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BOTANY

Anatomical Structure and Phytochemical Analysis of the Leaf, Stem, and Root of a Hydrangea Bush (*Hydrangea arborescens* L.)

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Abstract—The relevance of this study is due to the insufficient knowledge of the biological characteristics of hydrangeas. The aim of this research was to identify the anatomical and diagnostic features, as well as the chemical composition, of the arborescent hydrangea (*Hydrangea arborescens* L.) from the collection of the Southern Ural Botanical Garden-Institute, Ural Federal Research Center, Russian Academy of Sciences. As a result of this research, it was found that the diagnostically significant features of the leaf of this type of hydrangea are the structure of the cells of the epidermis of the upper and lower sides of the leaf, the presence of an anomocytic stomatal apparatus on the lower side of the leaf, crystalline inclusions of calcium oxalate in the form of raphides on the upper and lower sides of the leaf, and simple hairs with a coarse warty surface, multicellular base, and simple sinuous hairs on the underside of the leaf. Phytochemical analysis of the leaves, stems, and roots confirmed the presence of coumarins, ascorbic acid, and tannins in them, as well as of flavonoids in leaves. It has been established that the highest content of ascorbic acid ($0.28 \pm 0.014\%$) and tannins ($7.13 \pm 0.639\%$) is observed in the leaves of *Hydrangea arborescens*; coumarins in terms of umbelliferone are in the roots ($0.41 \pm 0.007\%$); and the content of flavonoids in the leaves was $1.14 \pm 0.048\%$.

Keywords: *Hydrangea arborescens* L., leaf, stem, root, anatomy, coumarins, ascorbic acid, tannins, flavonoids
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INTRODUCTION

Hydrangeas (Hydrangeaceae Dumort.) are a deciduous, sometimes climbing shrub, rarely tree, with exfoliating bark; alternate, petiolate leaves; and corymbose or paniculate inflorescences (Saakov, 1959; Reed and Rinehart, 2009). According to the latest data, the genus includes about 200 species (Karpun, 2005), distributed mainly in temperate, rarely subtropical, regions of the Northern Hemisphere (Rusanov, 1955; Takhtadzhyan, 1987; Malyarovskaya, 2011a). On the territory of the Russian Federation, two types of hydrangeas grow under natural conditions—paniculate (*Hydrangea paniculata* Siebold) and petiolate (*Hydrangea petiolaris* Siebold et Zucc.) (Pilipenko, 1954; Putenikhin, 2007).

Hydrangeas are very popular in ornamental gardening, where they are valued for their large beautiful flowers and long flowering period (Lancaster and Wesley, 2008; Conolly et al., 2010; Korkulenko, 2012). Despite the great popularity and great species diversity of the genus, some biological features of hydrangeas remain insufficiently studied. There are fragmentary data in the literature on the study of the initial stages of individual development (Mazurenko and Khokhryakov, 1977; Murzabulatova et al., 2021), microclonal and vegetative reproduction (Akhmetova and Pirogova, 2016; Akhmetova and Molkanova, 2019), and winter hardiness and phenology of hydrangeas in various climatic zones (Malyugin, 1981; Martynov, 2009; Murzabulatova et al., 2018). The most complete description of the morphology of hydrangeas is given in the works of A. Rehder (Rehder, 1940), F.S. Pilipenko (1954), and E. McClintock (1957). As for anatomical studies, in some cases, the literature provides a detailed description of the anatomy of individual species and, in others, the individual parts of plants (Solreder, 1908; Burkett, 1931; Tsyrendorzhieva, 2006; Malyarovskaya, 2011b; Nekhaichenko et al., 2014; Mandzhigoladze et al., 2016; Boyko, 2020). In addition, there are no data on the study of the anatomical structure of hydrangea roots in the literature. There are also very few recent publications on the study of hydrangea morphology (Koksheeva and Nekhaichenko, 2021).

