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COMPARATIVE PHARMAKOGNOSTICAL STUDY OF ROOTS OF ROSA MAJALIS HERRM. AND ROSA CANINA L.

Tetiana Oproshanska, Olga Khvorost, Kateryna Skrebtsova, Konradas Vitkevicius

*The **aim** is to conduct a comparative pharmacognostical study of the series of roots of Rosa (R.) majalis Herrm. and Rosa (R.) canina L. with the establishment of diagnostic features of morphological and anatomical structure and boundary limits of numerical indicators of raw materials.*

***Materials and methods.** The fresh and dry raw materials were used to study the macroscopic microscopic features by microscope Delta optical BioLight 300 (Poland). Determination of total polyphenols was performed by spectrophotometry (on a spectrophotometer Optizen POP (Korea)) and HPLC (chromatograph an Agilent 1200 3 D LC System Technologies (USA)).*

***Results.** The morphological (nature of the surface (periderm) and fracture) and anatomical (color of cell walls and their cavities; location of the sclerenchyma; the presence of a crystalline coating of the sclerenchyma at the root of R. canina; of various elements of the remains of the tetraarchic conducting bundle in the center of the root) diagnostical features of roots of R. majalis and R. Canina were established.*

Comparing the numerical values of loss on drying (not more than 10 %), total ash (not more than 5 %), extractable matter (not less than 9 %) and the quantitative content of total polyphenols (not less than 4 %) it was determined that both types of raw materials according to these indicators are almost indistinguishable.

***Conclusions.** Loss on drying, total ash, extractable matter and content of total polyphenols of the root of R. majalis and R. canina do not have significant differences, that is why the root of both plant species can be used as medicinal plant raw materials such as "Rose root". The obtained data will be used in further research when creating methods of quality control of plant raw materials and phytomedicines*

***Keywords:** root, Rosa, diagnostical features, morphological and anatomical structure, numerical indicators*

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1. Introduction

The genus of Rose belongs to the family Rosaceae and includes from 140 to 400 species that grow in the temperate and subtropical zone of the northern hemisphere [1–3]. The most species of the genus are cultivated (*Rosa (R.) majalis* Herrm., *R. canina* L., *R. alba* L., *R. rugosa* Thunb., *R. centifolia* L. etc.) [4]. Plants of the genus are widely used in medicine, cooking and horticulture. Fruits of different species of the genus of Rose are used in medicine as a source of vitamin C, the content of which in the raw material ranges from 1 % (*R. canina*) [5] to 17 % in the fruits of *R. rugosa*. The fruits also contain organic acids, flavonoids, catechins, minerals, pectin (*R. majalis*, *R. canina*) [6–8], fatty acids (*R. canina*, *R. rugosa*) [7], carotenoids (*R. canina*, *R. corymbigera* Borkh., *R. nisami* Sosn., *R. teberdensis* Chrshan.) [9], anthocyanins (*R. spinosissima* L., *R. hraciana* Tamam.) [2]. The main active ingredient of fruits are vitamin C and carotenoids [6]. The flowers of such species of the genus of *Rosa* as *R. damascene* Mill., *R. gallica* L., *R. moschata* Herrm., and *R. centifolia* are sources of essential oil [10].

According to the monograph "Rosa fruit^{N}" of State Pharmacopoeia of Ukraine (2.1) [11] as a plant raw material can be used fruit of *R. rugosa, R. majalis, R. acicularis Lindl., R. davurica Pall., R. beggeriana Schrenk., R. fedischenkoana Regel., R. kokanika (Regel) Regel ex Juz., R. canina, R. corymdifera Borkh., R. micraniha Smith., R. psammophila Chrshan., R. tomentosa Smith., R. zangezura* P. Jarosch and other species. The plant raw material «Rosa fruit» is used for preparing the multivitamin decoction, drugs "Holosas", "Carotolin", "Echinasal", "Phytodent" and "Rose hip syrup" which are used as choleretic, anti-inflammatory, wound healing agents [6, 12]. The fruits are also part of the plant collection: "Immunophyte", "Detoxify", "Phytocystol", "Arfazetin", vitamin collection № 2 and dietary supplements (Vitamin C with rose hips, C-500 With Rose Hips, Rose Hips), which exhibit vitamin, choleretic, diuretic, anti-inflammatory effects, improve metabolism and increase hormone synthesis [12].

Monographs on different plant species on Rose hips are available in the European Pharmacopoeia, the Pharmacopoeia of Japan, China, Korea, Belarus and Russia Federation.

