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Effect of an Extractant on the Composition of the Lipophilic Constituents of the Extracts of *Rhodiola rosea* L. and on the Extracts' Activity

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Abstract—The composition of the lipophilic components of the *Rhodiola rosea* L. plant was studied. Acidic and neutral components were identified by gas chromatography–mass spectrometry. With methyl-*tert*-butyl ether (MTBE) as an extractant instead of the volatile solvent diethyl ether, lipophilic extract was obtained. Methyl-*tert*-butyl ether used as an extraction solvent for raw materials has all the advantages of diethyl ether, being free of its disadvantages. It does not form peroxides or produce elevated partial gas pressure due to its higher boiling point. As a result, comparison with databases identified some triterpene, phenolic, and aliphatic acids with chain lengths 12 to 30 carbon atoms, including saturated, unsaturated, and dibasic acids. In addition to the components known from the literature, more than 50 triterpene and aliphatic compounds were detected in the unsaponifiable residue and acidic fractions for the first time. The hexane extract and the product obtained by the stepwise extraction by MTBE after the extraction of low-polarity compounds with hexane were investigated in a similar way. In the case of an extract obtained using MTBE after the extraction of low-polarity components with hexane, extraction of benzoic and cinnamic acids was more efficient compared to the exhaustive extraction of MTBE. These acids are absent from the hexane extract. Ethanol extraction was also carried out to test bioactivity: exhaustive and after hexane and MTBE extraction. Extracts obtained using MTBE and ethanol showed antiviral activity against the Ebola pseudovirus.

Keywords: gold root, *Rhodiola rosea* L., extractive substances, methyl-*tert*-butyl ether, phenolic acids

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INTRODUCTION

Rhodiola rosea L. of the Crassulaceae family (golden root) is included in traditional and official medicine due to its powerful adaptogenic effect [1]. It is also used as a tea substitute and an additive in tonic drinks. The rhizomes and roots of the plant are mainly used as medicinal raw materials. In addition to adaptogenic activity, it exhibits general tonic, anti-inflammatory, wound healing, diuretic, and antipyretic properties. It is used for treatment of vegetovascular dystonia, asthenia, neurasthenia, overwork, fevers of various etiologies, diseases of the reproductive system in both men and women, diarrhea, dysentery, tuberculosis, paradontosis, respiratory infections, diabetes, scurvy, and anemia [1]. Antioxidant, antitumor, cardioprotective, antiviral, antidiabetic activity was also revealed [2–4]. To achieve a therapeutic effect, mainly water, water–alcohol, and alcohol extracts are used [1]. Pharmacological studies of *Rh. rosea* L., conducted under the guidance of Prof. Krylov, showed pronounced stimulating and adaptogenic effects, comparable with the effect of preparations from plants of the Araliaceae family. Researchers from the Soviet Union and Russia made a significant contribution to the study of the plant and received international recognition [2, 5–11]. The plant is also widely studied in Mongolia, where it is used in official and traditional medicine [12]. In official medicine, the golden root is used in the form of a liquid extract (extractum rhodiolae fluidum). This is a 1 : 1 alcoholic extract from roots and rhizomes in 40% ethanol. The extract is used for asthenic conditions, increased fatigue, neurasthenic conditions, and vegetovascular dystonia. The main active ingredients of the rhizomes are phenolic com-

