



Roots of *Rosa majalis* Herrm. as a source of antioxidants and anti-influenza agents

Evgeniya A. Karpova¹ · Vera A. Kostikova¹ · Elena P. Khramova¹ · Tatiana M. Shaldaeva¹ · Olga Yu. Vasil'eva¹ · Natalia A. Mazurkova² · Ekaterina I. Filippova² · Oleg Yu. Mazurkov² · Elena V. Makarevich²

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Abstract

Rosa majalis Herrm. (Rosaceae) is a shrub widespread throughout Eurasia. In West Siberia, it is cultivated as an ornamental plant and raw material for the manufacture of fruit multivitamin concentrates. Meanwhile, metabolites and biological activities of its roots are poorly studied. The aim of this investigation was to evaluate the potential of ethyl acetate (EtOAc), butanol (ButOH), aqueous ethanol (EtOH), and aqueous (H₂O) extracts of *R. majalis* roots as sources of antioxidant and anti-influenza compounds. Spectrophotometry and HPLC were used for metabolite quantification. Antioxidant activities were determined via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The antiviral activities were assessed as half-maximal inhibitory concentrations (IC₅₀). The EtOAc extract manifested the highest concentrations of phenolic compounds (53.79 mg g⁻¹ of dry extract) and saponins (48.92 mg g⁻¹). Total gallic acid was the main phenolic compound in all extracts (24.98–38.68 mg g⁻¹). Catechin, free ellagic acid, and total ellagic acid had the highest levels in the EtOAc, H₂O, and EtOH extracts, respectively (30.83, 22.38, and 34.95 mg g⁻¹). The EtOAc extract showed the highest free-radical scavenging activity (2.31 trolox equivalents) and anti-influenza effects (IC₅₀ 61.8 and 50.3 μg mL⁻¹) against A/Aichi/2/68 (H3N2) and A/Chicken/05/2005 (H5N1), respectively. Our findings indicate good potential of these extracts for drug design against free-radical damage and influenza virus.

Keywords *Rosa majalis* · Catechin · Ellagic acid · Saponin · Antioxidant activity · Anti-influenza activity

✉ Evgeniya A. Karpova
karyevg@gmail.com

Vera A. Kostikova
serebryakova-va@yandex.ru

Elena P. Khramova
elenakhramova2023@yandex.ru

Tatiana M. Shaldaeva
tshaldaeva@yandex.ru

Olga Yu. Vasil'eva
vasil.flowers@rambler.ru

Natalia A. Mazurkova
mazurkova@vector.nsc.ru

Ekaterina I. Filippova
filippova_ei@vector.nsc.ru

Oleg Yu. Mazurkov
mazurkov_oyu@vector.nsc.ru

Elena V. Makarevich
makarevich_ev@vector.nsc.ru

¹ Central Siberian Botanical Garden, Siberian Branch of Russian Academy of Sciences, 101 Zolotodolinskaya Str, Novosibirsk, Russian Federation 630090

² Federal Budgetary Research Institution, State Research Center of Virology and Biotechnology VECTOR, Federal Service for Surveillance on Consumer Rights Protection and Human Well-being, Koltsovo, Novosibirsk Oblast, Russian Federation 630559

1 Introduction

Rosa majalis Herrm. (*R. cinnamomea* L.) is a species of natural flora of most of Eurasia. According to The State Pharmacopeia of the Russian Federation (14 edition), the fruits of this species, along with other five rosehip species, are the source of ascorbic acid, carotenoids, and flavonoids, which are used for industrial production of vitamin teas, oil, and choleric drug Holosas (*Rosae fructus* 2018). Secondary metabolites of leaves and fruits of rosehip are well studied, including ecological and geographic variation, extraction methods, and antioxidant activity (Nowak and Gawlik-Dziki 2007; Safonova et al. 2012; Polumackanycz et al. 2020; Jiang et al. 2022). Diversity of phenolic acids has been revealed: caffeic, gallic, chlorogenic, rosmarinic, *p*-coumaric, sinapic, and benzoic acids; coumarins (esculin); ellagic acid and its derivatives; glycosides of flavones (luteolin, vitexin, and baicalein), flavonols (quercetin and kaempferol), and flavanones (naringenin). Meanwhile, studies on the roots are only beginning. Detailed identification and quantification of phenolic compounds in the roots of *R. heckeliana* have been performed by Çoruh and Özdoğan (2015). In that study, catechin, ellagic acid, and caffeic acid were found to be major phenolic compounds in these root samples. Catechins are also the most abundant phenolic compounds in the *Rosa acicularis* roots (Olennikov et al. 2021). Meanwhile, benzoic acid and 3-protocatechuic aldehyde are reported to be the main phenolic compounds in the root extract of *R. pimpinellifolia* (Güven et al. 2021).

Hydrolyzable tannins are considered constant components of plants of the family Rosaceae (Olennikov et al. 2021); among them, the diversity of ellagitannins has been revealed (Cai et al. 2005). Ellagic acid is attracting attention due to its antioxidant, anti-inflammatory, antimutagenic, and antiproliferative properties. It has exerted pharmacological effects in various *in vitro* and *in vivo* model systems. Furthermore, it has been well documented that ellagic acid possesses antiallergic, antiatherosclerotic, cardioprotective, hepatoprotective, nephroprotective, and neuroprotective properties (Sharifi-Rad et al. 2022). Ellagitannins usually reach high compared to leaves, flowers,

and roots (Cendrowski et al. 2017). Besides, ellagitannins have an antiviral effect on influenza virus replication (Saha et al. 2010). In the roots of plants from the family Rosaceae, condensed tannins have also been found (Olennikov et al. 2021).

Chemical structure and biological activities of saponins from the roots of representatives of the genus *Rosa* are poorly investigated. Anti-inflammatory activity of triterpenoid saponins from *Rosa rugosa* fruits was discovered a decade ago (An et al. 2011). In recent decades, a lot of studies on saponins of the other genus from the family Rosaceae (*Potentilla*) have been conducted, and their cardioprotective (Enayati et al. 2022), cytotoxic (Walia et al. 2016; Wu et al. 2022), antitumor (Guo and Gao 2013), and anti-hepatitis B virus (Zhao et al. 2008) properties have been revealed. In addition, triterpenoid saponins from some plants exert an antiviral effect on human coronavirus (Cheng et al. 2006).