Also, the Pharmacopoeia of China includes monographs "Flos Rosae Chinensis" and "Flos Rosae Ru-

gosae" which use raw materials of *R. chinensis* Jacq. and *R. rugosa* accordingly.

Roots of *R. majalis* and *R. canina* are used in folk medicine in Ukraine, Belarus, Russia and other European countries. Decoction of the root of *R. majalis* has astringent and antiseptic effects and uses to treat dyspepsia, cystitis, hypertension and heart disease. Decoction of the root of *R. canina* is normalized metabolism, have anti-inflammatory, choleretic, diuretic and bactericidal activity and uses to treat diseases of liver, kidney and heart, cystitis and hypertension [8]. The root of *R. laevigata* Michaux., *R. multiflora* Thunb. and *Rose cymosa* Tratt. which contains triterpenoids is used in China folk medicine [13–15]. There is a dietary supplement "Artofit" in the market of Ukraine one of the components of which is Rose roots. The supplement affects metabolism, promotes the dissolution and excretion of urate and oxalates, and it is recommended for use in gout, osteochondrosis, osteoarthritis, arthritis, radiculitis, uric acid diathesis and kidney stones [12]. The root of *R. roxburghii* Tratt. is used in Gui Zhou province of Chinese ethnomedicine as medicine for treatment various intestinal diseases [16].

Chinese clinics have made extensive use of the roots of *Rose cymosa* as a source of Chinese herb medicine Jinyinggen. Information about the chemical composition of the roots of *Rose cymosa* is insignificant and Chinese scientists have conducted a number of studies of ethanol extract. As a result, it was found that triterpenoids were dominated in the roots of *Rose cymosa* (found 27 compounds, including 2 undescribed triterpenoids). It was identified more than 10 substances with using thin-layer chromatography and high-performance liquid chromatography [17–19].

The roots of *R. multiflora* have been used as remedies for scabies, rheumatic arthralgia and stomatitis. It was studied an influence of root extract on atopic dermatitis [20].

The chemical composition of the roots of *R. majalis* and *R. canina* are little studied according to the literature. It is known that the roots contain triterpenoids, tannins, flavonoids, organic acids, ascorbic acid, amino acids, macro-and micronutrients [8]. Data on the morphology and anatomy and quantitative content of compounds are absent or different in different sources. Therefore, a comparative phytochemical characterization of the roots of *R. majalis* and *R. canina* as promising relatively new official species of medicinal plant raw materials are relevant.

The **aim** is to conduct a comparative pharmacognostical study of the series of roots of *R. majalis* and *R. canina* with the establishment of diagnostic features of morphological and anatomical structure and boundary limits of numerical indicators of raw materials.

2. Research planning (methodology)

The design of the experiment with a comparative phytochemical characterization of roots of *R. majalis* and *R. canina* included several steps (Fig. 1).