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pounds (phenol alcohols and their glycosides, cinnamon alcohol and its glycosides, flavonoids, tannins). Phenolic alcohol *p*-hydroxyphenylethanol (*p*-tyrosol) and its glycoside (rhodioloside, salidroside) are crystalline substances the pharmacological stimulating effect of which coincides with the properties of the total plant preparations. Of the glycosides of cinnamic alcohol, salidroside matches cinnamylarabinoglycoside (rosavin). Among flavonoids, quercetin, hyperoside, kaempferol, and quercitrin, as well as derivatives of tricine and herracetin, rare for other plants, were found [6, 7]. Tannins of the pyrogallic group have been found, together with free gallic acid. The rhizomes contain organic acids (oxalic, succinic, citric, and malic), lactones, essential oils (EOs) with a yield of up to 0.9%, and β-sitosterol. Krasnov carried out a systematic study of 21 species of the Crassulaceae family of the flora of the Soviet Union [9]. More than 50 natural compounds belonging to the groups of phenolic acids, hydroxycoumarins, catechins, flavanols, simple phenols and their glycosides, and bases (piperidine derivatives) have been isolated from alcohol extracts. Eight new compounds have been isolated: glycosides of herbacetin (3,5,7,8,4'-pentahydroxyflavone) and gossypetin (3,5,7,8,3',4'-hexahydroxyflavone); their structure has been established [6]. It has been proven for the first time that galloylarbutin, *p*-tyrosol, and herbacetin glycosides have capillary-strengthening, anti-inflammatory, and cytostatic activity and are of interest for the creation of drugs with a wide spectrum of action [2, 7–12].

Benzene derivatives were found in the raw materials of *Rhodiola rosea*: benzyl alcohol, benzaldehyde, benzyl propanol, benzyl benzoate, phenylethyl alcohol, citral, β-phenylethyl acetate, cuminyl acetate, cinnamic aldehyde, cinnamic alcohol, cinnamyl acetate, thymol, carvacrol, estragol, and acetovanillone [6, 9, 11, 13–15]. One of the main biologically active substances is rosiridin, a glucoside of the acyclic monoterpene alcohol rosiridol [6]. Later, other monoterpene glycosides were discovered [16]. Phenolic glycosides have been studied: 1,4-methoxycinnamyl-*O*-β-*D*-glucopyranoside, cinnamyl-(6'-*O*-β-xylopyranosyl)-*O*-β-glucopyranoside, rosin, rosarin, rosavin, sachaliside, 4-methoxycinnamyl-(6'-*O*-α-arabinopyranosyl)-*O*-β-glucopyranoside, picein, benzyl-*O*-β-glucopyranoside, monogroside, rhodiooctanoside, salidroside and its aglycone tyrosol first discovered in willow *Salix triandra* L., rosavidin, rhodioloside F, and (–)-rosiridol [6, 7, 9, 11, 17, 18].

A cyanogenic compounds lotaustraline [19] and phenylalkaloids [20] were found.

Phenolcarboxylic acids have been identified: gallic, rosmarinic, chlorogenic, caffeic, *trans-p*-hydroxycinnamic, methyl gallate, as well as flavonoids, including those in the aerial part of the plant [6, 7, 9, 11, 21–23].

Among the acids the following ones were found: oxalic, malic, succinic, citric, hexanoic, and octanoic [10] and higher fatty acids decanoic, dodecanoic, tetradecanoic, pentadecanoic, and hexadecanoic [14].

Information on neutral aliphatic compounds is limited to *n*-pentanol, *n*-hexanol, *n*-octanol, *n*-nonanol, *n*-decanol, *n*-dodecanol, *n*-decanal, *n*-hexanal, *n*-octanal, *trans*-2-octenal, *n*-nonanal, *trans*-2-nonenal, *n*-heptanal, heptan-2-one, 6-methyl-5-hepten-2-one, i.e. components of EO [13–15].

The identification of terpenoids is mainly limited to EO components [9, 13–15, 24–26]. Sterols are also insufficiently studied [6–12]. Even the most comprehensive review [6], citing more than 140 scientific materials, does not fully reflect the spectrum of lipophilic metabolites of *Rh. rosea* L. Many substances found in the plant in the last century were studied later using modern methods, their structure was confirmed, new data were obtained, including antiviral activity [3, 27–30].

Literature data show that the study of bioactive components was mainly focused on substances that are highly soluble in polar solvents. As a result, flavonoids, alkaloids, glycites, γ-lactones, carbohydrates, cyanogenic compounds, anthraquinones, phenols, phenolic glycosides, and phenolic acids were identified. Low polarity compounds are limited to some sterols and triterpenes, as well as EO components [6, 7, 9, 13]. The true content of hydrocarbons and alcohols with a chain length of more than 10, and acids of more than 16 carbon atoms in EOs, is greatly underestimated due to their low volatility [13–15, 25]. Therefore, our aim was to study the chemical composition of lipophilic components: sterols, triterpenoids, and aliphatic metabolites, as well as to evaluate the biological activity of extracts using a pseudovirus system.