The chemical composition of the hydrangea bush is not fully understood and requires detailed revision. According to the literature, the following compounds are contained in the plant material of hydrangeas: cou-

marins (umbelliferone, hydrangetin); flavonoids (quercetin, luteolin, kaempferol, cyanidin, rutin); in the seeds, alkaloid febrifungin; bitter glycosides; vitamins, saponins, carbohydrates, and essential oils (*Rastitel'nye resursy SSSR...*, 1987; Manjigoladze et al., 2016, 2019; Manjigoladze and Kuznetsova, 2021).

Hydrangea arborescens is not included in the State Pharmacopoeia and is not used in official medicine; however, this species is widely used in folk medicine and homeopathy. For medicinal purposes, the leaves, flowers, and roots are used as raw materials. *Hydrangea* bush roots have a moderate wound-healing, diuretic, anti-inflammatory, and antimicrobial effect, which promotes the elimination of toxins from the body through the kidneys (Manjigoladze et al., 2016).

For a complete understanding of the taxonomy of species, complex data on the study of the morphology and anatomy of all parts of the plant are needed. Data on the chemical composition of various parts of the plant make it possible to give a scientific justification for the use of this species for medicinal and other purposes. In this regard, the objectives of our research were to study the anatomical structure of the leaves, stems, and roots of *Hydrangea arborescens* from the collection of the South Ural Botanical Garden-Institute, Ufa Federal Research Center, Russian Academy of Sciences, as well as to conduct research on the study of the chemical composition of biologically active substances in the aboveground and underground parts of these plants.

MATERIALS AND METHODS

Samples of raw materials of *Hydrangea arborescens* from the collection of the South Ural Botanical Garden-Institute (SUBSI) served as the objects of study.

The hydrangea (*Hydrangea arborescens* L.) is a deciduous shrub; the leaves are ovate or elliptical, pointed or oblong-pointed at the apex, with a rounded or heart-shaped base, serrated, glabrous, rarely covered with small hairs below. Stems are erect, rounded, ribbed, leafy, light green, with opposite leaf arrangement. The root system is fibrous. Inflorescence corymbose, 5–15 cm in diameter; sterile flowers are white, large, fertile, with small, inconspicuous, petals quickly falling off. The seed box is drop-down; seeds are small, ovate-ellipsoidal. The area of natural distribution is North America. Collectible plants of this species were grown from seeds obtained in 2006 from the Botanical Garden of Vakratot (Hungary). The collection of samples was carried out during the flowering period on July 20, 2019.

Microscopic examination of the samples was carried out on temporary micropreparations prepared according to the method given in the State Pharmacopoeia (http://resource.rucml.ru/feml/pharmacopia/14_2/HTML/516/index.html#zoom=z).

The roots were pre-soaked in an alcohol–glycerol mixture (1 : 1), and the stems and leaves were boiled in 5% sodium hydroxide solution. Longitudinal and transverse sections of the stem and root were stained with a solution of phloroglucinol with concentrated hydrochloric acid to highlight the mechanical elements of tissues. Photographs of micropreparations were taken using a Micromed C-1 LED microscope.

The determination of the content of *coumarins* in the raw material of *Hydrangea arborescens* was carried out by the spectrophotometric method: 1.0 g of raw materials (accurately weighed), crushed to the size of particles passing through a sieve with a hole diameter of 1 mm, was placed in a flask with a capacity of 50 mL, filled with 20 mL of 96% ethyl alcohol, and extracted for 30 minutes in a boiling water bath under reflux. The resulting alcoholic extract was cooled at room temperature and filtered through a paper filter. The extraction was carried out four more times, adding 10 mL of 96% ethyl alcohol, as described above. The alcohol extracts were combined and evaporated to a volume of 5 ml. Next, the resulting extract was applied to a Sorbfil PTSH-P-A-UV chromatographic plate, 15 × 15 cm in size, dried in air, and placed in a chamber with a mixture of solvents of benzene–ethyl acetate (2 : 1). Chromatography was carried out in an ascending manner. When the solvent front had passed about 80–90% of the plate length from the start line, it was removed from the chamber, treated with 5% potassium hydroxide alcohol solution, dried at a temperature of 100–105°C for 3–5 min, and viewed under UV light. The colored zone of the sorbent was cut out, cleaned, placed in 10 mL centrifuge tubes, filled with 5 mL of chloroform, and centrifuged for 10 min at 3000 rpm. The supernatant was separated, and the test solution was obtained. The optical density of the resulting solution was measured on a spectrophotometer at a wavelength of 324 nm in a cuvette with a layer thickness of 10 cm. Chloroform was used as the reference solution. The content of the sum of coumarins in terms of umbelliferone in absolutely dry raw materials in percent (X) was calculated by the formula

