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STUDY OF PETIOLARY ANATOMICAL FEATURES OF COMMON OAK (*QUERCUS ROBUR* L.) AND RED OAK (*QUERCUS RUBRA* L.) AND THEIR SIGNIFICANCE FOR MICRODIAGNOSTICS OF RAW MATERIALS

Actuality. *The study of petiolar anatomy of Q. rubra and Q. robur is relevant for establishing interspecies anatomical differences of leaves; its results can expand the range of diagnostic features traditionally used in pharmacognostic analysis when establishing the identity and quality of raw materials.*

The aim of the work was *to compare the features of the petiolar anatomy of Q. rubra and Q. robur, to evaluate their diagnostic significance and the possibility of application in the pharmacognostic analysis of cut raw materials.*

Material and methods. *The objects of the study were samples of leaves of Q. rubra and Q. robur collected in September 2023 on the territory of the M.M. Gryshko National Botanical Garden of National Academy of Sciences of Ukraine. The leaves were collected from 3–5 trees, mostly from the sunny side, at a height of 2–2.5 m. Petioles for anatomical studies were fixed and preserved in 70 % alcohol. Cross sections were examined with a SUNNY XSM-20 6500 microscope, photographed with a Sigeta MCMOS 5100 5.1 MP digital camera, and processed with ToupView v. software. 3.7.*

Research results. *The results of the study of the anatomical cross sections of the Q. rubra and Q. robur petioles in the distal, medial and proximal parts showed diagnostically significant features that can be used for species identification. In the proximal and distal parts, the general structure of Q. rubra and Q. robur the petioles appeared to be the most similar, and the species differences were the best observed in the structure of the medial part. Clear species differences in the structure of the medial part of Q. rubra and Q. robur petioles are able to be used as diagnostic features during a pharmacognostic analysis to determine the identity of the cut raw material. According to histochemical reactions, the presence of wax-like substances, lignified tissues, tannins and starch was confirmed in petioles of both species.*

Conclusion. *It is shown that the analysis of features of the petiolar anatomy of species of the genus Quercus in pharmacognostic studies may become a convenient tool for determining the identity of species and standardization of medicinal plant raw materials, as well as being informative for the taxonomy of the genus Quercus and for elucidating the anatomical adaptations of leaves.*

Key words: *red oak, common oak, Quercus rubra, Quercus robur, petioles, petiolar anatomy, anatomometric indicators, microdiagnostics of raw materials.*

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ДОСЛІДЖЕННЯ ПЕТІОЛЯРНИХ АНАТОМІЧНИХ ОЗНАК ДУБА ЗВИЧАЙНОГО (*QUERCUS ROBUR* L.) І ДУБА ЧЕРВОНОГО (*QUERCUS RUBRA* L.) ТА ЇХ ЗНАЧЕННЯ ДЛЯ МІКРОДІАГНОСТИКИ СИРОВИНИ

Актуальність. *Дослідження петіолярної анатомії Q. rubra та Q. robur актуальне для встановлення міжвидових анатомічних відмінностей листків; його результати можуть розширити спектр діагностичних ознак, що традиційно використовуються у фармакогностичному аналізі для встановлення ідентичності та якості сировини.*

Мета дослідження. *Порівняти ознаки петіолярної анатомії Q. rubra та Q. robur, оцінити їх діагностичну значущість і можливість застосування під час проведення фармакогностичного аналізу різаної сировини.*

Матеріали та методи дослідження. *Об'єктами дослідження були зразки листків Q. rubra та Q. robur, зібрані у вересні 2023 року на території Національного ботанічного саду імені М. М. Гришка. Листки відбирали з 3–5 дерев, переважно із сонячної сторони, на висоті 2–2,5 м. Черешки для анатомічних досліджень фіксували та зберігали у 70 %-му спирті. Зрізи були досліджені за допомогою мікроскопа SUNNY XSM-20 6 500, сфотографовані цифровою камерою Sigeta MCMOS 5100 5.1 MP і опрацьовані за допомогою програмного забезпечення ToupView v. 3.7.*

Результати дослідження. *Результати дослідження анатомічних зрізів черешків Q. rubra та Q. robur у дистальній, медіальній і проксимальній частинах показали діагностично значимі ознаки, які можна використовувати для ідентифікації видів. У проксимальній і дистальній частинах загальний план будови черешків Q. rubra та Q. robur виявився найбільш подібним, а найкраще видові відмінності проявилися в будові медіальної частини. Чіткі видові відмінності будови медіальної частини черешків Q. rubra та Q. robur можуть бути використані як діагностичні ознаки під час проведення фармакогностичного аналізу для встановлення ідентичності різаної сировини. За гістохімічними реакціями в черешках обох видів підтверджено наявність воскоподібних речовин, лігніфікованих тканин, танінів і крохмалю.*

Висновки. *Показано, що аналіз ознак петіолярної анатомії видів роду Quercus у фармакогностичних дослідженнях може стати зручним інструментом для визначення тотожності видів і стандартизації лікарської рослинної сировини, а також є інформативним для таксономії роду Quercus і для з'ясування анатомічних адаптацій листків.*

Ключові слова: *дуб червоний, дуб звичайний, Quercus rubra, Quercus robur, черешки, петіолярна анатомія, анатомометричні показники, мікродіагностика сировини.*

Introduction. Natural compounds of plant origin and medicines based on them has a significant place in the modern medicine. The search and development of preparations of plant origin that stimulate the physiological functions of the human organism is one of the priority areas of scientific research (Lichota and Gwozdzinski, 2018). It is well-known that in the complex study of medicinal plant raw material the identification of its anatomical and morphological structure is an important stage. Diagnostic features in the taxonomy of angiosperms are the anatomical features of the generative and vegetative organs of plants (Deep morphology, 2003; Zamani et al., 2008; Faghir et al., 2016). In particular, for this purpose, the data on the structure of the node and leaf petiole are often used (Lee et al., 2010).