RESULTS AND DISCUSSION

When studying the MTBE extract, two fractions of acids were obtained: free and bound. Chromatographic separation of UR led to the production of six main fractions: hydrocarbons, ketones, three fractions of mixtures of aliphatic and triterpene alcohols, and sterols. The hexane extract and the product obtained by stepwise extraction by MTBE after the extraction of low-polarity compounds with hexane (MTBE/hexane) were studied in a similar way. Table 1 reflects the yields of the fractions obtained in the above experiments.

In addition, to test the biological activity, raw material extracts were obtained with ethanol (exhaustive extraction, 24.8% yield) and ethanol after raw material extraction with hexane and MTBE (18.9% yield). The composition of ethanol extracts was not studied in detail.

Based on the results of GC–MS analysis, the content of each component in the analyzed mixtures was calculated in mg/100 g of raw material (mg %).

Table 1. Yield of extracts and fractions obtained upon extraction of *Rh. rosea* L. rhizomes with low polarity solvents

Extractant	Extract, % to raw material weight	Free acids, % to extract weight	Bound acids, % to extract weight	HO, % to extract weight
Hexane	0.82	22.7	40.0	25.4
MTBE/Hexane	0.43	67.6	10.5	10.8
MTBE	1.25	21.9	27.8	32.4

Table 2. Content of carbohydrates in UR extract of *Rh. rosea* L. rhizomes related to the raw material weight (mg %)

Component	MTBE	Hexane	MTBE/hexane
Octadecane	0.4	0.3	–
Nonadecane	0.3	0.4	0.1
Eicosane	0.5	0.2	–
Heneicosane	0.8	1.0	0.2
Docosane	0.9	0.3	–
Tricosane	8.0	6.5	1.4
Tetracosane	1.3	0.6	–
Pentacosane	6.5	5.0	1.7
Hexacosane	0.9	1.0	–
Heptacosane	4.9	4.9	1.5
Octacosane	0.3	0.2	–
Nonacosane	5.3	5.2	1.3
Triacontane	0.1	0.2	–
Hentriacontane	2.2	3.8	1.0
Dotriacontane	0.1	0.1	–
Tritriacontane	5.4	6.2	1.0
Pentatriacontane	0.9	1.0	0.2
Squalene	0.6	0.3	0.1
Stigmasta-3,5-diene	2.0	0.5	0.5
28-Nor-17β-hopane	0.3	–	–
28-Nor-17α-hopane	0.3	–	–
17α-21β-Hopane	0.2	–	–

Analysis of acidic and neutral components of extractive substances from *Rh. rosea* L. obtained by saponification with alkaline treatment of extracts showed the following results. The content of extractive lipophilic substances is the highest with exhaustive extraction by MTBE (1.25%), nonsaponifiable substances in this case make up 40% of the weight of the extract. With stepwise extraction, the total yield is approximately the same as with exhaustive extraction, but the ratio of the separated fractions is different. For UR, additional fractionation and purification of groups of compounds were carried out. As a result, concentrates of hydrocarbons, ketones, aliphatic and terpene alcohols, including sterols and diols, were obtained. The contents of the identified components are summarized in Tables 2–8.

As follows from Table 2, aliphatic hydrocarbons with an odd number of carbon atoms are predominant in the fraction; the major ones include tricosane, pentacosane, heptacosane, nonacosane, and tritriacontane, accounting for 75% of the total weight of the fraction. Triterpene hydrocarbons are few and their contribution to the composition of UR is small. In hexane and MTBE/hexane extracts, only stigmasta-3,5-diene (0.5 mg %) was found.