Biological activity of plant extracts has been shown to depend on the profile of secondary metabolites, which in turn is determined by the solvent and extraction procedure. Solid–liquid extraction methods with different types of solvents are the most commonly used procedures to extract diverse valuable compounds from plant tissues (Stalikas 2007).

In the present study, we aimed to determine profiles of secondary metabolites of *R. majalis* roots in terms of composition of their ethyl acetate (EtOAc), butanol (ButOH), aqueous ethanol (EtOH), and aqueous (H₂O) extracts as sources of antioxidant and anti-influenza compounds.

2 Materials and methods

2.1 Plant material and extraction

The roots of *R. majalis* plants from “Collections of living plants indoors and outdoors” of the Central Siberian Botanical Garden of the Siberian Branch of the Russian Academy of Sciences (CSBG SB RAS; Novosibirsk, Russia) were used as plant material for the extraction of secondary metabolites. H₂O, EtOH 80% v/v, ButOH, and EtOAc extracts were prepared. For each extraction, dry powdered roots were treated three times with appropriate volumes of

Table 1 The main parameters of the extraction of secondary metabolites from *R. majalis* roots

Extract	Mass of dry roots (g)	Volumes of solvent ^a (mL)	Yield (g)	Yield (%) (w/w)
H ₂ O	25	1000, 700, 500	4.77	19.08
EtOH 80% v/v	50	1000, 700, 500	16.02	32.04
ButOH	10	800, 700, 500	1.60	15.97
EtOAc	25	900, 500, 500	1.61	6.44

^aVolumes for three successive treatments

solvents (Table 1) for 30 min in a water bath (65 °C) in a round bottom flask fitted with a reflux condenser.

Each combined extract was passed through a glass funnel filter with a sintered glass disk. After cooling, the filtrates were concentrated using a rotary evaporator (Ika RV3 V, Germany) to remove the solvent. After organic solvents' evaporation, the EtOH and ButOH extracts were treated with chloroform to remove fatty substances, and the H₂O extract was directly incubated with chloroform without concentration. Defatted extracts were dried in a vacuum drying cabinet (LP-402, Hungary) to obtain powder residues. When the EtOAc extract was made, the solvent from the combined extract was being removed until the beginning of precipitation. After that, sixfold volume of chloroform was added with stirring, and the resultant precipitate (the EtOAc extract) was dried in vacuum similarly to the above procedure and used for phytochemical profiling and estimation of biological activity (Table 1). The residual moisture content of the obtained extracts was 3.0–3.5%.

2.2 Spectrophotometric analyses

For the assessment of total catechin concentrations, a vanillin assay was performed. A standard curve was constructed with (+)-catechin from Sigma-Aldrich (Saint Louis, MO, USA) in the concentration range 0.001–0.02 mg mL⁻¹. Precisely weighed dry extract (10–15 mg) was dissolved in 10 mL of 96% ethanol, and 0.8 mL of the sample was placed into two tubes. Then, 4 mL of 1% (w/v) vanillin in concentrated hydrochloric acid was added into the tube with the sample, and an equal volume of concentrated hydrochloric acid was added into the other tube (control). Total volume of the reaction mixture was brought to 5 mL with concentrated hydrochloric acid. The reaction was carried out at room temperature (~20 °C) for 5 min. Absorbance of the extract and standard solution was measured at 502 nm (Sun et al. 1998; Kaidash et al. 2022) by means of an SF-56 spectrophotometer (Lomo, St. Petersburg, Russia). The results were expressed as milligrams of (+)-catechin equivalent per gram of dry extract.

Total flavonol concentration was estimated by a spectrophotometric method based on the reaction of complex formation between flavonols and aluminum chloride (Kaidash et al. 2022). An extract (0.1 mL) was placed into two 5 mL test tubes. Into the first tube, 0.2 mL of a 2% ethanol solution of aluminum chloride was added, and 1–2 drops of 30% acetic acid were added into the second, and the volumes of the reaction solutions were adjusted to 5 mL with 96% ethanol. Next, the solutions were shaken, and after 40 min, the absorbance of the first solution was registered at a wavelength of 415 nm on the spectrophotometer. The second solution served as a control sample. A calibration curve was built with rutin from Chemapol (Mumbai, MH,

India) in the concentration range 0.01–0.1 mg mL⁻¹. The results were expressed as milligrams of rutin equivalent per gram of dry extract.

Quantitation of tannins (hydrolyzable tannins) was performed by their reaction with ammonium molybdate (Fedoseeva 2005). An extract (10 mL) was transferred into a 100 mL volumetric flask, and 10 mL of a 2% aqueous solution of ammonium molybdate was added. The volume of the flask content was brought to the nominal volume with purified water, and the mixture was incubated for 15 min. The intensity of the resulting color was measured at 420 nm. A calibration curve was constructed with tannin (Sigma, St. Louis, MO, USA) in the range 0.01–0.1 mg mL⁻¹. The results were expressed as milligrams of tannin equivalent per gram of dry extract.

For determining the total level of phenolic acids, Arnow's test was performed (Jain et al. 2017). To 1 mL of an extract, 5 mL of distilled water was added. Into this solution, 1 mL of 0.5 M HCl, 1 mL of Arnow's reagent (10.0 g of sodium molybdate and 10.0 g of sodium nitrate in 100.0 mL of distilled water), and 1 mL of 1 M NaOH were added sequentially. The volume was brought to 10 mL using distilled water, and absorbance was measured at 490 nm. A blank sample was prepared too, which contained all reagents except the extract. A calibration curve was built using caffeic acid (Serva, Heidelberg, Germany) in the range 0.02–0.1 mg mL⁻¹. The results were expressed as milligrams of caffeic acid equivalent per gram of dry extract.

The total phenolic concentration was determined with the help of the Folin–Ciocalteu reagent (Blainski et al. 2013). An extract (0.5 mL) was placed in a 5 mL volumetric flask, then 2.5 mL of the Folin–Ciocalteu reagent (diluted 1:10 with distilled water, v/v) and 2 mL of a 7.5% aqueous solution of sodium carbonate were added, and the flask was shaken well. In a blank sample, the reagents were combined with distilled water instead of the extract. The mixture was kept at 45 °C in a water bath for 15 min. Absorbance was measured at 765 nm (Kaidash et al. 2022). A standard curve was constructed with gallic acid from Sigma-Aldrich (Saint Louis, MO, USA) in the concentration range 0.002–0.01 mg mL⁻¹. The results were expressed in milligrams of gallic acid equivalent per gram of dry extract.