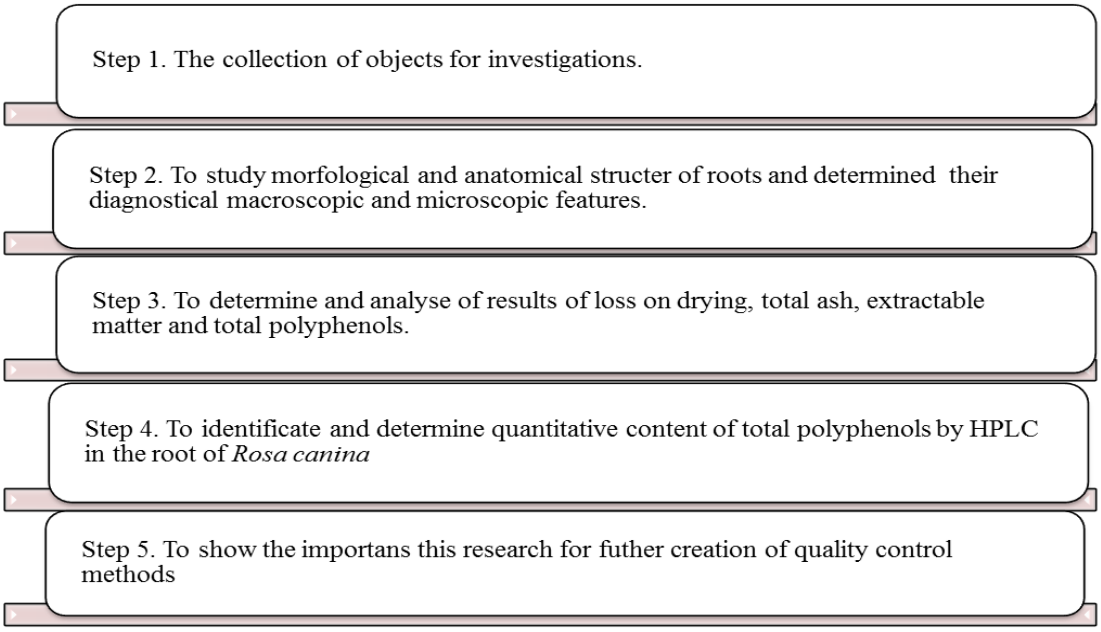


Fig. 1. Stage of the comparative pharmacognostical study of the series of roots of *R. majalis* and *R. canina*

3. Materials and methods

Roots of *R. majalis* and *R. canina* were harvested at the end of the growing season in 2019 in different regions of Ukraine (Table 1).

The study was carried out on the basis of the National University of Pharmacy.

The harvested plant raw material was dried in a fruit and vegetable dryer (Scarlett SC-FD421004, China), at a temperature of 45–50°C. Fresh and dry root fragments (up to 10 cm long) were used to study the anatomical structure. It was powdered on the grinder LZM-1 (Ukraine) plant raw materials (according to methods) for establishment numerical indicators and determine of total polyphenols.

The fresh and dry raw materials were used to study the macroscopic features, which were examined

under a magnifying glass and microscope Delta optical BioLight 300 (Poland) at a magnification of 4 times to describe the fracture. The anatomical structure was studied from freshly harvested, fixed and dried raw materials. Surface preparations and cross-sections were prepared according to generally accepted methods [21]. The microscopes Delta optical BioLight 300 with camera 2Mpx (Poland) were used for magnification at 100 and 400 times.

Table 1

Date and regions of harvested of plant raw material

Date	Region of harvested	Coordinates
Root of *R. majalis*		
22.10.2019	Vinnytsia	48.219646, 28.668950
10.11.2019	Ternopil	49.069444, 26.160185
26.10.2019	Kharkiv	49.910261, 36.617300
31.10.2019	Lviv	49.987377, 24.071711
02.11.2019	Cherkasy	48.815560, 30.140253
01.11.2019	Poltava	49.741328, 34.484088
Root of *R. canina*		
22.10.2019	Vinnytsia	48.219646, 28.668950
10.11.2019	Ternopil	49.069444, 26.160185
26.10.2019	Kharkiv	49.910261, 36.617300
31.10.2019	Lviv	49.987377, 24.071711
02.11.2019	Cherkasy	48.815560, 30.140253
01.11.2019	Poltava	49.741328, 34.484088

Quantitative determination of total polyphenols was performed by spectrophotometric method on a spectrophotometer Optizen POP (Korea) according to monograph 2.8.14. "Determination of tannins in herbal drugs" [22].

Loss on drying, total ash and extractable matter were determined according to monographs 2.2.32 "Loss on drying" [23], 2.4.16 "Total ash" [24] and 2.8.16 "Dry residue of extracts" [22].

The component composition and quantitative content of total polyphenols was studied by HPLC with using a chromatograph an Agilent 1200 3 D LC System Technologies (USA).

Test solution:

1. Flavonoids: to 1 g (exact portion) of the powdered raw material in a 250 ml round bottomed flask add 50 ml of a mixture of 40 volumes of water *R* and 60 volumes of methanol *R*. Heat under a reflux condenser in a water-bath for 15 min. Cool and filter through cotton into a 100 ml volumetric flask. Rinse the cotton with a mixture of 40 volumes of water *R* and 60 volumes of methanol *R* and use this mixture of solvents to dilute the contents of the volumetric flask to exactly 100.0 ml. filter through a disposable membrane with a pore diameter of 0.45 μm.

2. Catechins: to 1 g (exact portion) of the powdered raw material in a 250 ml round bottomed flask add 50 ml of a mixture of 70 volumes of water *R* and 30 volumes of methanol *R*. Heat under a reflux condenser in a water-bath for 30 min. Cool and filter through cotton into a 100 ml volumetric flask. Rinse the cotton with a mixture of 70 volumes of water *R* and 30 volumes of methanol *R* and use this mixture of solvents to dilute the contents of the volumetric flask to exactly 100.0 ml. filter through a disposable membrane with a pore diameter of 0.45 μm.

Column:

– size: l = 0.250 m, d = 4.6 mm;

– stationary phase: suitable octadecylsilyl silica gel for chromatography *R* (5μm).