The genus oak (*Quercus* L.) from the Beech family (Fagaceae), includes about 500 species distributed in the Northern Hemisphere (Russell et al., 2020). Common oak (*Quercus robur* L.) is an the official medicinal species of the genus; medicinal plant raw material – bark, the quality of which is regulated by the relevant monograph of the State Pharmacopoeia of Ukraine "Oak Bark" (ДФУ, 2014). Preparations from the bark of *Quercus robur* have versatile pharmacological activity: anti-inflammatory, antioxidant, astringent, antispasmodic, antimicrobial, hypotensive, due to the presence of a wide range of biologically active substances (BAS): phenolic compounds, volatile substances, sterols, aliphatic alcohols, fatty acids, etc. (Bursal and Boğa, 2018; Bhatia et al., 2019; Likhanov et al., 2019; Ferianac, et al., 2020; Burlacu et al., 2020).

Red oak (*Quercus rubra* L.) is an ornamental species that was introduced to Europe from Northern America (Burkardt et al., 2022) and it has resistance to illness and actively invades new territories. According to the literature data, *Quercus rubra* raw materials (bark, fruits, leaves) contain 42 phenolic compounds belonging to different groups of compounds, including ellagotannins, halotannins, phenolic glycosides, derivatives of hydroxybenzoic and cinnamic acids. According to clinical and pharmacological studies, consumption of these compounds can reduce the risk of cardiovascular and inflammatory diseases, cancer, diabetes, microbial infection and age-related disorders (Oracz, et al., 2022, 2023; Konovalova et al., 2023). It is aware that *Quercus rubra* bark extract exhibits antioxidant and antibacterial properties, inhibits α-glucosidase and tyrosinase due to the presence of a significant amount of tannins and other phenolic components in this extracts (Morales, 2021; Tanase et al., 2022).

Quercus L. species have a high level of intraspecific variability of morphometric parameters of leaves, which can vary significantly in different individuals within the same population or even one tree and depend on seasonal and ontogenetic changes (Jensen et al., 1993; Penas et al., 1994; Bruschi et al., 2003; González-Rodríguez and Oyama, 2005; Nikolić et al., 2005). In particular, the variability of the morphological and anatomical features of *Q. robur* (Borazan and Babaç, 2003; Nikolić et al., 2005, 2006; Boratynski et al., 2008; Kryvoruchko and Bessonova, 2018; Martins et al., 2022) and *Q. rubra* (Jensen et al., 1993; Ashton and Berlyn, 1994; Nagel et al., 1998; Kryvoruchko and Bessonova, 2017, 2018) leaf structure is shown in different growing conditions (moisture, lighting, increased UV radiation, influence of urban technogenic conditions).

High phenotypic plasticity of leaf morphometric parameters in *Quercus* species reduces their taxonomic value (Penas et al., 1994; Schicchi et al., 2001; Río et al., 2014) and redirects the search vector for diagnostic peculiarities in the features of the anatomical structure. In the taxonomy of the genus *Quercus* various anatomical characteristics of the leaf blade, in particular the stomatal apparatus, were used as diagnostic ones (Ashton and Berlyn, 1994; Bussotti and Grossoni, 1997; Lou and Zhou, 2001; Panahi et al., 2012); peculiarities of epicuticular wax deposits (Luo and Zhou, 2001; Scareli-Santos et al., 2007; Panahi et al., 2012). The features of pubescence of leaves, in particular the presence of trichomes of certain types, were clarified as the most valuable for the identification of not only species and hybrids, but also subgenera, sections of *Quercus* (Hardin, 1979; Penas et al., 1994; Uzunova et al., 1997, Fortini et al., 2009; Deng et al., 2014).

The ecological conditions of the habitats primarily affect on the characteristics of leaf blades, and they determine the main features of the anatomical and morphological structure of petioles (Filartiga et al., 2022). Leaf petioles, as key organs that provide hydraulic connections between the stem and the leaf blade, are characterized by intra- and interspecies diversity of dimensional, morphological and anatomical characteristics that are interrelated with the anatomical and morphological structure of the leaf blade, which in turn is a manifestation of adaptation to specific growing conditions, primarily to the temperature regime and moisture supply (Niinemets and Fleck, 2002; Poorter and Rozendaal, 2008; Fortini et al., 2015; Klepsch et al., 2016; Brocious and Hacke, 2016; Louf et al., 2018; Filartiga et al., 2022). Despite the variability and high plasticity of the features of petiolar anatomy even within one taxon, such characteristics of the petiole as the contour of the cross section, the characteristics of the epidermis, collenchyma, the presence of cellular inclusions and especially the number and location of vascular bundles can be used as taxonomically significant when defining separate systematic groups (Kocsis and Borhidi, 2003; Noraini et al., 2016; Talip et al., 2017; Ganem et al., 2019; Palacios-Rios et al., 2019; Anu and Dan, 2020; Karaismailoğlu, 2020).