For quantification of total saponins, a direct spectrophotometric method was employed based on a reaction between triterpene saponins and sulfuric acid (Pisarev et al. 2009). A precisely weighed dry extract (0.5–1.0 g) was dissolved in 10 mL of a mixture of glacial acetic acid, hydrochloric acid, and purified water (3.5:1.0:5.5) for hydrolysis of glycosides via 2 h incubation in a boiling water bath. Then, the hydrolysate was cooled, diluted with distilled water in a 1:2 ratio, and filtered. The residue on the filter was rinsed with distilled water, put with the filter into a volumetric 25 mL flask, and dissolved in 25 mL of hot ethanol. To 1 mL of

this ethanol solution, 4 mL of concentrated sulfuric acid was added, and absorbance at 310 nm was measured after 10 min. Concentrated sulfuric acid served as a blank. To calculate saponin concentration, a calibration curve for oleanolic acid (Sigma-Aldrich, Saint Louis, MO, USA) was built in the range 0.01–0.4 mg mL⁻¹. The results were expressed as milligrams of oleanolic acid equivalent per gram of dry extract.

All the aforementioned assays were performed on three biological and three technical replicates.

To assess the levels of these metabolites in the roots, their concentrations were calculated from extract concentrations and weights of roots.

2.3 HPLC analysis

Samples for HPLC were preparing by dilution of 10 mg of a dry extract in 10 mL of 50% aqueous ethanol (v/v). The solutions were diluted with double-distilled water at a 1:3 (v/v) ratio and passed through a C 16 cartridge (BioChemMack, Moscow, Russia). HPLC analysis of the resultant extracts was performed on an Agilent 1200 instrument with a diode array detector and the ChemStation software for data processing (Agilent Technologies, USA). The chromatographic separation was performed at 25 °C on a Zorbax SB-C18 Column (4.6 × 150 mm, 5 μm internal diameter). The mobile phase consisted of MeOH (solvent A) and 0.1% orthophosphoric acid in water (solvent B).

Separation of phenolic compounds in the extracts was carried out via gradient 1 (Table 2). To characterize the flavonoid aglycone profile and evaluate the concentrations of bound forms of gallic and ellagic acids, extracts were hydrolyzed with 2N HCl for 1 h in a water bath. The hydrolyzed extracts were passed through a C 16 cartridge and separated via gradient 2 (Table 2). For separation of catechins, gradient 3 was utilized (Table 2). The flow rate for all these gradients was set to 1 mL min⁻¹. The sample injection volume was 10 μL, and absorbance was measured at 220, 255, 270, 290, 325, 340, 360, and 370 nm. The quantification of phenolic compounds was conducted by the external standard method. Identification of the known compounds was carried out by comparing retention times and UV spectra of the analyzed peaks with the peaks of standard samples of gallic acid from Fluka (Munich, Germany); ellagic acid, (±)-catechin, isoquercitrin, and rutin from Sigma-Aldrich (Saint Louis, MO, USA); and epigallocatechin gallate and L-epicatechin from Teavigo (Gevelsberg, Germany). Standard stock solutions at a concentration of 1 mg mL⁻¹ in methanol were employed to build calibration curves in the concentration range of 2–100 μg mL⁻¹. Quantification of unidentified hydroxybenzoic acid 9 was conducted based on the calibration curve of gallic acid, and concentration of flavonol glycoside 10 was determined by means of the isoquercitrin

Table 2 Gradient conditions for HPLC analysis of phenolic compounds in the extracts of *R. majalis* roots

Time (min)	A (%)	B (%)
Gradient 1		
0	32	68
27	33	67
38	46	54
50	56	44
54	100	0
56	32	68
Gradient 2		
0	50	50
15	52	48
17	100	0
20	50	50
Gradient 3		
0	22	78
30	70	30
32	100	0
36	22	78

A, MeOH; B, 0.1% orthophosphoric acid in water

calibration curve. Concentrations of the compounds in the extracts were expressed in milligrams per gram of dry mass of an extract. To assess the levels of the compounds in the roots, their concentrations were calculated in milligrams per gram of dry mass of the roots.

2.4 Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was carried out to evaluate the ability of the extracts to scavenge free radicals of DPPH (Kaidash et al. 2022). For the assay, a 2 mL aliquot of an extract was mixed with 3 mL of a DPPH solution (62 μg mL⁻¹ in ethanol). After 40 min incubation in the dark at room temperature, absorbance (A) was measured at 517 nm against a blank. Free-radical scavenging activity was calculated as percentage inhibition via the following formula:

$$I(\%) = (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100,$$

where A_{blank} is the absorbance of the control solution (containing all reagents except the tested extracts) and A_{sample} is the absorbance of the sample. The results were expressed as half-maximal inhibitory concentration (IC₅₀), defined as the antioxidant concentration that causes a 50% loss of DPPH radical concentration. These data were also converted to trolox equivalents. IC₅₀ values were calculated by means of GraphPad Prism v.6.01 (GraphPad Software, USA). Solutions of trolox

(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and ascorbic acid (concentrations 2.5–50.0 $\mu\text{g mL}^{-1}$) served as a positive control.

2.5 Antiviral assay

Influenza virus strains A/chicken/Kurgan/05/2005 (H5N1) and A/Aichi/2/68 (H3N2) were obtained from the State Collection of Viral Infections and Rickettsioses Agents at the State Research Center of Virology and Biotechnology VECTOR (Koltsovo, Novosibirsk Oblast, Russia). All experiments with infectious materials were conducted at the State Research Center of Virology and Biotechnology VECTOR (Teplyakova et al. 2012). The virus strains were cultivated in 10-day embryonated chicken eggs. The virus concentration in the samples was determined by their titration in cultured Madin-Darby canine kidney (MDCK) cells according to the Spearman–Kärber algorithm (Spearman 1908; Kärber 1931).