Mobile phase:

– mobile phase A: 0,005 N solution of orthophosphoric acid *R* for flavonoids and 0.1 % trifluoroacetic acid *R*, 5 % acetonitrile *R*, water *R* (pH=2.08) for catechins (Table 2);

– mobile phase B: acetonitrile *R* for flavonoids and 0.1 % trifluoroacetic acid *R*, acetonitrile *R* for catechins (Table 2).

Table 2

Parameters of the gradient mode of elution of flavonoids and catechins

Time (min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
Flavonoids		
0	88	12
30	75	25
33	75	25
38	70	30
40	60	40
41	20	80
49	88	12
Catechins		
0	0	100
8	88	12
10	88	12
15	75	25
20	75	25
25	25	75
28	25	75
29	0	100

Flow rate: 0.8 ml/min for flavonoids and 0.1 ml/min for catechins.

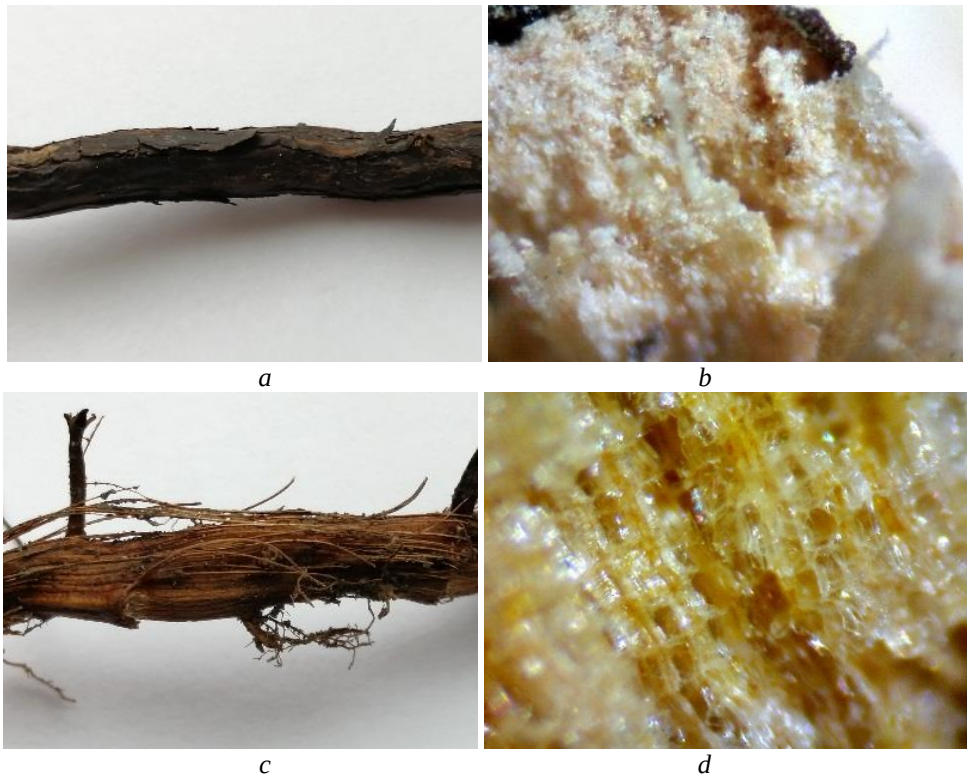
Detection: spectrophotometer at 255 nm for flavonoids and 280 nm for catechins.

Injection: 10 μl for flavonoids and 20 μl for catechins.

Retention time: 60 min for flavonoids and 40 min for catechins.

