragment of the HMBC spectrum (c) and structural formula ((d), arrows indicate some correlations in the HMBC spectrum) of compound **1** {luteolin-7-*O*-(3″,6″-di-*O*-acetyl)-β-*D*-glucopyranoside}.

in the region of 5.10 ppm (δ_H, d, J = 8.1 Hz), which is typical for the β-anomer of glucose [20]. NMR spectra were close to those of luteolin-7-*O*-β-*D*-glucopyranoside (**2**) [16] with the exception of additional signals of acetyl groups in the spectra of ^1H (δ_H 1.95/2.07) and ^{13}C (δ_C 20.5/21.4 ppm, 170.0/172.5 ppm) (Table 1). The signals of protons H-3″ and H-6″ of glucopyranose in **1** were shifted to a weak down in comparison with those of **2** (δ_H 3.40 → 4.98 for H-3″; 3.57/3.79 → 4.22/4.59 for H-6″), which was also observed for C-3″ carbon signals (δ_C 77.0 → 78.2) and C-6″ (δ_C 60.8 → 63.9) with simultaneous high-field shift of signals of neighboring carbon atoms (δ_C C-2″ 74.5 → 72.8; C-4″ 70.9 → 69.5; C-5″ 78.0 → 75.6). These features of the NMR spectra indicated the presence of substitution in C-3″ and C-6″ glucopyranose [20], which confirmed the existing correlations in the HMBC spectrum between the signals of the proton H-3″ c δ_H 4.98 ppm and H-6″with δ_H 4.22/4.59 ppm and carbons of acetyl carbonyls with δ_C 172.5 and 170.0 ppm, respectively (Fig. 1c).

Thus, compound **1** was a diacetylated analogue of **2**, which determined the structure of luteolin-7-*O*-(3″,6″-di-*O*-acetyl)-β-*D*-glucopyranoside, which is a new natural flavonoid (Fig. 1d).

One natural acetate of luteolin-7-*O*-glucoside is known—luteolin-7-*O*-(6″-*O*-acetyl)-glucoside isolated from *Salix gilgiana* Seemen (Salicaceae) [17]. The presence of acetic acid fragment in luteolin glycosides was also detected in luteolin-3′-*O*-glucuronide (3″- and 4″-*O*-monoacetates), luteolin-7-*O*-(2″-*O*-apiosyl)-xyloside (3‴-*O*-acetate), luteolin-7-*O*-(2″-*O*-allosyl)-