$$X = \frac{\mathrm{D} \times 5 \times 5 \times 100}{955.47m \times 0.05(100 - W)},$$

where D is the optical density of the test solution; 955.47 is the specific absorption index of umbelliferone in ethyl alcohol at a wavelength of 324 nm; m is a sample of raw materials (g); and W is the weight loss on drying (%).

The determination of the content of *ascorbic acid* was carried out by the titrimetric method according to the method given in the State Pharmacopoeia (http://resource.rucml.ru/feml/pharmacopia/14_2/

HTML/516/index.html#zoom=z) based on the ability of ascorbic acid to reduce sodium 2,6-dichlorophenolindophenolate.

The determination of the content of *tannins* was carried out by the titrimetric method according to the method given in the State Pharmacopoeia (http://resource.rucml.ru/feml/pharmacopia/14_2/HTML/516/index.html#zoom=z), based on the oxidation of tannins of potassium permanganate in an acidic medium in the presence of an indicator and an indigo sulfonic acid catalyst.

Determination of the content of *flavonoids* was carried out by the method of differential spectrophotometry with the addition of a complexing additive—an alcoholic solution of aluminum chloride and at an analytical wavelength of 410 nm: 1.0 g (accurately weighed), crushed to particle sizes passing through a sieve with a hole diameter of 1 mm, was placed in a 100-mL flask with a thin section, 100 mL of 50% ethyl alcohol was added and weighed to within ±0.01 g. The flask with the contents was attached to a reflux condenser and heated in a boiling water bath within 60 min. After cooling to room temperature, the flask was weighed and its content was adjusted with 50% alcohol to the initial mass, stirred, and filtered through a paper filter (test solution A). 2.0 mL of solution A was placed in a 25 mL volumetric flask, 2 mL of a 5% alcohol solution of aluminum chloride and 1 mL of 3% acetic acid were added. The volume of the solution was adjusted to the mark with 70% ethyl alcohol and mixed (solution B). The optical density of the resulting solution B was measured after 30 min on a spectrophotometer at a wavelength of 410 nm in a cuvette with a layer thickness of 10 mm. As a reference solution, a solution consisting of 2 mL of test solution A, 1 drop of acetic acid diluted 30%, brought to the mark with 70% ethyl alcohol in a 25 mL volumetric flask, was used.

At the same time, under the same conditions, the optical density of a solution of a standard sample (RS) complex of rutin with aluminum chloride was measured: two 25 mL volumetric flasks were filled with 2.0 mL of a rutin SS solution; 2 mL of a 5% alcohol solution of aluminum chloride in 95% alcohol, and in the other, 1 mL of 3% acetic acid and brought to the mark with the corresponding alcohol, mixed, and after 30 min the optical density was measured.

The content of the sum of flavonoids in terms of rutin in absolutely dry raw materials in percent (X) was calculated by the formula

$$X = \frac{Aa_o \times 100 \times 25 \times 1 \times 100 \times 100}{A_o a \times 100 \times 25 \times 2(100 - W)} = \frac{Aa_o \times 100 \times 100}{A_o a \times 2(100 - W)},$$

where A is the optical density of solution B of the test solution; A_o is the optical density of the solution B CO of rutin; a is the weight of raw materials, g; a_o is the sample of CO rutin, g; W is weight loss during drying of raw materials, %.

Statistical processing of the obtained results was carried out by standard methods of variation statistics using the Excel 7.0, Statistica 5.0, and Statistica 6.0 programs using Student's t-test (http://resource.rucml.ru/feml/pharmacopia/14_2/HTML/516/index.html#zoom=z).