Comparison of the mass spectra of aliphatic ketones with the database indicates the predominance of compounds with a keto group in the second position in the fraction. In hexane and MTBE extracts, after extraction with hexane, ketones were outside the detection limits. The total content of triterpene ketones (21.1 mg %) is an order of magnitude higher

Table 3. Content of ketones in UR of MTBE extract of *Rh. rosea* L. rhizomes related to the raw material weight, mg %

Component	Content in raw material	Component	Content in raw material
2-Tricosanone	0.4	2-Heptacosanone	0.6
2-Tetracosanone	0.3	2-Octacosanone	0.1
2-Pentacosanone	0.3	2-Nonacosanone	0.2
2-Hexacosanone	0.2	10-Nonacosanone	0.3
Tarax-14-en-3-one	2.9	α-Amirenone	5.1
Isomultiflorenone	7.0	Lupenone	2.5
β-Amirenone	1.1	Stigmasta-3,5-dien-7-one	2.5

Table 4. Content of alcohols and sterols in NO extracts of *Rh. rosea* L. rhizomes related to the raw material weight, mg %

Component	MTBE	Hexane	MTBE/Hexane
Hexadecanol	0.8	–	–
Octadecanol	0.9	–	–
Nonadecanol	1.0	–	–
Eicosanol	1.7	1.3	0.9
Heneicosanol	1.0	–	–
Docosanol	17.8	19.6	0.6
Tricosanol	0.9	0.9	–
Tetracosanol	42.7	53.9	0.4
Pentacosanol	0.7	1.6	–
Hexacosanol	39.7	56.8	0.4
Heptacosanol	0.3	–	–
Octacosanol	3.3	4.9	0.3
Triacontanol	0.1	–	–
Phytol	0.1	–	0.3
Cholesterol	0.8	0.8	0.2
Campesterol	23.8	23.9	1.5
β-Sitosterol	168.7	79.6	70.1
β-Stigmastanol	3.1	5.1	1.1
β-Amirin	0.7	1.6	1.2
α-Amirin	1.8	1.8	1.3
Brassicasterol	2.6	–	–
Stigmasterol	1.5	–	4.3
Isomultiflorenol	1.4	–	–
Isosvertenol	1.2	–	0.9
Stigmast-7-en-3-ol	2.0	1.7	1.0
Ergostanol	0.9	1.6	–
Ergost-7-en-3-ol	0.4	–	–
Stigmast-7,16,25-trien-3-ol	0.6	–	–
24-Methylene-cycloartanol	0.1	–	–
Lupeol	0.2		0.3

than that of aliphatic ketones (2.4 mg %). In hexane and MTBE/hexane extracts, only the triterpene ketone stigmasta-3,5-dien-7-one was found (1.3 and 1.2 mg %, respectively).

As follows from Table 4, *n*-alkanols with an even number of carbon atoms are predominant among the aliphatic components. The main ones include docosanol, tetracosanol, and hexacosanol accounting for up to 90% of the total weight of alkanols. The main component of the sterol group, β-sitosterol, was discovered earlier [6, 7, 9]; data on the detection of β-amirin were known [7]; the remaining components were detected in raw materials for the first time. In addition to the alcohols listed in Table 4, small amounts of geraniol, geranylgeraniol, myrtenol, and cinnamon alcohol were found; these compounds were identified in raw materials earlier [7]. Manool, manoyl oxide, and epimanoyl oxide, also previously identified in URs, were identified [25]. We also identified methyl ursolate in the sterol fraction.

As follows from Tables 5 and 6, the main components are palmitic, linoleic and lignoceric acids. The qualitative and quantitative composition of the extracts differs due to the different solubility of the components in hexane and MTBE.

Analysis of the extract obtained with MTBE after the extraction of low-polarity components with hexane (MTBE/hexane) revealed a more efficient extraction of benzoic and cinnamic acids compared to the exhaustive extraction with MTBE. These acids are absent in the hexane extract. It should be noted that they were found exclusively in the fractions of free acids.

In addition, betulonic acid was found in these extracts (2.2 and 2.0 mg%, respectively). In the extract obtained by MTBE after the extraction of low-polarity components with hexane, a component was found that coincides in spectrum with nor-5,6-secocholest-2-en-6-ovoic-1-oxoacid. The correctness of the identification can be confirmed only after the production of a sufficient amount of this component for the use of alternative methods of analysis.