Literature sources on histological sections of leaves of different *Quercus* species for taxonomic purposes are very limited (Río et al., 2014; Shahbaz et al., 2015; Hürkul and Yayla, 2021). The study of petiolar anatomy of *Q. rubra* and *Q. robur* is relevant for identification interspecies anatomical differences of leaves; such results can expand the range of diagnostic features that are traditionally used in pharmacognostic analysis during determining the identity and quality of raw leaf materials.

The aim of the work is to compare the characteristics of the petiolar anatomy of *Q. rubra* and *Q. robur*, to assess their diagnostic value and the possibilities of application in the pharmacognostic analysis of cut raw materials.

Materials and methods of the study. Leaf samples of *Q. rubra* and *Q. robur* were collected in September 2023 on the territory of the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine. To study the anatomical structure of petioles for each species, 5–10 mature average leaves, normally developed and intact, were randomly selected. Leaves were collected from 3–5 trees, mostly from the sunny side, at a height of 2–2.5 m. Petioles for anatomical studies were fixed and preserved in 70 % alcohol.

Cross sections of the petioles were made by hand with a razor. During staining, petiole sections were kept for 1 min. in 0.1 % (w/v) aqueous solution of safranin, then washed with 70 % ethanol, followed by staining with 1 % (w/v) aqueous solution of Astra Blue for 10 min. and washed with distilled water (Kraus et al., 1998).

All cross sections were examined with a SUNNY XSM-20 6500 microscope, photographed with a Sigeta MCMOS 5100 5.1 MP digital camera, and processed with ToupView v. software. 3.7.

When studying the anatomical structure of the petioles, the geometry of the cross section, features of the epidermis (cell sizes, thickness of the outer cell wall together with the cuticle) were analysed; presence of trichomes; degree of collenchyma development; the thickness of the mechanical coating, the diameter of the fibers and the thickness of their cell walls; the nature of the arrangement of vascular bundles and the presence of inter-bundle areas; the thickness of the phloem and xylem, the diameter of the vessels in the latter and the presence of fibers; peculiarities of the distribution of crystalline inclusions in tissues, their size and shape.

Quantitative anatomical measurements for each species were performed using Image J software. The sample for anatomometric measurements was at least 25–100 values; arithmetic mean (M) and standard deviation (±SD) were calculated.

Common histochemical reactions were used to detect: lipophilic compounds – with Sudan III; phenolic compounds – with iron (III) chloride 2 %; lignified structures – with a 1 % alcohol solution of phloroglucinol followed by concentrated HCl; starch – with Lugol's solution (Kovalov et al., 2014).

Research results and their discussion. A statistical evaluation of the features of the anatomical structure of *Q. robur* and *Q. rubra* petioles is given in table 1.

In cross section, the proximal part (base, lower) of *Q. robur* petiole on the abaxial side is more or less rounded in outline (Fig. 1: A.1), and in *Q. rubra* it is distinctly U-shaped (Fig. 1: B.1); on the adaxial side, the contour of the petiole of *Q. robur* is almost flat or with a shallow notch, in *Q. rubra* – with a distinct notch.

In both species, petioles in the basal part in the tangential direction (in width) are similar in size (*Q. robur* – 2952.51 ± 357.6; *Q. rubra* – 2856.14 ± 374.55), but in the dorsoventral part (in height) they are larger in *Q. rubra* (2214.03 ± 328.38) than in *Q. robur* (1878.30 ± 236.5).

The medial (middle) part of *Q. robur* petiole of in cross section becomes more rounded, but remains compressed in the dorsoventral direction; in the adaxial part, the contour of the petioles of *Q. robur* is convex or straight, paired ribs also become noticeable in the view of small rounded-triangular lateral ridges (Fig. 1: A.2). Transverse sections of *Q. rubra* petioles in the medial part are rounded in outline, without ribs (Fig. 1: B.2). The average dimensions (H × W) of petioles cross sections in the medial part are slightly larger in *Q. rubra* (1392.07 ± 155.61 × 1281.17 ± 115.28) than in *Q. robur* (1143.02 ± 195.39 × 1238.28 ± 148.87).

The distal (upper) part of the *Q. robur* petiole is similar in shape to the medial part, but smaller in size; in *Q. rubra* its contour is distinctly convex on the abaxial side and almost straight on the adaxial side, the lateral ribs are developed. The average dimensions (H×W) of the cross section of the petiole in the upper part are slightly larger in *Q. rubra* (1252.4 ± 86.42 × 1271.10 ± 134.3) than in *Q. robur* (912.50 ± 144.6 × 1147.70 ± 275.1); in both species, the width is slightly longer than the height.