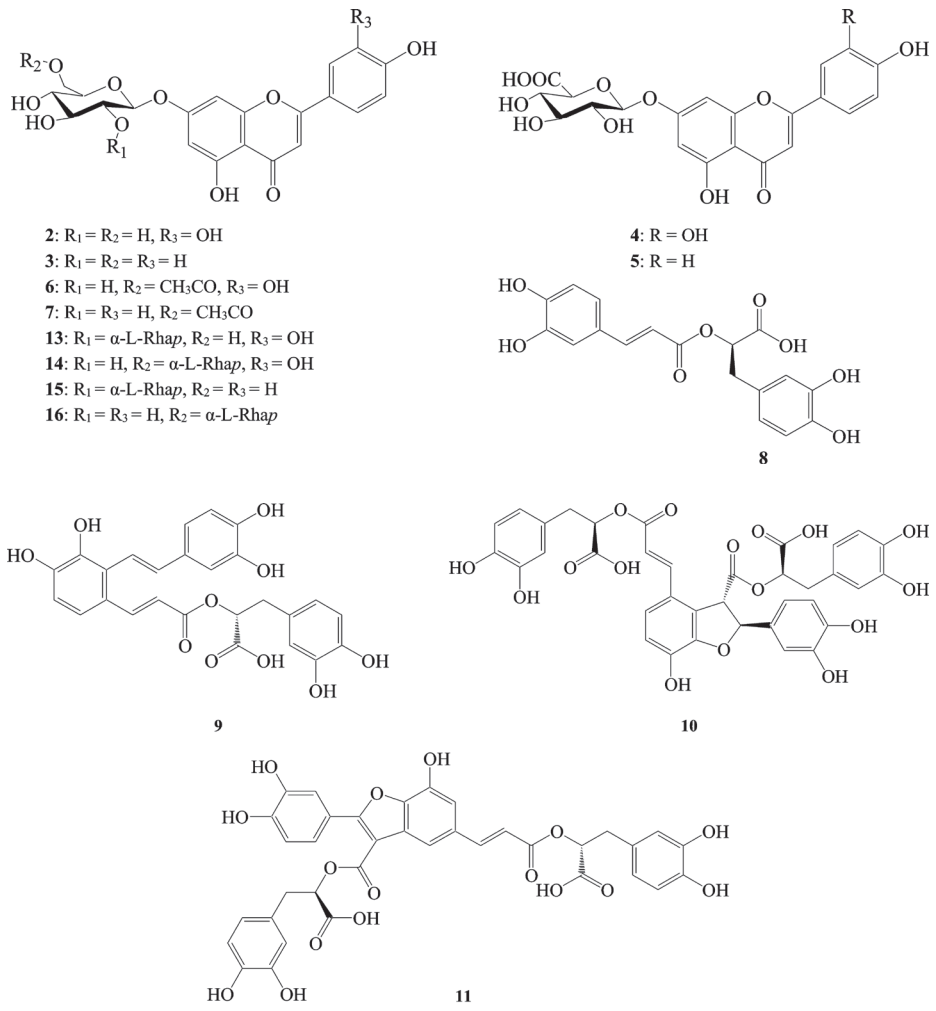

Fig. 2. Structural formulas of compounds **2–16** isolated from *N. multifida leaves*. α-L-Rha *p*—α-L-rhamnopyranose.

glucoside (6‴-*O*-acetate) and luteolin-7-*O*-sophoroside (6‴-*O*-acetate) [21].

Among the known compounds in the leaves of *N. multifida*, luteolin-7-*O*-glucoside (**2**), apigenin-7-*O*-glucoside (**3**), luteolin-7-*O*-glucuronide (**4**), apigenin-7-*O*-glucuronide (**5**), luteolin-7-*O*-(6″-*O*-acetyl)-glucoside (**6**), apigenin-7-*O*-(6″-*O*-acetyl)-glucoside (**7**), rosemary acid (**8**), salvianolic acid A (**9**), salvianolic acid B (**10**), schizotenuin A (**11**), nepetamultin A (**12**), luteolin-7-*O*-neohesperidoside (**13**), luteolin-7-*O*-rutinoside (**14**), apigenin-7-*O*-neohesperidoside (**15**) and apigenin-7-*O*-rutinoside (**16**) were identified (Fig. 2). The presence of compounds **2–12** was previously shown in the flowers of *N. multifida* [10, 11], and **13–16** were detected for the first time for the species. The studied species belongs to the subsection Schizonepeta Benth. the genus *Nepeta*, which in addition to *N. multifida* includes *N. annua* Pall. and *N. tenuifolia* Benth. In the composition of phenolic compounds of *N. tenuifolia*, **2**, **3**, **4**, **8**, **11**, and **14** were found, and in *N. annua*—**2** [5], which indicates the widespread distribution of luteolin, apigenin and rosmarinic acid derivatives in the species of this subsection.

The study of the quantitative profile of phenolic compounds of *N. multifida* leaves was carried out using HPLC-UV on raw material samples collected during various phases of vegetation (Fig. 3).

Table 2. The content of some compounds in the leaves of *N. multifida*, mg/g of air-dry raw materials (S.D.)