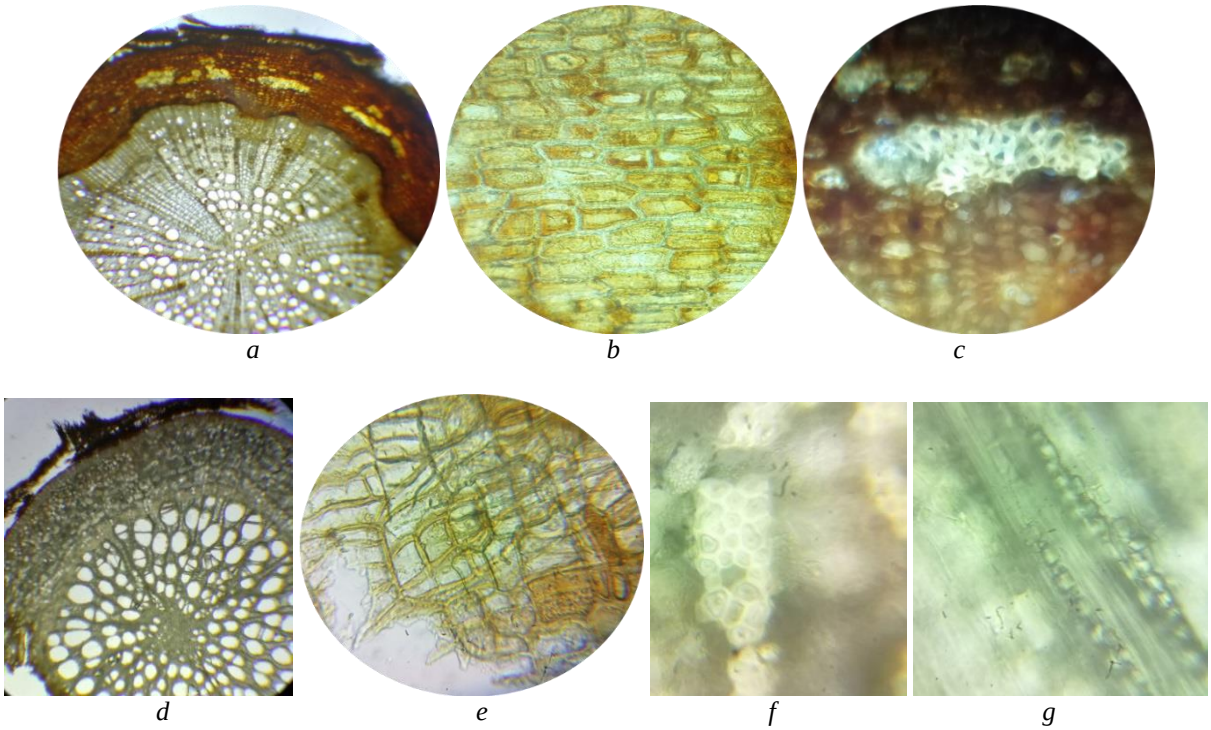
4. Results

Macroscopic features of raw materials: the roots of *R. majalis* (Fig. 2, *a*) and *R. canina* (Fig. 2, *c*) have the form of cut pieces of different lengths, twisted, cylindrical. The surface of the bark of the root of *R. majalis* is longitudinally wrinkled, the wrinkles are shallow, and the root of *R. canina* is deeply longitudinally wrinkled. The fracture of the root of *R. majalis* is granular, light brown or corporeal (Fig. 2, *b*), and the root of *R. canina* is fibrous and corporeal (Fig. 2, *d*). The cortex part is thin, the cambium does not stand out under a magnifying glass, the central part is radial. Anatomical features: structure of roots of *R. majalis* (Fig. 3, *a*) and *R. canina* (Fig. 3, *d*) is secondary beamless.



a *b* *c* *d*

Fig. 2 Morphological features: *a* – a form and surface of the root of *R. majalis*; *b* – a fracture of the root of *R. majalis*; *c* – a form and surface of the root of *R. canina*; *d* – a fracture of the root of *R. canina*



a *b* *c* *d* *e* *f* *g*

Fig. 3 Anatomical features: *a* – the structure of the root of *R. majalis*; *b* – covering tissue of the root of *R. majalis*; *c* – bast fibers of the root of *R. majalis*; *d* – the anatomical structure of the root of *R. canina*; *e* – covering tissue of the root of *R. canina*; *f* – bast fibers of the root of *R. canina*; *g* – a crystalline coating of bast fibers of the root of *R. canina*

Covering tissue is periderm. The external part of the periderm is the cork which consists of 4–5 (*R. majalis*) and 8 (*R. canina*) and more layers. The cork of 2 years old root may peel off. The cells of the cork are parenchymal, thick-walled; the cavities of the cells are dark brown in the roots of *R. majalis* (Fig. 3, *b*) and cell walls and some cavities are light brown in the roots of *R. canina* (Fig. 3, *e*).

Among the parenchymal cells of the cortex there are groups of cells (over 30 cells) of mechanical tissue – sclerenchyma with yellow cell walls in the roots of *R. majalis* (Fig. 3, *c*) and lemon-yellow cell walls in the roots of *R. canina* (Fig. 3, *f*). The sclerenchyma are arranged in 2–3 circles in the roots of *R. canina* and the cross section shows that they have a crystalline coating, which is represented by single tetrahedral crystals of calcium oxalate (Fig. 3, *g*).