The epidermis of petioles of both species is single-layered, consisting of cubic (in *Q. robur*) or cubic-oval *(Q. rubra*) cells (Fig. 2: B.1,2,5), the sizes of which do not differ substantially in different parts of the petiole. The epidermal cell width is larger in *Q. rubra* (13.39 ± 4.94–15.09 ± 5.03), the height is larger in *Q. robur* (8.42 ± 1.78–11.98 ± 2.05), the cuticle layer is also more developed in *Q. robur* (8.25 ± 1.19–10.52 ± 2.13). Solitary and bundle trichomes are observed on petioles of both species, mainly on the adaxial side in the upper surface groove.

Under the epidermis there is the primary cortex, the thickness of which in the distal part of the petiole of both species decreases slightly compared to the medial and proximal, but remains more developed on the abaxial side (Fig. 1: A.1–3, B.1–3). In the medial and distal parts of the petiole, the layer of the primary cortex on the adaxial side is more developed in *Q. robur*; on the abaxial side it is almost the same in both species. The proximal section of *Q. rubra* petiole has a larger diameter and, accordingly, a larger bark thickness, especially on the abaxial side (650.52 ± 97.31) (in *Q. robur* – 456.70 ± 169.00).

The outer layer of the cortex is represented by collenchyma, mainly with rounded, rounded-oval or square cells (Fig. 2: B.1). Collenchyma may include 5 to 12 layers of cells in *Q. rubra* and 4 to 10 layers in *Q. robur*; in both species it reaches its greatest thickness in the ribs and on the adaxial side. In both species, the sizes of collenchyma cells in petioles are larger on the abaxial side.