Compound	Vegetation phase (harvest period, number of plants)*				
	The beginning of the growing season (June 2–5, *n* = 30)	Vegetation (June 28–30, *n* = 28)	Flowering (July 15-–8, *n* = 45)	Fruiting (August 10–14, *n* = 38)	Extinction (August 28–31, *n* = 22)
Luteolin-7-*O*-glucuronide	2.03 (0.28)	10.37 (0.93)	14.10 (1.55)	14.18 (1.63)	8.22 (0.98)
Luteolin-7-*O*-glucoside	<0.01	<0.01	0.28 (0.02)	0.38 (0.04)	3.67 (0.44)
Luteolin-7-*O*-(6″-*O*-acetyl)-glucoside	0.53 (0.07)	2.88 (0.25)	6.55 (0.78)	6.93 (0.62)	3.76 (0.45)
Luteolin-7-*O*-(3″,6″-di-*O*-acetyl)-β-D-glucopyranoside	n.d.	n.d.	0.22 (0.02)	<0.01	n.d.
Luteolin-7-*O*-rutinoside	n.d.	n.d.	<0.01	<0.01	n.d.
Apigenin-7-*O*-glucuronide	<0.01	0.82 (0.07)	2.14 (0.25)	1.85 (0.16)	0.53 (0.04)
Apigenin-7-*O*-glucoside	n.d.	<0.01	0.02 (0.00)	<0.01	n.d.
Apigenin-7-*O*-(6″-*O*-acetyl)-glucoside	n.d.	n.d.	<0.01	<0.01	n.d.
Rosemary acid	8.36 (0.92)	22.14 (1.99)	35.71 (5.36)	32.68 (3.92)	14.01 (1.96)
Salvianolic acid A	<0.01	0.59 (0.43)	1.78 (0.16)	2.35 (0.19)	2.06 (0.23)
Salvianolic acid B	<0.01	<0.01	0.53 (0.05)	1.40 (0.15)	0.99 (0.11)
Schizotenuin A	5.29 (0.47)	7.53 (0.92)	8.36 (1.17)	9.56 (1.05)	9.24 (0.83)
The total content of phenolic compounds, including	16.21	44.33	69.69	69.33	42.48
luteolin glycosides	2.56	13.25	21.15	21.49	15.65
apigenin glycosides	<0.01	0.82	2.16	1.85	0.53
flavone-*O*-glycosides	2.56	14.07	23.31	23.34	16.18
hydroxycinnamates	13.65	30.26	46.38	45.99	26.30

* n.d.—not detected.