The vessels of the primary xylem of the remnant of the tetraarchic conducting bundle are at the center of root of *R. majalis* (Fig. 3, *a*), while the sclerenchyma which is part of the remains of the tetraarchic conducting bundle is in the center of root of *R. canina* (Fig. 3, *d*).

The secondary xylem is permeated with narrow single-row and wide two-three-row core rays. The leading xylem system is represented by large and small reticular vessels. Elements of conductive and mechanical tissue are dominated in the secondary xylem of the roots of *R. majalis*, and conductive tissue is dominated in the in the secondary xylem of roots of *R. canina*.

The results of determination of loss on drying, total ash, extractable matter and total polyphenols in different series of root of *R. majalis* and *R. canina* are given in Table 3.

Table 3

Determination of loss on drying, total ash, extractable matter and total polyphenols in the root of *R. majalis* and *R. canina* (m=5, in terms of absolutely dry raw materials)

The place of collection of plant raw material	Loss on drying (%)		Total ash (%)		Extractable matter (%)		Total polyphenols (%)	
	R. majalis	*R. canina*	*R. majalis*	*R. canina*	*R. majalis*	*R. canina*	*R. majalis*	*R. canina*
Vinnytsia region	9.57±0.38	9.88±0.40	4.53±0.15	4.62±0.15	9.47±0.36	10.12±0,37	4.37±0,15	4.50±0,15
Ternopil region	9.59±0.38	9.84±0.40	4.57±0.15	4.59±0.15	9.50±0.36	10.19±0,36	4.39±0,15	4.53±0,15
Kharkiv region	9.43±0.39	9.81±0.41	4.50±0.15	4.63±0.15	9.44±0.36	10.14±0,37	4.40±0,15	4.54±0,15
Lviv region	9.50±0.38	9.91±0.41	4.54±0.15	4.58±0.14	9.45±0.36	10.10±0,37	4.36±0,15	4.48±0,16
Poltava region	9.34±0.37	9.79±0.40	4.53±0.14	4.56±0.15	9.47±0.36	10.14±0,37	4.37±0,15	4.50±0,15
Cherkasy region	9.52±0.38	9.80±0.40	4.56±0.15	4.56±0.15	9.51±0.37	10.16±0,37	4.39±0,14	4.52±0,15

As can be seen from Table 3, the loss on drying for the root of *R. majalis* ranged from 9.34±0.37 % (place of harvesting Poltava region) to 9.59±0.38 % (place of harvesting Ternopil region). For the root of *R. canina* this indicator was slightly higher and ranged from 9.79±0.40 % (place of harvesting Ternopil region) to 9.91±0.41 % (place of harvesting Lviv region). Total ash for the roots of both species of Rose was almost the same and ranged from 4.50±0.15 % in *R. majalis* (place of harvesting Kharkiv region) to 4.63±0.15 % in *R. canina* (place of harvesting Kharkiv region). The highest content of extractable matter was found in the root of *R. majalis*, which was harvested in Cherkasy region (9.51±0.37 %) and in the root of *R. canina* from the place of harvesting in Ternopil region (10.19±0.36 %). At the same time with the lowest content of extractable matter of the root of *R. majalis* and *R. canina* were harvested in Kharkiv region and Lviv region respectively.

According to the literature, the plant raw material contains polyphenols, so the next step in a comprehensive study of raw materials was to determine the quantitative content of total polyphenols with using a spectrophotometry as pharmacopoeial method of analysis (Table 3).

The content of total polyphenols in the root of *R. canina* is slightly higher than in the root of *R. majalis*. The following is observed: the highest content is inherent in raw materials of both types, which was harvested in the Kharkiv region, and the lowest – in the Lviv region. So, at the root of *R. majalis* this indicator ranged from 4.36±0.15 to 4.40±0.15 %, and at the root of *R. canina* from 4.48±0.16 % to 4.54±0.15 %.

As we can see the results determination of loss on drying, total ash, extractable matter and total polyphenols in the roots of *R. majalis* and *R. canina*, which were harvested in different regions of Ukraine are very similar. We can connect this fact only with the same conditions of harvested and drying, type of soil where plants grows and that it was at the same year.

Due to the fact, that the total polyphenols is slightly higher in *R. canina*, therefore the identification of them and their quantification by HPLC was performed in the root of *R. canina* (Kharkiv region) The results are shown in Table 4 and HPLC chromatograms in Fig. 4.