Table 1

Anatomometric parameters of *Quercus robur* and *Quercus rubra* petioles

Parameters		Distal part of petiole		Medial part of petiole		Proximal part of petiole	
		Q. robur	*Q. rubra*	*Q. robur*	*Q. rubra*	*Q. robur*	*Q. rubra*
Diameter of cross sections	height, µm	912.50 ± 144.6 663.51–1780.8	1252.4 ± 86.42 1090.4–1364.5	1143.02 ± 195.39 874.72–2187.93	1392.07 ± 155.61 1056.21–1855.3	1878.30 ± 236.5 975.88–2527.2	2214.03 ± 328.38 1598.97–2626.6
	width, µm	1147.70 ± 275.1 765.03–1780.3	1271.10 ± 134.3 1088.9–1550.2	1238.28 ± 148.87 713.82–1898.15	1281.17 ± 115.28 997.89–1757.70	2952.51 ± 357.6 1612.6-3785.6	2856,14 ± 374.55 1947.32–3387.1
Epidermal cells	width, µm	10.97 ± 3.56 4.21–17.40	14.10 ± 3.40 8.85–19.91	11.08 ± 2.18 8.57–19.05	14.68 ± 3.80 8.52–23.74	11.83 ± 2.42 5.52–18.69	15.09 ± 5.03 5.91–25.43
	height without cuticle, µm	10.57 ± 1.44 7.94–14.66	7.86 ± 0.89 6.78–9.49	10.88 ± 1.58 8.48–14.34	8.63 ± 1.97 5.92–13.27	11.98 ± 2.05 8.89–15.31	9.72 ± 2.32 5.23-14.93
The thickness of the outer cell wall of the epidermis with the cuticle, µm		8.35 ± 1.87 4.84–12.25	7.15 ± 1.40 3.98–10.72	8.52 ± 2.04 4.62–11.62	7.85 ± 1.78 4.05–10.97	10.52 ± 2.13 6.95–16.55	7.87 ± 1.39 5.48–11.00
The thickness of the primary cortex layer, µm	on the adaxial side	163.65 ± 31.54 100.45–217.43	157.68 ± 19.37 97.91–219.93	174.20 ± 51.15 106.89–310.97	173.97 ± 39.37 88.87–278.74	238.45 ± 72.84 141.15–437.89	347.28 ± 116.92 182.94-605.54
	on the abaxial side	181.12 ± 52.69 152.7–300.81	198.50 ± 30.31 119.62–309.66	198.21 ± 76.73 172.64–341.98	204.47 ± 40.40 114.73–313.02	456.70 ± 169.00 261.34–929.96	650.52 ± 97.31 484.10-807.33
The thickness of the cortex collenchyma layer, µm	on the adaxial side	81.94 ± 19.65 47.72–128.72	70.22 ± 13.01 41.58–96.16	100.51 ± 31.89 81.28–178.73	74.23 ± 19.68 46.01–113.96	119.00 ± 38.68 65.07–180.39	191.58 ± 51.83 90.29-297.63
	on the abaxial side	59.17 ± 10.16 46.05–85.38	48.36 ± 12.32 30.58–77.04	97.90 ± 19.92 72.47–170.80	50.07 ± 10.56 31.05–79.50	101.00 ± 28.16 68.73–166.14	179.58 ± 60.66 88.09-292.49
Collenchyma cell wall thickness, µm		2.90 ± 0.38 1.88–6.84	2.53 ± 0.87 1.93–6.16	3,5 ± 0.42 2.16–6.48	3.06 ± 0.74 1.61–4.26	3.11 ± 1.52 1.51–8.74	2.59 ± 1.20 1.88–7.81
Diameter of collenchyma cells, µm	on the adaxial side	10.47 ± 1.82 3.02–14.11	9.12 ± 1.80 3.27–13.96	20.95 ± 7.47 9.80–37.17	13.94 ± 2.90 9.29–20.63	10.31 ± 2.36 5.33–18.38	13.03 ± 2.65 7.13-18.83
	on the abaxial side	16.62 ± 4.08 8.68–24.32	13.15 ± 3.11 7.19–22.37	26.82 ± 7.09 13.47–45.16	22.53 ± 5.00 12.81–32.88	18.94 ± 4.53 8.29–29.01	19.17 ± 5.13 7.06-31.46
Diameter of parenchyma cells, µm	on the adaxial side	14.21 ± 2.24 7.67–15.63	15.23 ± 4.54 8.07–25.40	16.27 ± 2.17 6.18–18.57	16.28 ± 3.54 7.32–29.47	22.36 ± 14.13 6.21–64.43	11.30–2.47 7.22–19.93
	on the abaxial side	25.88 ± 5.16 14.53–48.01	30.18 ± 9.20 17.50–61.52	30.95–9.53 12.83–61.69	31.51 ± 9.40 10.44–65.48	34.92 ± 10.96 17.72–64.43	41.66 ± 11.07 17–73.71
The thickness of the sclerenchyma sheath, µm		48.73 ± 11.37 30.45–56.23	79.84 ± 16.99 48.41–115.44	56.52 ± 23.11 32.39–112.97	98.97 ± 19.94 39.30–122.03	131.61 ± 38.22 47.23–202.97	133.00 ± 32.15 78.50–219.35
Diameter of sclerenchymal fibers, µm		14.71 ± 4.40 3.56–35.28	15.22 ± 5.43 3.94–36.62	16.80 ± 5.23 7.13–36.90	16.50 ± 7.60 3.70–38.97	18.38 ± 5.15 7.06–38.70	19.31 ± 5.52 8.64–39.91
The thickness of the walls of sclerenchyma cells, µm		2.91 ± 1.47 1.05–5.99	2.97 ± 0.82 0.98–6.63	3.57 ± 0.57 1.71–5.74	3.58 ± 0.79 1.84–6.55	4.12 ± 1.17 2.01–8.66	4.87 ± 0.78 2.30–7.45
Phloem thickness, µm		61.05 ± 20.35 33.21–112.93	60.75 ± 8.92 26.39–69.09	61.96 ± 14.71 30.46–91.40	60.07 ± 19.42 24.56–107.86	64.31 ± 19.04 35.04–100.38	63.20 ± 17.35 23.00–105.23
Xylem thickness, µm		110.38 ± 16.69 62.04–175.86	125.26 ± 30.77 63.23–219.57	124.38 ± 28.02 71.11–201.42	126.09 ± 30.24 75.21–205.15	144.85 ± 51.83 85.69–262.03	181.29 ± 45.31 94.21–276.92
Vessel diameter, µm		23.28 ± 5.38 11.07–35.11	29.53 ± 9.66 12.94–50.58	24.55 ± 6.58 7.54–36.22	31.72 ± 7.61 17.55–56.23	23.97 ± 6.34 11.10–32.64	24.89 ± 5.08 13.22–36.54
Diameter of core parenchymal cells, µm		16.02 ± 6.46 5.42–33.07	19.44 ± 5.27 8.38–30.27	17.27 ± 6.44 5.10–45.89	20.12 ± 7.79 8.79–46.78	22.88 ± 9.25 9.05–55.66	23.06 ± 4.94 10.64–57.96
Diameter of crystals in the parenchyma of the primary cortex, µm		21.08 ± 4.44 12.88–30.75	23.38 ± 5.84 10.98–39.23	26.48 ± 7.97 9.49–45.94	28.58 ± 8.11 10.11–46.63	25.70 ± 4.03 17.55–35.32	27.42 ± 5.17 18.73–39.453
Diameter of crystals in the core, µm		17.75 ± 5.96 8.68–30.00	15.94 ± 4.39 6.33–25.89	15.33 ± 3.24 10.38–27.37	17.28 ± 4.14 7.77–36.83	19.44 ± 5.27 10.74–39.85	25.37 ± 5.64 13.86–40.18