Capric, lauric, myristic, pentadecanoic, and palmitic acids appear in the literature [7, 13–15, 25]. They were found in the composition of EOs; therefore, components with a chain length of more than 16 carbon atoms were not included in the composition of the studied fraction due to low volatility. Caffeic and gallic acids were also identified earlier [7, 8, 10], the remaining acidic components were found in this raw material for the first time.

In the work, an assessment of the antiviral activity of the obtained extracts was carried out. For this, preparations of Ebola pseudoviruses based on the vesicular stomatitis virus, which is defective in the gene of the surface protein G, were used. This model allows to identify substances that inhibit viral entry into cells.

Table 5. Content of aliphatic free and bound acids in the MTBE extract of *Rh. rosea* L. rhizome related to the raw material weight, mg %

Component	Free acids	Bound acids
Suberic (octanedioic)	4.2	
Azelaic (nonanedioic)	12.1	
Lauric	0.6	1.5
Myristic	1.0	4.9
Pentadecenoic	0.1	2.3
Pentadecanoic	0.1	0.7
Palmitic	52.6	65.0
Palmitoleic	0.6	0.5
Margaric	0.6	1.3
Oleic	9.9	17.8
Linolenic	9.4	41.2
Linoleic	38.8	102.0
Stearic	6.1	6.7
Conjugated linolenic (9,11,13-octadecatrienoic, 3 isomers)	0.1	3.4
Isolinoleic	0.1	0.3
Nonadecanoic		2.8
Eicosa-11,14-dienoic		1.5
Arachidic	4.9	8.6
Heneicosanoic	0.9	1.2
Behenic	8.8	12.2
Tricosanoic	3.4	5.7
Lignocerinic	25.4	24.8
Pentacosanoic	1.8	1.4
Phellogenic (docosandioic)		0.3
Cerotic	21.4	17.4
Montanic	2.5	1.7
Melissic	1.0	0.5

As follows from the data presented in Table 8, the extracts obtained with ethanol showed the highest activity. For the ethanol extract obtained after extraction with hexane and MTBE, the lowest toxicity of 235 ± 4 μg/mL was observed, while the IC_{50} reached a value of 69 ± 6 μg/mL. Interestingly, the activity of the extracts for both alcohol and MTBE increased if the raw material was preextracted with hexane. Possibly, the detected activity is explained by phenolcarboxylic acids, whose concentration in the extract has been shown to increase after preliminary extraction with hexane.

Table 6. Content of aliphatic free and bound acids in extracts of *Rh. rosea* L. rhizome obtained upon stepwise extraction related to the raw material weight, mg %

Component	Hexane		MTBE/Hexane	
	free acids	bound acids	free acids	bound acids
Azelaic (nonanedioic)			13.2	0.3
Lauric	0.7	3.9	0.4	0.4
Myristic	1.7	6.9	1.0	0.9
Pentadecenuic		3.6	0.5	
Pentadecanoic		1.1	1.3	0.4
Palmitic	71.2	64.8	36.9	11.7
Palmitoleic	2.0	1.9		0.4
Margaric	0.6	0.9	1.9	0.8
Oleic	15.2	23.6	8.4	2.3
Linolenic	6.7	27.8	3.6	3.0
Linoleic	34.0	94.0	21.8	8.8
Stearic	5.2	7.0	4.5	1.8
Conjugated linolenic (9,11,13-octadecatrienic, 3 isomers)	6.2	3.1		0.2
Eicosa-11,14-dienoic	1.3	1.7		
Arachidic	4.1	9.5	4.4	0.7
Heneicosanoic	0.7	1.4	1.9	0.3
Behenic	4.4	13.1	47.7	6.5
Tricosanoic	0.9	2.9	8.0	1.8
Lignoceric	7.0	23.9	65.9	2.0
Pentacosanoic		1.3	3.2	0.1
Cerotic	4.2	11.9	3.6	0.2
Heptacosanoic	0.3	0.5		
Montanic	0.4	0.7		
Melissic	0.2	0.5		