RESULTS AND DISCUSSION

The Anatomical Structure of the Leaf, Stem, and Root of Hydrangea arborescens

The leaf. Microscopic analysis of the leaves of *Hydrangea arborescens* showed that the upper epidermis of the leaf blade consists of polygonal cells, the walls of which are slightly thickened, and the lower epidermis is represented by sinuous cells (Fig. 1). Previously, other authors (Manjigoladze et al., 2016) also indicated that the structure of the upper and lower epidermis of the leaf of the hydrangea bush is different. The anomocytic stomatal apparatus (the stomata is surrounded by several cells) is located on the underside of the leaf blade (Fig. 2). On the lower and upper sides of the leaf blade, calcium oxalate inclusions are located in the form of needle-shaped raphide crystals, which are located over the entire surface of the leaf blade (Fig. 3). *Hydrangea arborescens* is characterized by the presence of unicellular hairs with a roughly warty surface, forming a rosette of six cells at the attachment site, as well as curved simple hairs. The hairs are arranged along the vein (Figs. 4, 5). According to T.Yu. Manjigoladze et al. (2016), covering hairs are outgrowths of epidermal cells. In other types of hydrangea, hairs of a similar structure were not found on the leaves. For example, panicle hydrangea (*Hydrangea paniculata* Siebold) unicellular proboscis

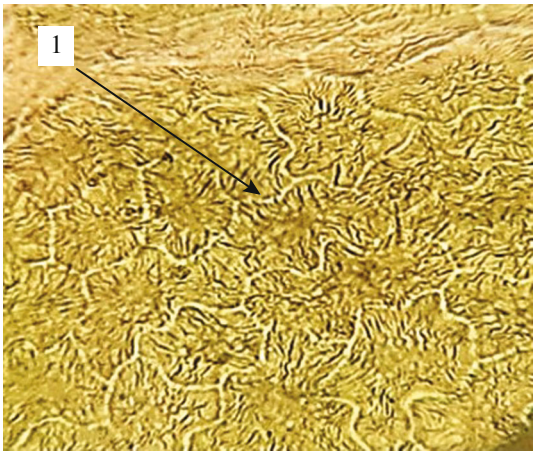

Fig. 1. The upper epidermis of a hydrangea bush leaf (×100): 1, polygonal cells with slightly thickened walls.

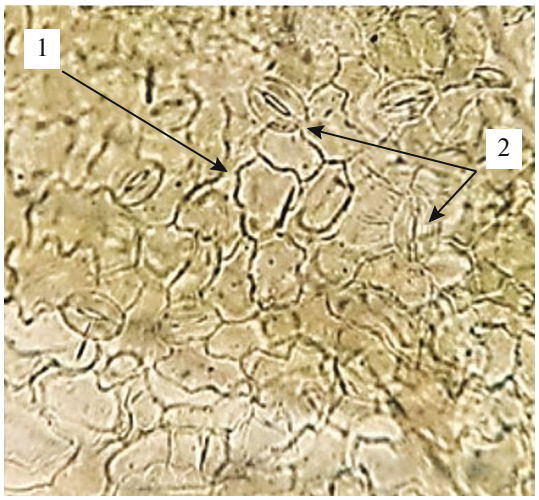

Fig. 2. The lower epidermis of a hydrangea bush leaf (×100): 1, sinuous cells of the epidermis; 2, anomocytic stomata.

hairs with spines over the entire surface are noted (Nekhaichenko et al., 2014).

The stem. On the cross section of the stem of the hydrangea bush, a secondary structure, a bundle type, open collateral bundles, bark, and a central cylinder are noted. The cortex consists of integumentary tissue—epidermis, collenchyma, and parenchyma cells. In the work of Manjigoladze et al. (2016), it is specified that the bark of the hydrangea bush stem consists of the corner collenchyma, chlorenchyma (oval cells with chloroplasts), and columnar lignified parenchyma cells. The central cylinder consists of elements of the phloem, cambium layer, secondary and primary xylem, and core parenchyma cells (Fig. 6).

The root. We have not found any literature data on the study of the anatomical structure of the hydrangea bush root. According to our studies, the structure of the root is secondary, and the cortex consists of the integumentary tissue, represented by periderm and parenchyma cells. The central cylinder consists of elements of the conducting system, represented by the secondary phloem, the primary and secondary xylem, separated by the cambium, and also the medullary rays (Fig. 7).