The antiviral activity of the *R. majalis* root extracts was determined as IC_{50} , i.e., the concentration of the extract in the culture medium at which 50% of MDCK cells are preserved in the infected monolayer. The cytotoxic activity of the extracts was evaluated in a monolayer of cultured MDCK cells in 96-well plates containing the RPMI 1640 medium (Biolot, Russia) supplemented with 5% of fetal bovine serum (HyClone, USA). To determine the cytotoxicity, 10 mg of a dry extract was dissolved in 1 mL of RPMI 1640 without serum and diluted 5-, 10-, 20-, 40-, 100-, and 1000-fold with the same medium. Aliquots of each dilution (100 μL) were placed in plate wells (four wells for each dilution) and incubated at 37 °C, 5% CO_2 , and 100% humidity for 2 days. Cytotoxic effects of the extracts were assessed under an inverted microscope. Maximum nontoxic concentration was defined as a concentration at which 100% of the MDCK cells stayed alive in the monolayer, and a half-maximal toxic concentration (TC_{50}) meant the concentration of the extract causing the death of 50% of cells in the monolayer in each well. The selectivity index of the extract (SI) was calculated as the $\text{TC}_{50}/\text{IC}_{50}$ ratio (Al Jabri et al. 1996).

2.6 Statistical analysis

Concentrations of total of catechins, flavonols, tannins, phenolic acids, and phenolic compounds and of individual phenolic compounds in the roots and in the root extracts were expressed in milligrams per gram of dry mass of the roots or dry root extract, respectively. IC_{50} in the DPPH assay is presented as micrograms of dry extract per milliliter of the solvent ($\mu\text{g mL}^{-1}$). The dry matter percentage was estimated by the gravimetric method.

The data were analyzed in the STATISTICA 10.0 software (Statsoft Inc., Tulsa, OK, USA), are reported as

mean \pm standard error from three biological and three technical replicates, and were compared by Duncan's multiple-range test. Differences between the means were considered statistically significant at $P < 0.05$.

3 Results

3.1 Secondary metabolite contents and antioxidant activity

Each of the four obtained extracts had a distinct profile of secondary metabolites. The highest yield of dry matter from extracted roots was shown by the EtOH extract (32.04%). It significantly exceeded this parameter of the H_2O extract, exceeded this parameter of the ButOH extract by more than twofold, and exceeded that of the EtOAc extract by more than fivefold (Table 1). Meanwhile, concentration of total catechin in the EtOAc extract (43.23 mg g^{-1}) was the highest and exceeded this parameter in the other extracts by threefold to fourfold (Table 3). The lowest catechin content was found in the EtOH extract. The ButOH extract had the greatest content of flavonols (9.13 mg g^{-1}). It was greater than that in the H_2O extract by almost twofold and greater than that in the EtOH and EtOAc extracts by approximately 5.5-fold.

Tannin content varied slightly among the extracts. The maximum (48.19 mg g^{-1} ; in the EtOAc extract) exceeded the minimum (31.83 mg g^{-1} ; in the EtOH extract) by 1.5-fold. Phenolic acids, just as catechins, were most abundant in the EtOAc extract (57.56 mg g^{-1}). In the H_2O and EtOH extracts, phenolic acid levels were lower than the aforementioned maximum by approximately twofold, and in the ButOH extract, they were lower > 5 times. The total phenolic content corresponded to the concentration of phenolic acids; both parameters were the highest in the EtOAc extract (53.79 and 57.56 mg g^{-1}) and the lowest in the ButOH extract (16.46 and 11.40 mg g^{-1}). The saponin content was also consistent with the total phenolic content: the saponin content had a maximum in the EtOAc extract (48.92 mg g^{-1}) and a minimum in the ButOH extract (16.13 mg g^{-1}).

Thus, the EtOAc extract showed abundance of most classes of the tested secondary metabolites except for flavonols, whose concentration was the highest in the ButOH extract.

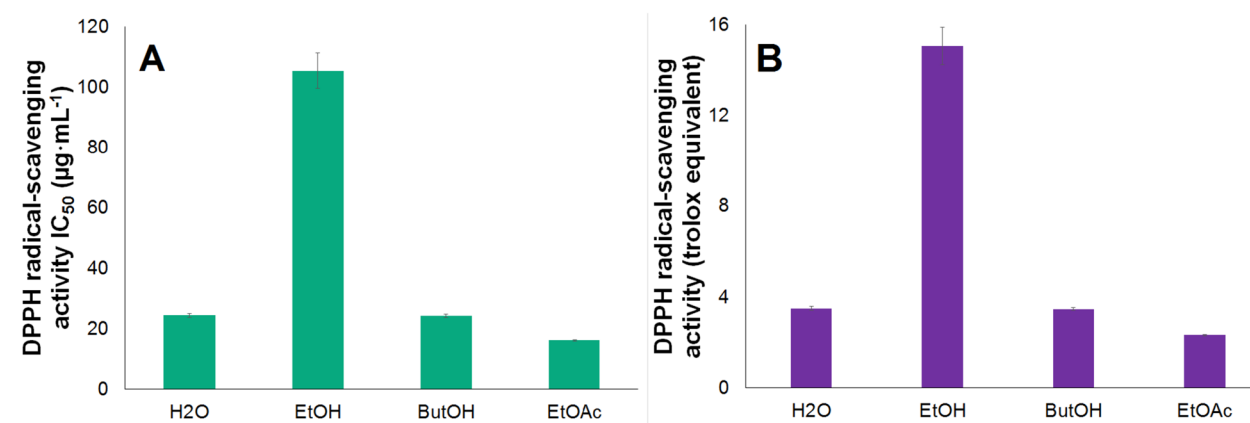
The EtOAc extract also manifested the highest DPPH radical scavenging activity (16.18 $\mu\text{g mL}^{-1}$), whereas the H_2O and ButOH extracts were slightly inferior (24.40 and 24.24 $\mu\text{g mL}^{-1}$, respectively), and the EtOH extract contained the lowest radical scavenging activity (105.33 $\mu\text{g mL}^{-1}$) (Fig. 1).