Table 7. Content of phenolcarbonic acids in the extracts of *Rh. rosea* L. rhizome related to the raw material weight, mg %

Component/Extractant	MTBE	MTBE/Hexane
Salicylic	3.1	3.0
Anisic	0.8	0.3
4-Methoxybenzylacetic		11.7
Veratric	1.0	11.0
2,4-Dimethoxybenzoic		5.2
Homoveratric		2.2
Gallic		3.8
Caffeic	2.5 (2 isomers 2 : 3)	2.7 (2 isomers 2 : 3)

EXPERIMENTAL

Extraction and analysis of extracts. The raw material of *Rhodiola rosea* L. rhizome was harvested in the phase of dying of flower shoots in August 2020 in the Altai Territory and dried at room temperature in a room without direct sunlight. Air-dry raw materials were grounded on a screw grinder and sieved through a sieve with 2-mm holes. Extracts for research were obtained in two different ways: exhaustive extraction with solvents of different polarity in a Soxhlet trap and stepwise with increasing polarity at each stage of

Table 8. Activity of *Rh. rosea* L. rhizome extracts against Ebola pseudoviruses

Method of extraction	CC_{50}, μg/mL	$IC_{50\ Ebola}$, μg/mL	SI_{Ebola}
MTBE (exhaustive extraction)	≤8	120 ± 17	–
MTBE (extraction after hexane)	202 ± 15	150 ± 15	1.3
Ethanol (exhaustive extraction)	217 ± 3	105 ± 10	2.1
Ethanol (extraction after hexane and MTBE)	235 ± 4	69 ± 6	3.4
Hexane	207 ± 20	140 ± 20	1.5
Sertralin (control)	124 ± 11	0.2 ± 0.02	620

extraction. Hexane, MTBE, and ethanol were used as extractants that sufficiently fully extract lipophilic compounds. In stepwise extraction, the same solvents were used without unloading the raw material from the nozzle when changing the solvent. At the first stage, the extract obtained by exhaustive extraction of raw materials was studied in detail. A sample of the raw material was loaded into a Soxhlet apparatus and extracted with MTBE for 20 h (3 × (6–7) h). The extract yield was 1.25% to the raw material by weight. Processing of the extract and chromatographic purification of the components of the unsaponifiable residue (UR) were carried out similarly [31–33]. Sample preparation for GC-MS analysis included isolation of free acids with an alkaline extractant (2% aqueous solution of sodium hydroxide) and hydrolysis of the extract, freed from free acids, with a 15%-aqueous–alcoholic solution of potassium hydroxide. Three fractions were obtained: free acids, bound acids, and unsaponifiable residue (UR). Acidic components were methylated with diazomethane; neutral components of the UR were subjected to chromatographic separation on a silica gel column using hexane with diethyl ether fraction increasing from 0 to 50% as eluent. Fractions were collected into 7–8-mL vials. Fractions were pooled in accordance with the results of thin layer chromatography on Sorbfil and Silufol plates. To develop the chromatogram, a mixture of hexane with MTBE was used; MTBE content was varied from 10 to 50%. A mixture of vanillin with sulfuric acid and ethanol in a ratio of 1 : 10 : 90 was used as a developing reagent, followed by heating. Analysis of the fractions was carried out using GC-MS at the Chemical Research Center for Collective Use, Siberian Branch, Russian Academy of Sciences. Low polar compounds (hydrocarbons, ketones) were analyzed without derivatization. The most representative fractions enriched with triterpene components were acetylated with acetic anhydride in pyridine. GC-MS spectra were recorded on a Hewlett Packard G 1800 A instrument, consisting of an HP 5890 series II gas chromatograph and an HP 5971 mass selective detector. The column was 30 m × 0.25 mm × 0.25 μm with the HP-5MS adsorbent (5% diphenyl, 95% dimethylsiloxane). The carrier gas was helium (1 mL/min). Column temperature: 2 min at 50°C, then temperature increase at a rate of 10°C per minute up to 300°C, 30 min at 300°C. The evaporator temperature was 280°C, the ion source temperature was 170°C. FAMEs were identified using the NIST 08 mass spectrum library in the same way as in [31–33]. The hexane extract and the product obtained by stepwise extraction of MTBE after the extraction of low polar compounds with hexane were studied in a similar way.