Table 4

HPLC study of polyphenols in the root of *R. canina*

Name of compound	Retention time (min)	Quntitative content (%)
Flavonoids		
Rutin	15.81	0.06
Quercetin-3-D-glucoside	19.53	0.01
Apigenin	44.51	0.02
Sum of flavonoids		0.09
Catechins		
Galic acid	7.55	0.02
Gallocatechin	9.67	0.58
Epigallocatechin	12.01	2.83
Catechin	12.92	0.95
Epicatechin	15.07	0.41
Epicatechin gallate	33.83	0.10
Sum of catechins		4.89

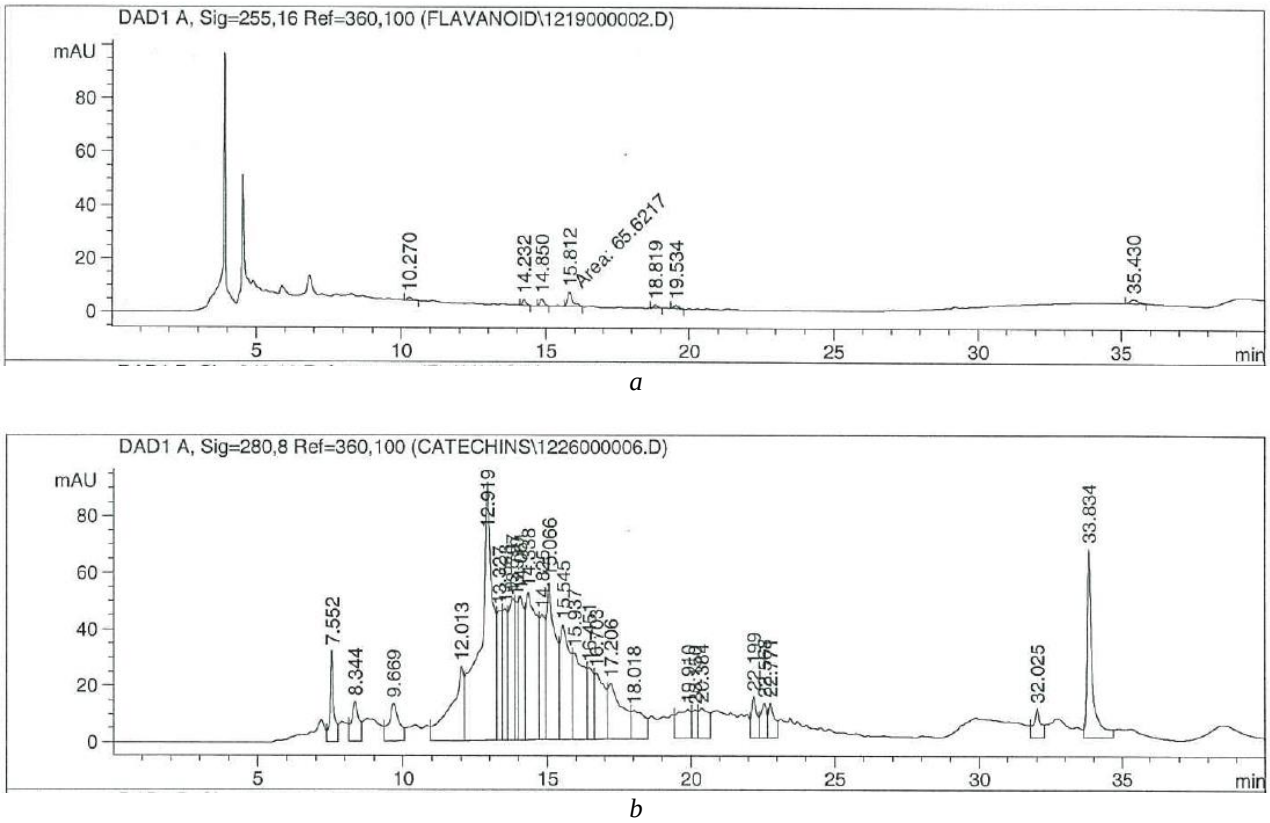


Fig. 4. HPLC chromatogram of polyphenols in the root of *R. canina*: *a* – flavonoids; *b* – catechins

It was identified and determined the quantitative content of three flavonoids in the root of *R. canina*: 2 glycosides (rutin and quercetin-3-D-glucoside) and 1 aglycone (apigenin). The quantitative content of flavonoids was dominated by rutin (2/3 of the total content of flavonoids). In addition, 6 compounds of catechin nature were identified in the raw material and their quantitative content was determined (the total content was 4.89 %). The root of *R. canina* contains the highest content of epigallocatechin (almost 3 % of the total content of catechins) from identified catechins.