Evaluation of contents these secondary metabolites in dry mass of the roots enabled us to compare recovery

Table 3 Secondary metabolite contents in dry extracts of the roots (E) and in dry mass of the roots (R) of *R. majalis*

Content (mg g ⁻¹)	Extracts			
	H ₂ O	EtOH	ButOH	EtOAc
Catechins				
E	15.33 ± 0.03b	11.39 ± 0.00d	13.72 ± 0.02c	43.23 ± 0.02a
R	2.92 ± 0.01b	3.65 ± 0.00a	2.20 ± 0.00b	2.78 ± 0.00ab
Flavonols				
E	2.37 ± 0.04b	1.42 ± 0.01d	9.14 ± 0.08a	1.68 ± 0.01c
R	0.45 ± 0.01b	0.45 ± 0.00b	1.46 ± 0.01a	0.11 ± 0.00c
Tannins				
E	35.56 ± 0.92b	31.83 ± 0.76c	32.07 ± 0.87c	48.19 ± 1.12a
R	6.78 ± 0.18b	10.20 ± 0.24a	5.13 ± 0.14c	3.10 ± 0.07d
Phenolic acids				
E	20.37 ± 0.68c	24.95 ± 0.93b	11.40 ± 0.23d	57.56 ± 1.48a
R	3.89 ± 0.13b	8.00 ± 0.30a	1.82 ± 0.04c	3.71 ± 0.09b
Total phenolic content				
E	18.12 ± 0.42c	25.56 ± 0.45b	16.46 ± 0.27d	53.79 ± 1.31a
R	3.46 ± 0.09b	8.19 ± 0.14a	2.63 ± 0.04c	3.46 ± 0.08b
Saponins				
E	16.76 ± 0.09c	42.79 ± 0.44b	16.13 ± 0.07d	48.92 ± 0.55a
R	3.20 ± 0.02b	13.71 ± 0.14a	2.58 ± 0.01c	3.15 ± 0.04b

E, concentration of a compound in milligrams per gram of dry mass of an extract; R, concentration of a compound in milligrams per gram of dry mass of the roots; Data are mean ± SE (n = 9). Means followed by the same letter within a row do not differ significantly ($P < 0.05$)

**Fig. 1** Radical scavenging activity of the extracts of the roots of *R. majalis*: half-maximal inhibitory concentrations of the extracts (IC₅₀) (A) and trolox equivalents (B). IC₅₀ values for trolox and ascorbic acid were 7.47 ± 0.01 and 8.69 ± 0.16 µg mL⁻¹, respectively

levels (completeness of the extraction) among the solvents (Table 3). Aqueous ethanol yielded the highest recovery of the majority of the assayed compounds (catechins, tannins, phenolic acids, total phenolics, and saponins), whereas butanol most completely extracted flavonols.

Chromatographic profiling confirmed the EtOAc extract as containing the highest level of phenolic compounds (Table 4). In this extract, the highest concentrations of gallic acid, including its free form (38.68 and 2.79 mg g⁻¹, respectively), and catechin (30.83 mg g⁻¹) were revealed.

They were the main phenolic compounds in this extract and, along with total ellagic acid, contributed to the total phenolic content of all tested extracts the most. Total ellagic acid was dominant in the EtOH extract (34.95 mg g⁻¹). Meanwhile, ellagic acid in free form and total gallic acid were major phenolic compounds in the H₂O extract (22.38 and 32.64 mg g⁻¹, respectively). This extract also showed higher—as compared to the other extracts—levels of ellagic acid glycoside (16.10 mg g⁻¹). Noteworthy is the presence of two minor phenolic compounds in the ButOH and EtOAc

Table 4 Concentrations of phenolic compounds in the extracts of *R. majalis* roots (mg g⁻¹ of dry extract) (E) and in dry mass of the roots (R)

No	Compound	<i>t_R</i> (min)*	λ_{\max} (nm)	Extracts			
				H ₂ O	EtOH 80% (v/v)	ButOH	EtOAc
1	Free gallic acid						
	E	2.3 ²	216, 272	2.71 ± 0.18a	2.69 ± 0.14a	0.28 ± 0.02b	2.79 ± 0.17a
	R			0.52 ± 0.03b	0.86 ± 0.04a	0.05 ± 0.00d	0.18 ± 0.01c
	Total gallic acid**						
2	E	1.6 ³	216, 272	32.64 ± 1.11b	24.98 ± 0.93c	35.51 ± 1.10ab	38.68 ± 0.99a
	R			2.10 ± 0.07d	4.00 ± 0.15c	11.38 ± 0.35a	7.38 ± 0.19b
	Catechin						
	E	4.7 ²	280	10.37 ± 0.86c	9.44 ± 0.77d	18.43 ± 0.85b	30.83 ± 1.26a
3	R			1.98 ± 0.16b	3.02 ± 0.25a	2.95 ± 0.14a	1.99 ± 0.08b
	Epigallocatechin gallate						
	E	6.6 ²	280	2.21 ± 0.19a	0.51 ± 0.03d	0.62 ± 0.03c	1.68 ± 0.11b
	R			0.42 ± 0.04a	0.16 ± 0.01b	0.10 ± 0.00c	0.11 ± 0.01c
4	L-epicatechin						
	E	7.6 ²	280	1.97 ± 0.09b	3.57 ± 0.21a	1.99 ± 0.02b	2.02 ± 0.15b
	R			0.38 ± 0.02b	1.14 ± 0.07a	0.32 ± 0.00c	0.13 ± 0.01d
	Ellagic acid glycoside						
5	E	18.7 ¹	256, 306 sh., 350 sh., 367	16.10 ± 1.11a	5.60 ± 0.38c	9.81 ± 0.74b	13.62 ± 1.03b
	R			3.07 ± 0.21a	1.79 ± 0.12b	1.57 ± 0.12b	0.88 ± 0.07c
	Quercetin 3- <i>O</i> -glucoside (isoquercitrin)						
	E	19.5 ¹	268, 362	Nd	Nd	Nd	0.55 ± 0.06a
6	R			Nd	Nd	Nd	0.04 ± 0.00a
	Quercetin 3- <i>O</i> -rutinoside (rutin)						
	E	20.4 ¹	259, 266 sh., 299 sh., 359	Nd	Nd	Nd	0.71 ± 0.06a
	R			Nd	Nd	Nd	0.05 ± 0.00a
7	Free ellagic acid						
	E	22.4 ¹	254, 366	22.38 ± 1.85a	9.69 ± 0.75b	5.08 ± 0.42c	9.28 ± 0.64d
	R			4.27 ± 0.35a	3.11 ± 0.24b	0.81 ± 0.06c	0.60 ± 0.05d
	Total ellagic acid**						
8	E	3.8 ³	254, 366	32.33 ± 1.47a	34.95 ± 2.58a	33.91 ± 2.12a	8.10 ± 0.33b
	R			10.36 ± 0.47c	11.20 ± 0.83a	5.43 ± 0.34b	0.52 ± 0.02d
	Hydroxybenzoic acid						
	E	34.1 ¹	216, 290	9.44 ± 0.73b	Nd	4.87 ± 0.29b	11.91 ± 0.97a
9	R			1.80 ± 0.14a	Nd	0.78 ± 0.05b	0.77 ± 0.06c
	Flavonol glycoside						
	E	35.9 ¹	266, 360	Nd	Nd	0.57 ± 0.04a	0.39 ± 0.02b
	R			Nd	Nd	0.09 ± 0.01a	0.02 ± 0.00b