The anatomical structure of the stem and root of *Hydrangea arborescens* is characterized as typical for woody plants; no peculiarities were revealed during this study.

The Content of Biologically Active Substances in Hydrangea arborescens

An analysis of the literature showed that, in previous phytochemical studies of hydrangea bush raw materials, mainly qualitative methods were used to detect the presence of certain biologically active substances in various parts of the plant (Mandzhigoladze et al., 2016, 2019; Mandzhigoladze and Kuznetsova, 2021). There are practically no literature data on the quantitative composition of raw materials, with the exception of the work by Mandzhigoladze and Kuznetsova (2021), where the content of flavonoids was determined in terms of rutin in the dosage form of granules with liquid hydrangea bush extract. Our research, which is the subject of this work, is aimed at determining the amount of biologically active substances in the leaves, stems, and roots of the hydrangea bush.

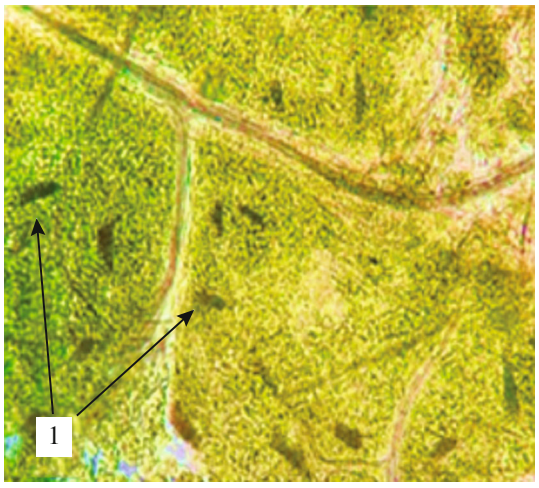

Fig. 3. Upper epidermis of *Hydrangea arborescens* leaf, inclusions (×100): 1, crystalline inclusions in the form of acicular raphide crystals.

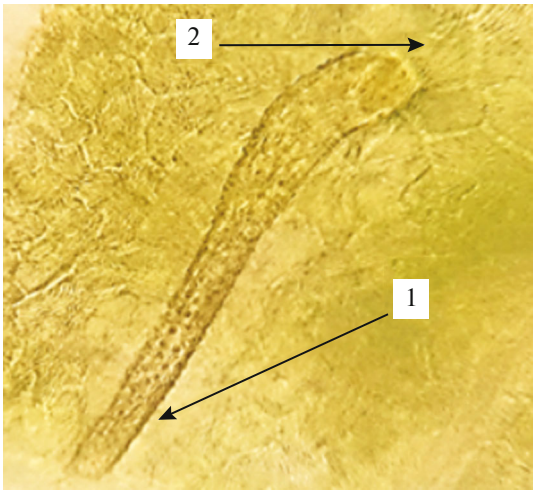

Fig. 4. The lower epidermis of a hydrangea bush leaf (×100): 1, simple warty hair; 2, multicellular base forming a rosette.

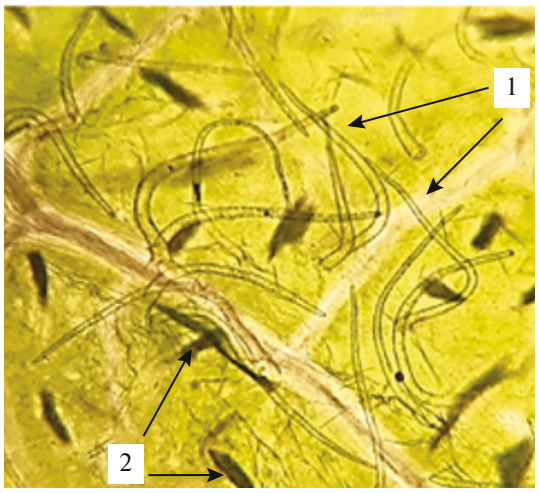

Fig. 5. The lower epidermis of a hydrangea bush leaf (×100): 1, simple curved hairs; 2, crystalline inclusions of calcium oxalate in the form of acicular raphide crystals.