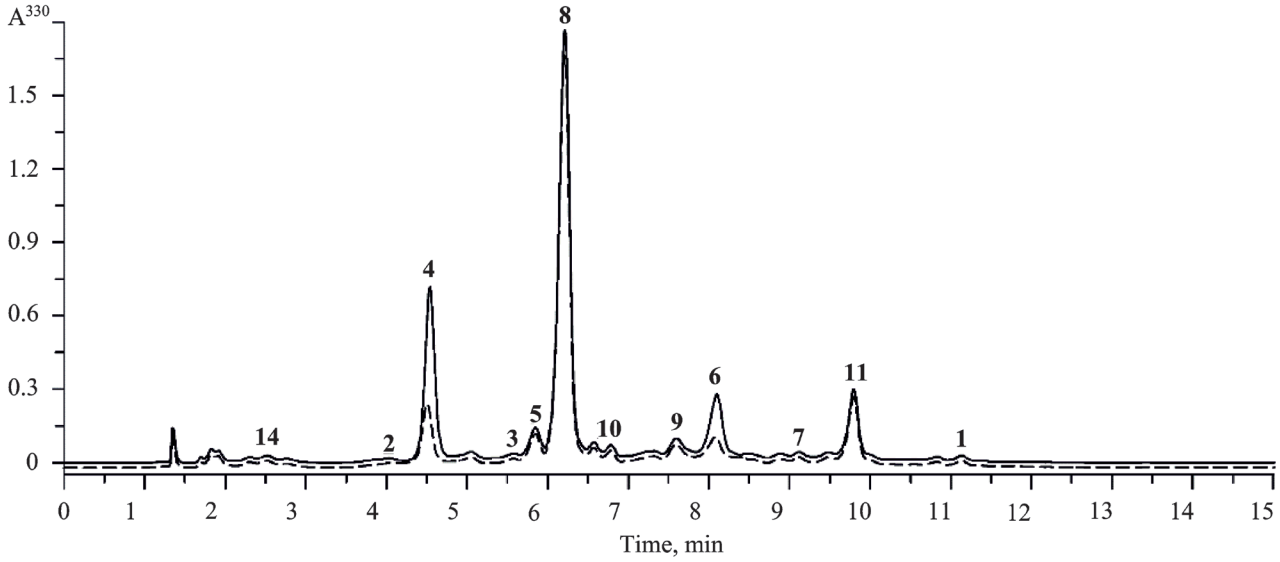

Fig. 3. Chromatogram (HPLC-UV) of *N. multifida* leaf extract (λ 330 nm; raw material sample collected in the flowering phase) before (straight line) and after incubation with acetylcholinesterase from *Electrophorus electricus* (dotted line). The numbers indicate the position of the compounds: **1**, luteolin-7-*O*-(3″,6″-di-*O*-acetyl)-β-*D*-glucopyranoside; **2**, luteolin-7-*O*-glucoside; **3**, apigenin-7-*O*-glucoside; **4**, luteolin-7-*O*-glucuronide; **5**, apigenin-7-*O*-glucuronide; **6**, luteolin-7-*O*-(6″-*O*-acetyl)-glucoside; **7**, apigenin-7-*O*-(6″-*O*-acetyl)-glucoside; **8**, rosemary acid; **9**, salvianolic acid A; **10**, salvianolic acid B; **11**, schizotenuine A; **14**, luteolin-7-*O*-rutinoside.

Analysis of the content of twelve compounds revealed a change in the total concentrations of phenolic compounds during seasonal plant growth from 16.21 to 69.69 mg/g (Table 2). The dominant compound of *N. multifida* leaves was rosmarinic acid, the content of which varied from 8.36 mg/g at the beginning of the growing season to the highest in the flowering and fruiting phases (32.68–35.71 mg/g), decreasing to 14.01 mg/g by the end of the growing season. The content of other hydroxycinnamates, including schizotenuine A, salvianolic acids A and B, was 5.29–9.56, 0.59–2.35, and 0.53–1.40 mg/g, respectively, and the highest concentration of this group of phenols was detected in the flowering phase (46.38 mg/g). The main group of flavonoid compounds were luteolin glucosides, with luteolin-7-*O*-glucuronide and its 6″-*O*-acetyl derivative being the main flavones of *N. multifida leaves*. The nature of the accumulation of compounds was similar: there was an increase from the beginning of the growing season to the phases of flowering and fruiting and a decrease by the end of the growing season. The variation in the concentrations of luteolin-7-*O*-glucuronide and luteolin-7-*O*-(6″-*O*-acetyl)-glucoside was 2.03–14.18 and 0.53–6.93 mg/g, respectively. The content of apigenin glycosides in *N. multifida leaves* did not exceed 2.16 mg/g. The obtained results indicated that the optimal time for collecting *N. multifida leaves* is the flowering and fruiting period of the plant.

A study of the biological activity of *N. multifida* leaf extract showed the presence of an inhibitory effect on acetylcholinesterase with a concentration of half-maximal inhibition of the enzyme (IC_{50}) 105.33 ± 4.73 μg/mL. Chromatographic analysis of the extract before and after incubation with acetylcholinesterase from *Electrophorus electricus* revealed a significant decrease in the peak area of two compounds – luteolin-7-*O*-glucuronide (**4**) and luteolin-7-*O*-(6″-*O*-acetyl)-glucoside (**6**), which indicated the formation of insoluble complexes between the enzyme and flavonoids (Fig. 3). The IC_{50} values for pure compounds **4** and **6** were 32.10 ± 1.61 and 35.14 ± 1.65 μg/mL, respectively. Minor luteolin glycosides also demonstrated pronounced inhibition of the enzyme—luteolin-7-*O*-(3″,6″-di-*O*-acetyl)-β-D-glucopyranoside (**1**; IC_{50} 43.69 ± 1.74 μg/mL), luteolin-7-