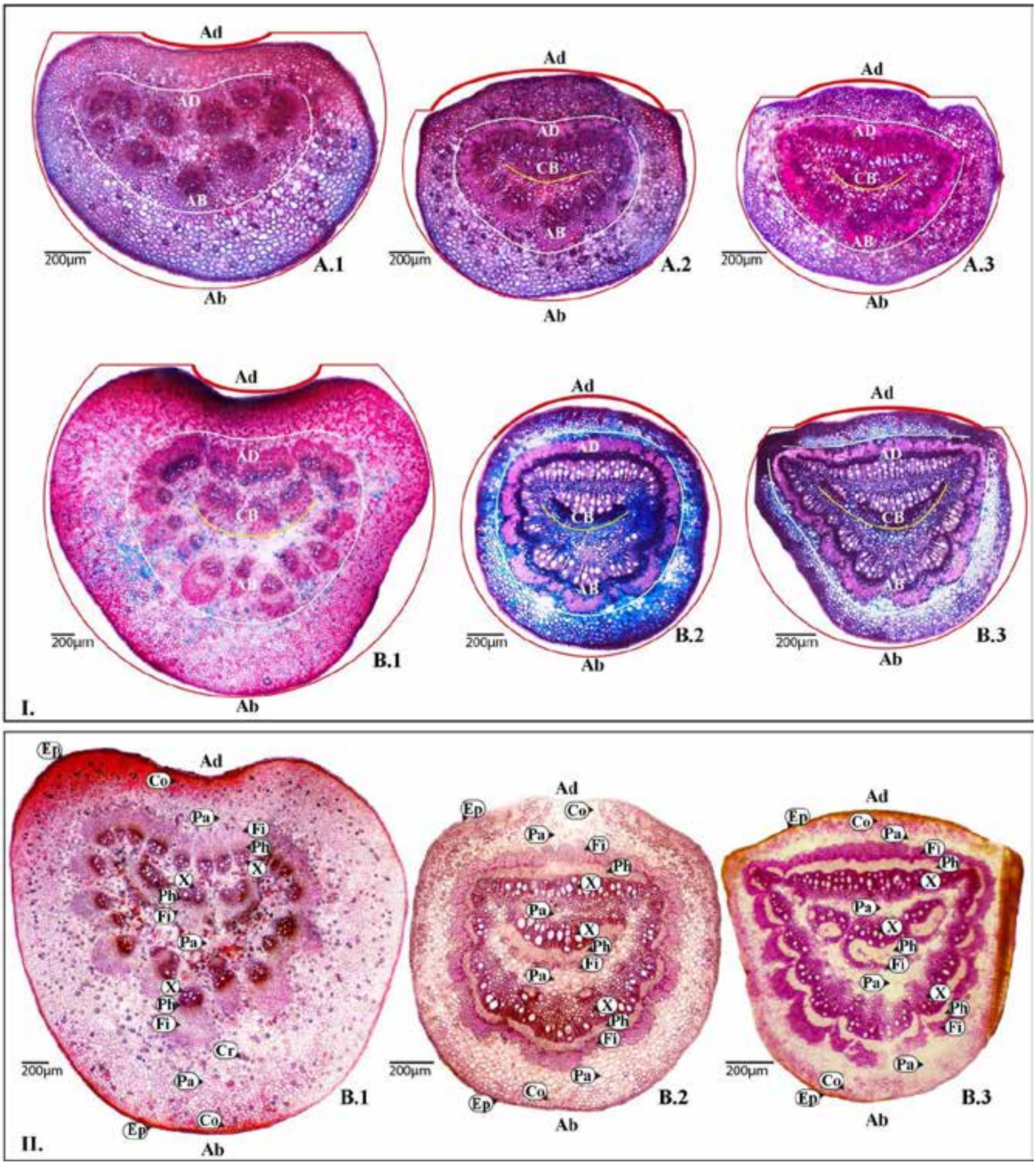

Fig. 1. Cross section through the petiole of A. – *Quercus robur*, B. – *Quercus rubra:*

1 – proximal; 2 – medial; 3 – distal parts.

I – Staining Safranin/Astra Blue, II – Staining Phloroglucinol/HCl.

Ad – adaxial side, Ab – abaxial side.

Ep – epidermis, Co – collenchyma, Pa – parenchyma, Fi – bundle-sheath of sclerenchyma fibres, Ph – phloem, X – xylem, Cr – calcium oxalate crystal.

Petiole outline in the cross section (red line).

Primary vascular tissue (white line): AD – adaxial block; AB – abaxial block.

Secondary vascular tissue (yellow line): CB – central block.