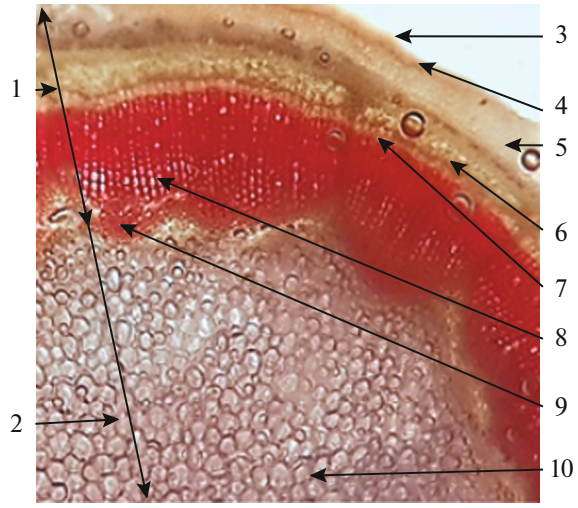

Fig. 6. Cross section of the stem, secondary structure of the stem, beam type (×100): 1, cortex; 2, central cylinder; 3, epidermis; 4, collenchyma; 5, parenchyma; 6, phloem; 7, cambium; 8, secondary xylem; 9, primary xylem; 10, core parenchyma.

Coumarins. To study the content of coumarins in the raw materials of *Hydrangea arborescens,* the method of spectrometric determination was used. Alcohol extracts were obtained from the raw materials studied, then the solutions were applied to plates and chromatographed in an ascending way in a solvent system; the resulting spots were cleaned and dissolved in chloroform. The resulting solutions were spectrophotometrically measured at a wavelength of 324 nm (Fig. 8). The content of the sum of coumarins was determined in terms of umbelliferone. The results of this study of the quantitative content of coumarins are presented in Table 1. The analysis showed that the content of coumarins in terms of umbelliferone is 0.41 ± 0.007% in the roots, 0.25 ± 0.002% in the stems, and 0.22 ± 0.002% in the leaves. The highest content of coumarins is observed in the roots of the hydrangea bush.

The metrological characteristics of the method for the quantitative determination of coumarins in the raw materials of *Hydrangea arborescens* showed that, with three independent samples, the error in the average result for leaves does not exceed 4.88%, while that for stems is 5.12% and that for roots is 4.92%.

Vitamin C. Quantitative determination of the content of ascorbic acid was carried out by redox titration. Sodium 2,6-dichlorophenolindophenolate was used as a titrant. At the equivalence point, a pink change in the color of the solution was observed due to the reduction of sodium 2,6-dichlorophenolindophenolate in an acid medium. The results of determining the amount of ascorbic acid are presented in Table 2.

Analyzing the results obtained, it can be noted that the highest content of ascorbic acid was observed in the leaves at 0.28 ± 0.014%; the smallest value in the roots is 0.20 ± 0.014%.

Quantitative determination of ascorbic acid in *Hydrangea arborescens* showed that, in three independent samples, the error of the mean result is approximately the same and does not exceed 5.33% in the leaves, 5.85% in the stems, and 6.84% in the roots.

Tannins. The determination of the content of tannins was carried out by the titrimetric method of redox titration with a solution of potassium permanganate (0.02 mol/l) in the presence of an indicator of indigo

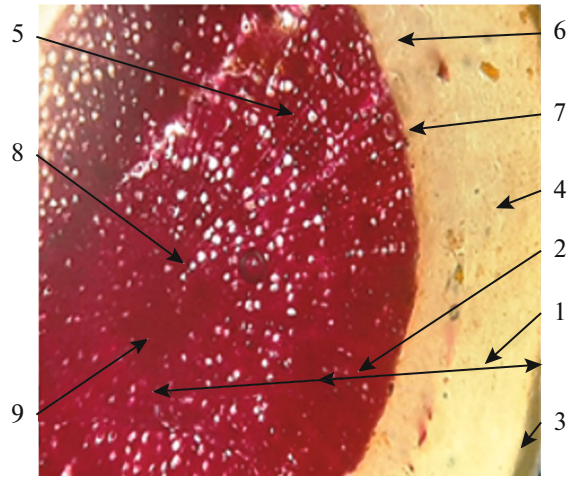

Fig. 7. Root cross section, secondary structure (×100): 1, bark; 2, central cylinder; 3, periderm; 4, parenchyma; 5, core ray; 6, secondary phloem; 7, cambium; 8, secondary xylem; 9, primary xylem.