O-glucoside (**2**; IC_{50} 29.03 ± 1.39 μg/mL), luteolin-7-*O*-neohesperidoside (**13**; IC_{50} 57.45 ± 2.98 μg/mL) and luteolin-7-*O*-rutinoside (**14**; IC_{50} 58.36 ± 2.80 μg/mL). Apigenin derivatives (**3**, **5**, **7**) and hydroxycinnamates were less active (**8–11**), showing efficacy with IC_{50} >200 μg/mL. Thus, luteolin glycosides are the components responsible for the manifestation of the anti-acetylcholinesterase effect of *N. multifida leaf extract*. It was previously shown that luteolin [22], luteolin-7-*O*-glucoside [23] and luteolin-7-*O*-rutinoside [24] have an inhibitory effect on cholinesterases, which is due to the influence of the *ortho*-di-hydroxy-substituted ring B in the structure of these flavonoids. The presence of luteolin glycosides is characteristic of many *Nepeta* species, as well as representatives of the Nepetinae subtribe of the Lamiaceae family [5], which is probably a chemical sign of the presence of anticholinesterase activity in these species.

CONCLUSIONS

Nepeta multifida leaves contain benzofuran lignans from the rosemary acid group, as well as luteolin and apigenin *O*-glycosides, including the new flavonoid luteolin-7-*O*-(3″,6″-di-*O*-acetyl)-β-*D*-glucopyranoside.

The accumulation of phenolic compounds in the leaves of *N. multifida* depends on the phase of plant development, with the highest content revealed during flowering and fruiting.

Luteolin glycosides found in *N. multifida leaves* have an inhibitory effect on acetylcholinesterase.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATES

This article does not contain any studies involving patients or animals as test objects.

Informed consent was not required for this article.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

AUTHOR CONTRIBUTION

The authors NIK and DNO—selected the literature data on the review topic, contributed to manuscript preparation.

All authors participated in the discussions.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Amirzargar, N., Heidari-Soureshjani, S., Yang, Q., Abbaszadeh, S., and Khaksarian, M., *Nat. Prod. J.*, 2020, vol. 10, pp. 550–565. https://doi.org/10.2174/2210315509666190820103658
2. Pineda-Ramírez, N., Calzada, F., Alquisiras-Burgos, I., Medina-Campos, O.N., Pedraza-Chaverri, J., Ortiz-Plata, A., Pinzón Estrada, E., Torres, I., and Aguilera, P., *Antioxidants*, 2020, vol. 9, 253. https://doi.org/10.3390/antiox9030253
3. Hanafy, D.M., Prenzler, P.D., Burrows, G.E., Gurusinghe, S., Thejer, B.M., Obied, H.K., and Hill, R.A., *Nutrients*, 2020, vol. 12, Article ID: 1366. https://doi.org/10.3390/nu12051366
4. Topcu, G. and Kusman, T., *Bezmialem Sci*., 2014, vol. 1, pp. 1–25. https://doi.org/10.14235/bs.2014.233
5. Sharma, A., Cooper, R., Bhardwaj, G., and Cannoo, D.S., *J. Ethnopharmacol*., 2021, vol. 268, Article ID: 113679. https://doi.org/10.1016/j.jep.2020.113679
6. *Flora of Siberia. Pyrolaceae-Lamiaceae*, Ed. Malyshev, L.I. CRC Press: Boca-Raton, USA, 2006, vol. 11, 310 p.
7. *Flora of Central Siberia*, Ed. Malyshev, Z.D. and Peshkova, G.A. Novosibirsk, 1979, 536 p.
8. Liu, Z.L., Chu, S.S., and Jiang, G.H., *J. Sci. Food Agricult*., 2011, vol. 91, pp. 905–909. https://doi.org/10.1002/jsfa.4263