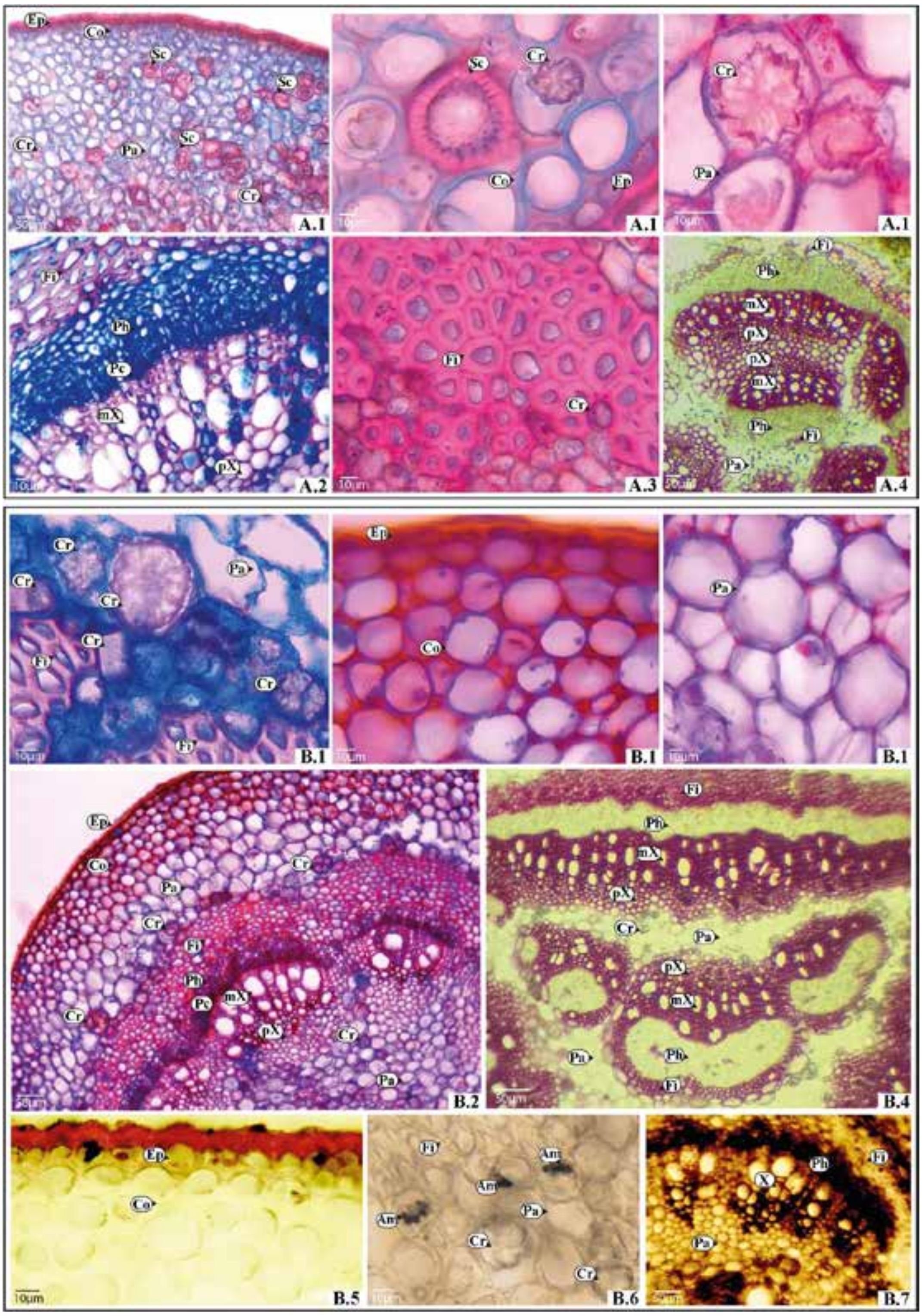

Fig. 2. Anatomical structure of the leaf petiole in the medial part of A. – *Quercus robur*, B. – *Quercus rubra*:

1 – primary cortex; 2 – primary vascular tissue (abaxial block); 3 – sclerenchyma fibres; 4 – primary vascular tissue (adaxial block) and secondary vascular tissue (central block); 5 – orange-red colour of the cuticle in reaction with Sudan III; 6 – dark blue colour of amyloplasts in reaction with Lugol's solution; 7 – black colour of phenolic compounds of xylem and phloem parenchyma in reaction with iron chloride (III).

Sc – sclereids; mX – metaxylem; pX – protoxylem; Pc – procambium; Am – amyloplast.

The other components of the legend are same as in Fig. 1.

In the medial and distal parts of *Q. robur* petioles, especially on the adaxial side, the thickness of the collenchyma layer, the size of the cells, and the thickness of their membranes are larger than in *Q. rubra* (Fig. 1: A.2,3, B.2,3); in the proximal part, *Q. rubra* petioles have more developed collenchyma (Fig. 1: A.1, B.1), which is probably related to their larger linear dimensions.

The storage parenchyma of the primary cortex is located between the collenchyma and the mechanical sheath of the vascular system (Fig. 1: A.1–3, B.1–3; Fig. 2: A.1, B.1). The number of cortical parenchyma cell layers decreases in both species in the distal (on the adaxial side in *Q. rubra* – 8–10, and in *Q. robur* – 12–15; on the abaxial side in *Q. rubra* – 9–14, and in *Q. robur* – 7–10) part of petioles compared to the proximal one (on the adaxial side in *Q. rubra* – 20–25, and in *Q. robur* – 13–20; on the abaxial side in *Q. rubra* – 23–30, and in *Q. robur* – 15–20). In both species, the number of cortex parenchyma layers is greater on the abaxial side. *Q. rubra* has the largest number of cortex parenchyma layers in the proximal part of petioles. In the medial and distal parts on the adaxial side, there are more layers of parenchyma cells in *Q. robur*; on the abaxial side – in *Q. rubra*.

In petioles of both species, cells of the parenchyma of the primary cortex don't decrease from the base to the apex significantly, and have smaller sizes on the adaxial side; in *Q. rubra* in the medial and distal cross sections, the sizes of parenchymal cells, especially on the abaxial side, were larger than in *Q. robur*; in the proximal cross sections of *Q. rubra* petioles from the adaxial side, parenchyma cells are the smallest.

The cells of the parenchyma of the primary cortex are round, oval or irregular in shape, the largest of them (41.66 ± 11.07 – in *Q. rubra* and 34.92 ± 10.96 – in *Q. robur*) are concentrated at the base of petioles on the abaxial side, near the sheath of mechanical tissue (Fig. 2: A.1, B.1).

In the primary cortex of *Q. robur* sclereids were identified in all parts of the petiole, especially in the proximal and medial parts (Fig. 2: A.1).

Scattered druses and less often prismatic crystals of calcium oxalate are visualized in the tissues of the primary cortex, they reach the highest concentration in the inner layers of the cortex, creating a crystal-bearing coating around the ring of mechanical tissues surrounding the vascular system (Fig. 2: A.1, B.1). In both species, crystals occur in all parts of the petiole, but the largest number of them is concentrated in its base. In cross sections of petioles, crystalline inclusions are localized in greater numbers in the abaxial cortex, where they reach the largest sizes. The parenchymal tissues of *Q. rubra* petioles are characterized by a greater saturation of crystalline inclusions and their larger sizes, especially in the medial part of the petioles (in *Q. rubra* – 28.58 ± 8.11; in *Q. robur* – 26.48 ± 7.97).