6. Discussion of research results

The definition of morphological and anatomical features of raw materials is important for the identification of medicinal plant raw materials. Description of these diagnostic features is a necessary section of monographs on plant raw materials. Roots of *R. majalis* and *R. canina* is little studied plant raw materials which are used in folk medicine. There is information about studying anatomical structure root of *R. canina* [25]. The comparative analysis of literature and the results are given in Table 5.

Table 5

Differences in the anatomical structure of the roots of *R. canina*

Features	Literature	Obtained results
Covering tissue	name	name and describing cells
Crystals	absent	single crystals forms a crystalline coating of sclerenhima
Secondary xylem	present	the dominant tissue is indicated
Primary xylem	present	the specified tissue which is located in the center (sclerenchyma).

According to Table 5 a distinctive feature of our results is the presence of a crystalline coating of the sclerenchyma. Also there are a detailed description cells of covering and conductive tissue and the dominant tissue of the secondary and primary xylem. In addition, a comparative morphological and anatomical study of roots of *R. majalis* and *R. canina* was carried out and diagnostic features were established.

Distinctive macroscopic features include the nature of the surface (periderm) (longitudinally wrinkled, the wrinkles are shallow in the root of *R. majalis* and deeply longitudinally wrinkled in the root of *R. canina*) is of the root and the nature of the fracture (granular in the root of *R. majalis* and fibrous in the root of *R. canina*).

Distinctive anatomical features of the roots of *R. majalis* and *R. canina* are:

– the color and position of the cell walls of cork and their cavities (colorless cell walls and dark brown cavities in the root of *R. majalis* and light brown cell walls and some cavities in the root of *R. canina*);

– color and location of cells of sclerenchyma: in *R. majalis* are yellow and arranged in one layer, in *R. canina* are lemon-yellow and arranged in 2-3 layers;

– the presence of a crystalline coating of sclerenchyma in the root of *R. canina*;

– prevalence in the xylem of elements of conductive and mechanical tissue in the root of *R. majalis* and conductive tissue in the root of *R. canina*;

– the presence of various elements of the remains of the tetraarchic conducting bundle in the center of the root: vessels of the primary xylem in *R. majalis* and sclerenchyma in *R. canina*.

According to pharmacopoeial requirements, the quality of raw materials is determined by numerical indicators, so the definition of their limits is important in the development of methods for quality control of raw materials.

Comparing the numerical values of loss on drying (not more than 10 %), total ash (not more than 5 %), extractable matter (not less than 9 %) and the quantitative content of total polyphenols (not less than 4 %) it was determined that both types of raw materials according to these indicators are almost indistinguishable. The result of HPLC shows that the root of *R. canina* contains almost 3 % of epigallocatechin from of the total content of catechins, which can be used as marker for identification raw materials by thin-layer chromatography.

Study limitations. A limitation of the study could be considered the lack of identification of polyphenols and their quantification by HPLC of the root of *R. majalis* which makes it impossible to compare the qualitative composition and quantitative content of this group of compounds in two species of plant raw materials. Apart from these it is necessary to continue determination content of loss on drying, total ash, extractable matter and the quantitative content of total polyphenols in more series of plant raw materials and bordered harvested regions and determined the dynamics of accumulation for several years.

Prospects for further research. Promising areas for further research are to continue studying of roots of *R. majalis* and *R. canina* as a relatively new type of official medicinal plant raw materials for expanding the raw material base, creating quality control methods of their standardization and developing of phytomedicines.

7. Conclusions

1. For the first time a comparative phytochemical characterization of roots of *R. majalis* and *R. canina* was carried out, their macro- and microscopic common and distinctive features were established and the numerical indicators of plant raw materials were determined.

2. It was establishment morphological diagnostical features of roots of *R. majalis* та *R. canina*: the nature of the surface (periderm) and fracture of root.

3. It was establishment anatomical diagnostical features of roots of *R. majalis* та *R. canina*: color of cell walls and their cavities; location of the sclerenchyma; the presence of a crystalline coating of the sclerenchyma at the root of *R. canina*; of various elements of the remains of the tetraarchic conducting bundle in the center of the root.

4. Loss on drying, total ash, extractable matter and content of total polyphenols of the root of *R. majalis* and *R. canina* are almost indistinguishable, that is why the root of both plant species can be used as medicinal plant raw materials such as “Rose root”.

5. The obtained data will be used in further research when creating methods of quality control of plant raw materials “Rose root” and phytomedicines.

Conflict of interest

The authors declare that there are no conflicts of interests.

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