Data are mean ± SE (n=9). Means followed by the same letter within a row do not differ significantly ($P < 0.05$)

t_R retention time, *Nd* not detected

*The superscripted number indicates the name of the gradient

**Total gallic and ellagic acid concentrations were calculated after acid hydrolysis

extracts, which were absent in the H₂O and EtOH extracts: hydroxybenzoic acid 9 (4.87 and 11.91 mg g⁻¹) and flavonol glycoside 10 (0.57 and 0.39 mg g⁻¹, respectively). Only in these extracts, in contrast to H₂O and EtOH ones, individual flavonol glycosides were found. Total flavonol concentration was the highest in the EtOAc extract (1.65 mg g⁻¹), which contained all three flavonol glycosides (isoquercitrin, rutin,

and flavonol glycoside 10). Of these compounds, only flavonol glycoside 10 was detectable in the ButOH extract.

3.2 The antiviral assay

The extracts differed in their ability to inhibit the replication of the tested strains of viruses. All the extracts manifested

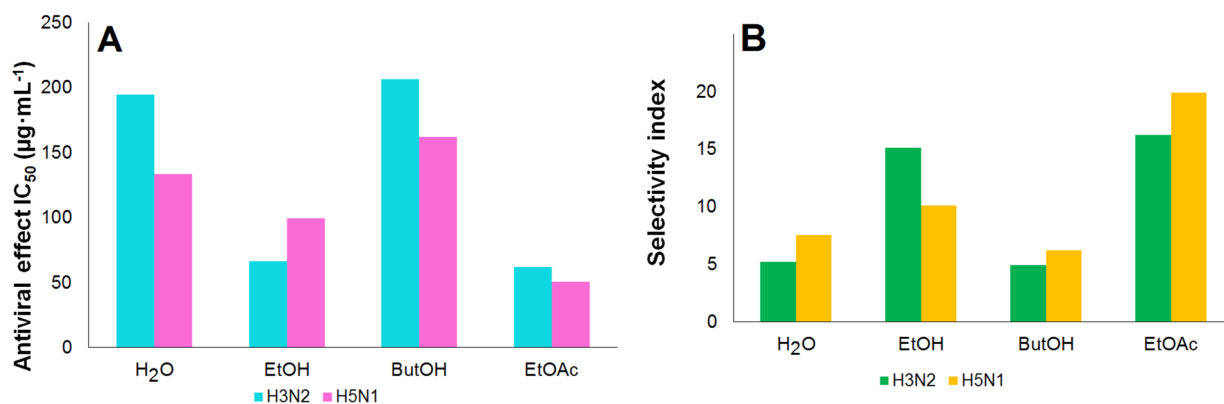


Fig. 2 Antiviral effects of *R. majalis* root extracts against influenza virus subtypes H3N2 (strain A/Aichi/2/68) and H5N1 (strain A/chicken/Kurgan/05/2005): half-maximal inhibitory concentrations of

the extracts in the antiviral assay (IC_{50}) (A) and selectivity indexes (B). Half-maximal toxic concentrations (TC_{50}) of all the extracts are $>1000 \mu\text{g mL}^{-1}$.

extremely low toxicity to MDCK cells (Fig. 2). IC_{50} values against both tested influenza virus subtypes significantly varied among these extracts. The EtOAc, ButOH, and H₂O extracts inhibited the H5N1 subtype with lower IC_{50} than the H3N2 subtype, whereas the EtOH extract inhibited subtype H3N2 more effectively than H5N1. The EtOAc extract showed the strongest activity toward both virus subtypes, and the ButOH extract showed the weakest one. The SI of the EtOAc extract was also the highest (16.2 and $19.9 \mu\text{g mL}^{-1}$ for H3N2 and H5N1, respectively).

The EtOH extract also had a pronounced antiviral effect against virus subtype H3N2, with IC_{50} of $66.1 \mu\text{g mL}^{-1}$ and the SI of 15.1.

4 Discussion

In this study, possible therapeutic compounds and the phenolic profile of *R. majalis* roots and their extracts were determined for the first time. To date, there has been lack of information about biologically active compounds of *R. majalis*, especially in the roots. The total phenolic content of *R. majalis* roots from some regions of Ukraine has been determined earlier (Oproshanska et al. 2021). These values ranged from 43.7 to 44.0 mg g^{-1} of absolutely dry mass of roots. These levels are substantially higher in comparison with the total phenolic content in our samples of *R. majalis* roots (8.19 mg g^{-1}) (Table 3). This difference is most likely due to climatic factors and quantification techniques. In that work, the researchers did not present a phenolic profile of *R. majalis* roots. In the roots of *R. canina*, just like in our samples of *R. majalis* roots, catechins were the most abundant. Meanwhile, in *R. canina* roots, epigallocatechin was the main phenolic constituent, in contrast to our samples, where catechin prevailed.