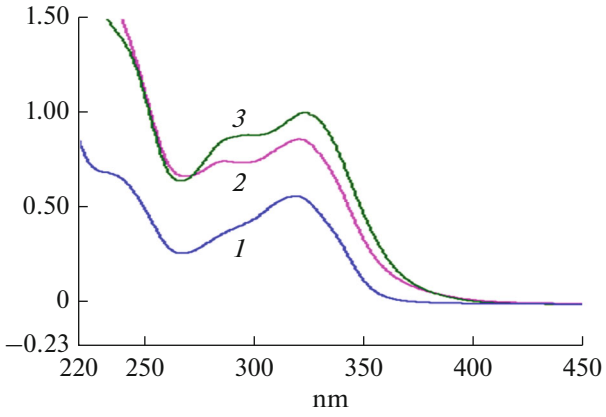

Fig. 8. Spectra of chloroform extracts of hydrangea at λ_{max} = 324 nm: (*1*) leaves; (*2*) stems; (*3*) roots.

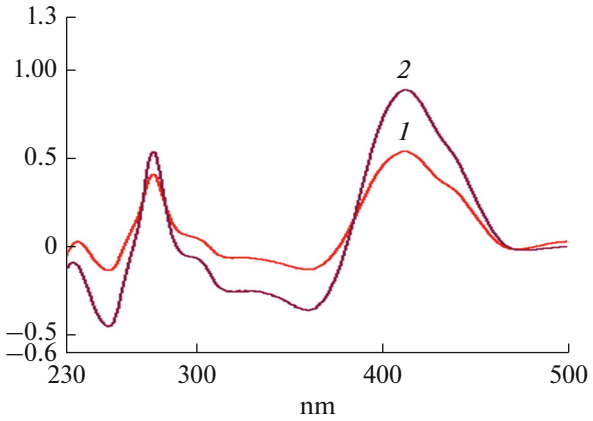

Fig. 9. Differential spectra of alcohol extracts from the leaves of *Hydrangea arborescens* (*1*) and CO rutin (*2*) λ_{max} = 410 nm.

sulfonic acid to a golden yellow color. The results of the determination are presented in Table 3.

The analysis conducted showed that the highest content of tannins is observed in the leaves (7.13 ± 0.639%), while the lowest content in the roots is 0.50 ± 0.048%.

The metrological characteristics of the method for the quantitative determination of tannins in the raw material of *Hydrangea arborescens* showed that, with three independent samples, the error in the average result of leaves does not exceed 8.96%, that of stems is 8.13%, and that of roots is 9.63%.

Flavonoids. The preliminary chromatographic and densitometric research methods showed that the roots and stems do not contain flavonoids; therefore, the quantitative determination of this group of biologically active substances was carried out only in the leaves. For the quantitative determination of flavonoids in the leaves of *Hydrangea arborescens,* a method based on the formation of a complex of flavonoids with an alcoholic solution of aluminum chloride(III) and the presence of characteristic absorption maxima was used. When measuring the optical density of the test solution in the region of 410 nm, an absorption maximum was observed, which coincided with the absorption maximum of a standard sample of a solution of rutin with aluminum chloride (Fig. 9). The results of the

Table 1. Determination of the results of coumarins in the raw material of *Hydrangea arborescens*