9. Liu, J.-T., Yu, J.-C., Jiang, H.-M., Zhang, L.-Y., Zhao, X.-J., and Fan, S.-D., *Chin. J. Chem.*, 2008, vol. 26, pp. 1129–1132. https://doi.org/10.1002/cjoc.200890201
10. Olennikov, D.N., *Chem. Nat. Comp*., 2021, vol. 57, pp. 818–822. https://doi.org/10.1007/s10600-021-03488-7
11. Kashchenko, N.I. and Olennikov, D.N., *Chem. Nat. Comp.*, 2022, vol. 58, pp. 274–278. https://doi.org/10.1007/s10600-022-03658-1
12. Batorova, S.M., Yakovlev, G.P., and Aseyeva, T.A., *Reference Book of Medicinal Plants of Traditional Tibetan Medicine*, Novosibirsk, 2013, 293 p.
13. Razuvayeva, Ya.G., Kharzheyev, D.V., Toropova, A.A., and Olennikov, D.N., *Voprosy Biologicheskoy, Meditsinskoy i Farmatsevticheskoy Khimii*, 2018, vol. 21, pp. 5–10. https://doi.org/10.29296/25877313-2018-07-02
14. Razuvaeva, Y.G., Toropova, A.A., Olennikov, D.N., and Kharzheev, D.V., *Nat. Prod. Rep*., 2021, vol. 36, pp. 3105–3109. https://doi.org/10.1080/14786419.2021.1935932
15. Olennikov, D.N., Nikolaev, V.M., and Chirikova, N.K., *Antioxidants*, 2021, vol. 10, Article ID: 863. https://doi.org/10.3390/antiox10060863
16. Malikov, V.M. and Yuldashev, M.P., *Chem. Nat. Comp*., 2002, vol. 38, pp. 358–406. https://doi.org/10.1023/A:1021638411150
17. Mizuno, M., Kato, M., Iinuma, M., Tanaka, T., Kimura, A., Ohashi, H., and Sakai, H., *Phytochemistry*, 1987, vol. 26, pp. 2418–2420. https://doi.org/10.1016/S0031-9422(00)84739-1
18. Matsuta, M., Kanita, R., Saito, Y., and Yamashita, A., *Nat. Med*., 1996, vol. 50, pp. 204–211.
19. Olennikov, D.N., Chirikova, N.K., Kashchenko, N.I., Nikolaev, V.M., Kim, S.-W., and Vennos, C., *Front. Pharmacol*., 2018, vol. 9, Article ID: 756. https://doi.org/10.3389/fphar.2018.00756.
20. Bock, K. and Pedersen, C., *Adv. Carbohydr. Chem. Biochem*., 1983, vol. 41 (C), pp. 27–66. https://doi.org/10.1016/S0065-2318(08)60055-4
21. *Flavonoids: Chemistry, Biochemistry, and Applications*, Eds. Andersen, Ø.M. and Markham, K.R. CRC Press, Boca Raton, USA, 2006, 1197 p.
22. Tundis, R., Bonesi, M., Menichini, F., Loizzo, M.R., Conforti, F., Statti, G., and Menichini, F., *Nat. Prod. Commun*., 2012, vol. 7, pp. 1015–1020. https://doi.org/10.1177/1934578x1200700814
23. Olennikov, D.N., Chirikova, N.K., and Vennos, C., *Nat. Prod. Commun*., 2017, vol. 12, pp. 55–56. https://doi.org/10.1177/1934578x1701200115
24. Orhan, I., Kartal, M., Tosun, F., and Şener, B., *Z. Naturforsch. C*, 2007, vol. 62, pp. 829–832. https://doi.org/10.1515/znc-2007-11-1210