In our samples, gallic and ellagic acids were other major phenolic compounds. The observed manifold increase in concentrations of gallic and ellagic acids in the extracts after hydrolysis indicates the presence derivatives of gallic and ellagic acids: glycosides and ellagitannins. Ellagitannins are abundant in plants of the family Rosaceae and are reported to produce gallic and ellagic acids as a result of acid hydrolysis (Yamada et al. 2018). Nonetheless, a comparison of levels of the class of phenolic compounds (Table 3) with concentrations of individual phenolic compounds (Table 4) suggests that the tannin content primarily corresponds to concentrations of catechin and total gallic acid. The highest tannin content was revealed in the EtOAc extract, which contains the lowest level of total ellagic acid and the highest level of total gallic acid. This pattern points to abundance of gallotannins in the roots. Dominance of gallotannins, which are related to ellagitannins, and negligible concentration of the latter in the roots of *Rosa* species have been demonstrated before by Olennikov et al. (2021). Meanwhile, ellagic acid in the roots of *R. acicularis* has been detected mainly in the glycosidic form (Olennikov et al. 2021). In our study, extracts of *R. majalis* roots, especially aqueous and ethyl acetate ones, also displayed a high level of ellagic acid glycoside (16.10 and 13.62 mg g^{-1}), with only 3.07 mg g^{-1} in the roots. Ellagitannins (tellimagrandins and rugosins) are common among species of the genus *Rosa* but have been found before primarily in other parts of the plants, outside the roots (Cunja et al. 2014; Olennikov et al. 2021). For the roots of *R. acicularis*, abundance of catechins and their oligomers has been demonstrated (Olennikov et al. 2021). Condensed tannins with potent free-radical scavenging activity have also been identified in the roots of *R. multiflora* (Park et al. 2014) and *R. cymosa* (Yoshida et al. 1993). Thus, in agreement with the literature, our results indicate that catechins and gallotannins are the main secondary metabolites in the roots of *Rosa* species, including *R. majalis*.

The recovery of each phenolic compound varied depending on the solvent. The decline of some compounds' concentrations after the extraction of the roots with the solvents (Table 3, 4) implies that aqueous ethanol extracted free gallic acid, catechin, L-epicatechin, and total ellagic acid better than the other solvents did. ButOH and EtOAc extracted total gallic acids the most completely and also extracted minor compounds 9 and 10. In this regard, water most completely extracted epigallocatechin gallate, ellagic acid glycoside, free gallic and ellagic acids, and hydroxybenzoic acid 9. In spite of a moderate recovery of many compounds in the EtOAc extract, concentrations of three main secondary metabolites (free gallic acid, total gallic acid, and catechin) in it were the highest. This finding explains the elevated antioxidant activity of this extract. The high free-radical scavenging activity of the aqueous and butanol extracts most likely is due to elevated levels of total gallic and ellagic acids. Ellagic acid glycoside showed high concentrations in the aqueous and ethyl acetate extracts and arguably contributed to the potent antioxidant effect. The weak antiradical effect of the aqueous ethanol extract coincided with reduced levels of total gallic acid, catechin, epigallocatechin gallate, and ellagic acid glycoside as well as the absence of flavonol glycosides. Several articles indicate a positive correlation between total phenolic or total flavonoid concentrations and antioxidant capacity of various extracts (Llorent-Martínez et al. 2017; Sarikurkcu et al. 2019; Karpova et al. 2020; Butkevičiūtė et al. 2022). Likewise, our results confirm potent antioxidant properties of gallic acid, ellagic acid, and catechins, particularly epigallocatechin gallate, which have been demonstrated earlier (Badhani et al. 2015; Bernatoniene and Kopustinskiene 2018; Tošović and Bren 2020).

Free ellagic acid and its glycoside apparently contributed to the antioxidant activity of the assayed extracts. The highest level of these compounds was found in the aqueous extract, which has high radical scavenging activity, and the lowest level was discovered in the aqueous ethanol extract, which manifested only a minimal radical scavenging effect. The total ellagic acid content of the extracts did not correspond to the antiradical effect but can be considered an indicator of the level of the active free form. Total ellagic acid concentration in the extracts was found to be at the same level as in root extracts of some other representatives of the family Rosaceae or surpassed it (Dias et al. 2015).

Pronounced anti-influenza activities of the ethyl acetate and aqueous ethanol extracts corresponded to increased levels of saponins. Triterpene saponins have been reported to possess strong anti-influenza effects (Mair et al. 2018; Hu et al. 2021). Saponins are surfactant compounds having a variety of biological activities important for plant ecology and human health (Moses et al. 2014). Triterpenoid saponins from *R. rugosa* have anti-inflammatory and antidiabetic activities (An et al. 2011; Thao et al. 2014). Saponins of *R.*

laevigata have shown antifungal activity (Yuan et al. 2008). Antiviral activity of triterpene saponins against hepatitis C virus has been revealed (Kong et al. 2013). Identification of individual saponins and of their biological activity is a good subject for further investigation.

Only a few species of the genus *Rosa* have been examined for secondary metabolites and biological activity of the roots. Contents of biologically active metabolites in the roots of the genus have substantially varied among species and due to some differences in the environment and in techniques of chemical analysis. Nonetheless, some similarities and differences are of interest. For instance, the ethyl acetate fraction of a methanol extract from the roots of *R. heckeliana*, just as the ethyl acetate extract from our samples of *R. majalis* roots, has shown the highest recovery of phenolic compounds as compared to a series of fractions, including n-butanol and aqueous ones (Çoruh and Özdoğan 2015). In that paper, the total phenolic content in this fraction is significantly lower (8.8 mg g^{-1}) compared to the ethyl acetate extract from the roots of *R. majalis* in our work (53.79 mg g^{-1}).

Metabolites in ethanolic and aqueous extracts of *R. persica* have been quantified by ultra-high-performance liquid chromatography coupled with electrospray ionization-photodiode array detection mass spectrometry (Koohestanian et al. 2022). In that study, gallic and chlorogenic acids were reported to be the most abundant phenolic compounds in these extracts. Their reported concentrations of gallic acid (0.46 and 0.18 mg g^{-1} in ethanolic and aqueous extracts, respectively) and catechin (0.035 and 0.011 mg g^{-1}) are significantly lower than those in the extract of *R. majalis* in our study. In the extracts of *R. persica*, ellagic acid has not been detected (Koohestanian et al. 2022). Consequently, *R. persica* and *R. majalis* significantly differ in the phenolic profile of the roots, including the main phenolic compounds. Additionally, free-radical scavenging activity detected in that study in the ethanolic extract of *R. persica* roots ($\text{IC}_{50} 19.22 \mu\text{g mL}^{-1}$) exceeds this activity in all the extracts of *R. majalis* in our work, except for the ethyl acetate extract ($\text{IC}_{50} 16.18 \mu\text{g mL}^{-1}$). The activity of the water extract of *R. persica* ($\text{IC}_{50} 86.00 \mu\text{g mL}^{-1}$) in that study is inferior to all the extracts of *R. majalis* in our work, except for the aqueous ethanol extract of *R. majalis* ($\text{IC}_{50} 105.33 \mu\text{g mL}^{-1}$).