Raw material	*n*	*f*	*X*	$X_{cf.}$	S_x	*P*, %	*t*(*P*,*f*)	Δ*X*	ε, %
Leaves	1	2	0.235	0.22	0.0028	95	4.30	0.0023	4.88
	2		0.200						
	3		0.225						
Stems	1	2	0.245	0.25	0.010	95	4.30	0.0028	5.12
	2		0.253						
	3		0.243						
Roots	1	2	0.412	0.41	0.009	95	4.30	0.0079	4.92
	2		0.398						
	3		0.405						

n is the number of measurements; *f* is the number of degrees of freedom (*f* = *n* – 1); *X* is an indicator of the content of coumarins for each measurement; $X_{cf.}$ is the average value of the coumarin content for three measurements; S_x is the standard deviation; *P* is the confidence level (95%); *t* (*P*,*f*) is Student's criterion; Δ*X* is the confidence interval; ε, % is the relative error (for Tables 1–4).

Table 2. Determination of the results of ascorbic acid in the raw material of *Hydrangea arborescens*

Raw material	*n*	*f*	*X*	$X_{cf.}$	S_x	*P*, %	*t*(*P*,*f*)	Δ*X*	ε, %
Leaves	1	2	0.272	0.28	0.0034	95	4.30	0.0147	5.33
	2		0.299						
	3		0.258						
Stems	1	2	0.233	0.24	0.0032	95	4.30	0.014	5.85
	2		0.240						
	3		0.247						
Roots	1	2	0.210	0.20	0.0032	95	4.30	0.013	6.84
	2		0.196						
	3		0.205						

Table 3. Determination of the results of tannins in the raw materials of the hydrangea bush

Raw material	*n*	*f*	*X*	$X_{cf.}$	S_x	*P*, %	*t*(*P*,*f*)	Δ*X*	ε,%
Leaves	1	2	7.578	7.13	0.1486	95	4.30	0.639	8.96
	2		7.132						
	3		6.988						
Stems	1	2	3.106	2.66	0.0503	95	4.30	0.216	8.13
	2		2.662						
	3		2.319						
Roots	1	2	0.220	0.50	0.0111	95	4.30	0.048	9.63
	2		0.650						
	3		0.421						

Table 4. Determination of the results of the amount of flavonoids in terms of rutin in the leaves of the hydrangea bush

Raw material	*n*	*f*	*X*	$X_{cf.}$	S_x	*P*, %	*t*(*P*,*f*)	Δ*X*	ε, %
Leaves	1	2	1.102	1.14	0.0113	95	4.30	0.048	4.31
	2		1.119						
	3		1.115						

determination are presented in Table 4. The content of total flavonoids in terms of rutin was 1.14 ± 0.048%. As mentioned above, there are data in the literature on determining the amount of flavonoids in terms of rutin in a medicinal product—granules with a liquid extract from the hydrangea bush (Manjigoladze and Kuznetsova, 2021). There the sum was 0.0225%.

The metrological characteristics of the method for the quantitative determination of flavonoids in the leaves of *Hydrangea arborescens* showed that, with three independent samples, the error in the average result of the leaves does not exceed 4.31%.

CONCLUSIONS

An anatomical study of the leaf, stem, and root of *Hydrangea arborescens* revealed diagnostically significant features that make it possible to distinguish this type of hydrangea from others, namely, the upper side of the leaf blade of *Hydrangea arborescens* is characterized by polygonal thickened epidermal cells, while the

lower side of the leaf has a sinuous-walled epidermis, anomocytic type of stomatal apparatus, simple hairs with a roughly warty surface and a multicellular base, simple sinuous hairs, and the presence of calcium oxalate inclusions in the form of needle-shaped raphide crystals both on the upper and lower sides of the leaf. In the anatomical structure of the stem and root, this species has no features; their structure has features characteristic of woody plants.

A phytochemical study of the leaf, stem, and root of *Hydrangea arborescens* made it possible to establish the presence of coumarins, ascorbic acid, tannins, and flavonoids in the leaves. The highest content of ascorbic acid ($0.28 \pm 0.014\%$) and tannins ($7.13 \pm 0.639\%$) is observed in the leaves of *Hydrangea arborescens,* while the content of coumarins in terms of umbelliferone in the roots is $0.41 \pm 0.007\%$. The content of rutin in the leaves of *Hydrangea arborescens* was $1.14 \pm 0.048\%$.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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