Determination of cytotoxicity of extracts against the 293FT cells. Stock solutions of extracts in DMSO (at a concentration of 500 μg/mL) were added to the growth medium to target cells of the 293FT line at various concentrations, from 12.5 to 500 μg/mL, for 48 h. After the incubation of the cells with the compounds, a tetrazolium dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) was added to cell cultures to a working concentration of 0.5 mg/mL and incubated for 4 h. The formazan residue was dissolved by adding 10% sodium dodecyl sulfate solution with 0.01 M HCl to the growth medium. The amount of formazan (proportional to the number of viable cells) was determined spectrophotometrically by measuring the absorbance at a light wavelength of 570 nm. The percentage of viable cells in cultures containing different concentrations of the studied extract X was determined relative to the control, which was a 293FT cell culture incubated in a growth medium with DMSO in the absence of the extract, using the formula:

$$\%\ \text{viable cells}[X] = OD[X]/OD[DMSO],$$

where OD is the corresponding optical density.

The CC_{50} value (50% cytotoxic concentration) was taken as the concentration of the extract at which 50% of the cells survived compared to the control.

Preparation of pseudoviruses based on recombinant vesicular stomatitis virus (VSV) exhibiting Ebola virus glycoprotein on the surface. To obtain pseudoviral progeny displaying the Ebola virus glycoprotein (Maing strain) on their surface, 293FT cells were first transfected with a plasmid containing the gene sequence of the ph-GPE glycoprotein (in the amount of 5 μg of the plasmid per 60-mm Petri dish). Twenty-four hours after transfection, producer cells were infected with 5 μL (~10^6 transducing units) of rVSV-ΔG-G. Four hours after infection, the infecting pseudovirus was washed off and the medium was

replaced with a fresh one. A pseudovirus preparation displaying the Ebola virus glycoprotein, rVSV-ΔG-GPE, was collected 24 h after infection. Pseudovirus preparations were stored at −80°C.

Determination of semi-inhibitory concentrations of extracts against rVSV-ΔG-GPE pseudoviruses, calculation of therapeutic index (SI) values. To determine the infectivity of pseudoviruses, target cells of the 293FT line were planted in a 96-well plate at a monolayer density of 80–90%. The infectivity of pseudoviruses in the presence of inhibitors and in control (non-inhibited) samples was determined by the luminescence index 24 h after infection. All measurements were made in triplicate with the determination of the mean value and standard deviation. For all studied extracts, the concentrations of 50% inhibition (IC_{50}) for rVSV-ΔG-GPE pseudoviruses were determined. Subsequently, the therapeutic index (SI), i.e., the ratio of compound toxicity and inhibitory activity against the virus (CC_{50}/IC_{50}), was calculated for each compound. Sertraline ((1*S*,4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthylamine) was selected as a reference compound inhibiting Ebola virus infection.

CONCLUSIONS

(1) The composition of extractive substances of *Rh. rosea* L. rhizome was studied by GC–MS. Over 100 compounds have been identified, most of which have been found in the raw material for the first time.

(2) Aliphatic and terpene compounds were found in the composition of neutral components: hydrocarbons, ketones, alcohols, including sterols, as well as cholesterol and stigmast-7-en-3-ol, which are rare in medicinal plants.

(3) An increase in the temperature of the analysis of acidic components made it possible to identify aliphatic components with a chain length of more than 16 carbon atoms, as well as triterpene betulonic acid. Bound and free components differ in qualitative and quantitative composition.

(4) The extract obtained with MTBE after the extraction of low-polarity components with hexane showed a more efficient extraction of benzoic and cinnamic acids compared to the exhaustive extraction by MTBE.

(5) Extracts of *Rh. rosea* L. obtained with MTBE and ethanol showed antiviral activity against Ebola pseudoviruses with an IC_{50} of 69 ± 6 μg/mL.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare no conflicts of interest. This article does not contain any research involving humans or animals as research objects.

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