The phenolic profile of the roots of *R. pimpinellifolia* has also been shown to differ from the profile of *R. majalis* roots (Güven et al. 2021). Ellagic acid has not been detected in a methanol extract just as in the extracts from the roots of *R. persica*. Concentration of gallic acid (0.11 mg g^{-1} of dry extract) in this extract is significantly lower than the one in our extracts of *R. majalis* (0.28 – 2.79 mg g^{-1} of dry extracts). The main phenolic compounds of *R. pimpinellifolia* roots were shown to be benzoic acid and protocatechuic aldehyde. The total phenolic content in the methanol extract of

R. pimpinellifolia roots (9.795 mg g⁻¹ of dry extract) (Güven et al. 2021) is substantially lower as compared to our extracts from the roots of *R. majalis* (16.46–53.79 mg g⁻¹).

Phenolic profiles of *R. rugosa* root extracts were found to feature gallic acid, rutin, hyperoside, and isoquercitrin as the main phenolic compounds (Olech et al. 2017). Concentrations of the sum of the individual flavones and flavonols in *R. rugosa* root extracts are lower (0.03 and 0.46 mg g⁻¹ of dry extract of infusion and tincture, respectively) in comparison with the sum of flavonol glycosides in our ButOH and EtOAc extracts of *R. majalis* roots (0.57 and 1.65 mg g⁻¹ of dry extract). Catechins and ellagic acid have not been detected in the root extracts of *R. rugosa* (Olech et al. 2017). Nevertheless, antiradical activities documented for these extracts (1.01 and 1.16 trolox equivalents for tincture and infusion, respectively) (Olech et al. 2012) are higher in comparison with the activity of all our extracts of *R. majalis* (2.31–15.05 trolox equivalents). Kim et al. (2022) demonstrated a lower level of antiradical activity in *R. rugosa* extracts from different organs (45.3–224.3 µg mL⁻¹).

Leaves and fruits of some *Rosa* species are used against influenza in folk medicine. Remedies from the roots of *R. canina* (Sargin 2021) and *R. damascena* (Boskabady et al. 2011) have also been mentioned in this regard. Low toxicity of our extracts of *R. majalis* roots seems to be in compliance with real-world requirements for antiviral medications. IC₅₀ values of our extracts indicate moderate anti-influenza activity against the tested virus strains. Lower IC₅₀ levels have been documented before for a methanol extract of *Dianthus superbus* var. *longicalycinus* against a similar strain: A/Vic/3/75 (H3N2) (6.61 µg mL⁻¹) (Gansukh et al. 2016). By contrast, higher IC₅₀ for plant extracts (> 100 µg mL⁻¹) has also been registered (Li et al. 2019). The strongest antiviral properties of our EtOAc and EtOH root extracts from *R. majalis*, which contain high levels of saponins, tannins, and phenolic acids (including free gallic acid), suggest that these compounds are anti-influenza active ingredients. This finding provides a basis for further investigation into this plant material as a source of antiviral agents. Antioxidant properties of catechins and ellagic acid have good prospects for a search for related activities, e.g., anti-inflammatory, anticarcinogenic, neuroprotective, antiatherosclerosis, anti-hypertensive, and cardioprotective (Tošović and Bren 2020).

5 Conclusion

Our results expand knowledge about secondary metabolites and biological activity of the roots of a poorly studied species from the genus *Rosa*: *R. majalis*. To profile biologically active compounds in detail, four solvents were used here for the extraction, and each extract showed a

distinct pattern and level of the extracted compounds. Aqueous ethanol perfectly extracted most of secondary metabolites from the roots, except flavonols. The EtOH extract contains considerable levels of tannins, total phenolic compounds, free gallic acid, L-epicatechin, total ellagic acid, and saponins (31.83, 25.56, 2.69, 3.57, 34.95, and 42.79 mg g⁻¹, respectively). In the meantime, the highest concentrations of the majority of these compounds were detected in the EtOAc extract (48.19, 53.79, 2.79, 2.02, 8.10, and 48.92 mg g⁻¹). Flavonols proved to be minor secondary metabolites in the roots and were extracted with butanol best of all. Individual flavonol glycosides were found only in the butanol and ethyl acetate extracts. The phenolic profiles indicate that total gallic acid is the main secondary metabolite in all the extracts (24.98–38.68 mg g⁻¹). Catechin is also abundant in the ethyl acetate extract (30.83 mg g⁻¹). Free ellagic acid is a major compound in the aqueous extract (22.38 mg g⁻¹), and total ellagic acid contributes appreciably to the total phenolic content in all the extracts (32.33–34.95 mg g⁻¹), except for the ethyl acetate extract (8.10 mg g⁻¹). High free-radical scavenging activity of the EtOAc, H₂O, and ButOH extracts suggests that catechins are potent antioxidants. These extracts can be regarded as sources of antioxidants. On the other hand, the pronounced anti-influenza activities of the EtOAc and EtOH extracts imply that saponins, along with phenolic compounds, contribute to antiviral properties. This paper uncovered good potential of the extracts of *R. majalis* roots as effective anti-influenza plant-based remedies with high antioxidant activity crucial for minimizing free-radical damage (including anti-inflammatory, neuroprotective, and anticancer properties).

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Author contributions EAK performed studies by means of HPLC, carried out statistical analyses, and wrote the manuscript; VAK performed studies by means of HPLC, drafted the manuscript, and provided critical readings of the manuscript; EPK designed the study and provided critical readings of the manuscript; TMS carried out the DPPH assay and spectrophotometric analyses; OYV collected the samples of the plants, designed the study, and revised the manuscript; NAM designed the antiviral assay and provided critical readings of the manuscript; EIF, OYM, and EVM performed the antiviral assay, prepared tables, and wrote the manuscript. All authors discussed the results and commented on the manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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