There are three blocks in the structure of the vascular system. Adaxial and abaxial blocks consist of primary vascular tissues; the central one is from the secondary ones (Fig. 1: A.1–3, B.1–3; Fig. 2: A.4, B.4). The vascular system is separated from the parenchyma of the cortex by the sheath of mechanical tissues (Fig. 2: B.2).

The bundles in the adaxial block are arranged linearly, along the adaxial side of the petiole; consist of layers of phloem (it adjacent to the ring of sclerenchyma), procambium is located in the middle, then primary xylem, which has differentiated metaxylem and protoxylem (Fig. 2: A.4, B.4). The central (Fig. 2: A.4, B.4) and abaxial (Fig. 2: A.2, B.2) blocks of vascular tissues consist of protoxylem, differentiated metaxylem, cambial/procambial cells and phloem. The abaxial block of vascular tissues is arched and separated from the central block by 5–8 layers of storage parenchyma with crystalline inclusions. Between the adaxial and central blocks of vascular tissues in *Q. rubra* there are 3–5 layers of storage parenchyma; in *Q. robur* the xylems of the central and adaxial sides are in contact (Fig. 2: A.4, B.4).

The structure of vascular system of *Q. rubra* and *Q. robur* petiole differs in cross sections of the proximal, medial, and distal parts (Fig. 1: A.1–3, B.1–3). One of the diagnostic features of the cross section of the petiole base is the absence of a central block of secondary vascular bundles in *Q. robur* (Fig. 1: A.1). Also, in the basal parts of petioles of both species, the primary vascular bundles in the adaxial and especially in the abaxial blocks don't fuse, they are separated by sections of parenchymal tissue (Fig. 1: A.1, B.1). The central block of secondary vascular bundles in *Q. rubra* in cross sections of the petiole base consists mainly of three separate bundles in which the xylem is oriented to the cortex, and the phloem to the pith (Fig. 1: B.1). Anatomical cross sections of *Q. rubra* and Q. *robur* petiole bases differ well in the number of vascular bundles, which are greater in *Q. rubra* (10–13 in the adaxial and abaxial blocks); in *Q. robur* – 6–8 bundles in both blocks. The vascular bundles in the abaxial block of both species have a semi-circular arrangement, and the adaxial ones have a linear arrangement (Fig. 1: A.1, B.1). The bundles of both blocks have the same type of structure: surrounded by sclerenchyma, containing phloem oriented towards the cortex and xylem oriented towards the pith; the procambial layer is located between the xylem and the phloem (Fig. 1: A.1, B.1). Bundles of vascular tissues at the base of the petiole are separated by parenchymal tissue with a high content of crystalline inclusions, especially in *Q. rubra*.

It should be noted that the cross sections of the proximal part of the petioles of both species can vary greatly in cross section sizes, degree of parenchyma development in the cortex, and dimensional indicators of the ground, vascular and mechanical tissues. The vascular system of *Q. rubra* may not contain a central vascular block and be similar to that of *Q. robur*. The stable diagnostic features of the proximal part of *Q. rubra* petioles remain the U-shaped contour and the greater number of conducting bundles in the abaxial and adaxial blocks of vascular tissues.

The structure of the vascular system of petioles in the medial part differs in *Q. rubra* by the complete fusion of vascular bundles in the adaxial and abaxial blocks and complete or partial fusion in the central one (Fig. 1: B.2; Fig. 2: B.4); in *Q. robur* – bundles in adaxial and abaxial blocks fuse completely or partially, the central block of secondary conducting bundles is visualized, or it may be absent (Fig. 1: A.2). The xylem of the central block of the leading bundles of *Q. rubra* is clearly separated by parenchymal tissue from the xylem of the adaxial block, *in Q. robur* the central and adaxial blocks are in contact (Fig. 2: A.4, B.4). The anatomical structure of the medial section of *Q. rubra* petiole is similar to the structure of the middle vein.

In the distal part cross sections of petioles of both species are similar to each other and similar to the structure of the middle veins (Fig. 1: A.3, B.3). The adaxial block of vascular tissues has a linear or almost linear arrangement, and not distinctly convex as in the middle veins. Vascular bundles in the adaxial and abaxial blocks are fused. The central block of vascular tissue is more developed in petioles of *Q. rubra*, separated from the adaxial block by parenchymal tissue. In the upper cross sections of the petioles of Q. *robur*, the central block is represented more often by one bundle, less often by several fused bundles; its xylem contacts the xylem of the adaxial block.

The sheath of sclerenchyma fibers around the vascular system is the most developed in petioles of *Q. rubra*; in both species its thickness decreases from the proximal to the distal part of the petiole (in *Q. rubra* from 133.00 ± 32.15 to 79.84 ± 16.99; in *Q. robur* from 131.61 ± 38.22 to 48.73 ± 11.37).

The sheath of sclerenchyma consists of 6–10 (in *Q. rubra*) or 5–8 (*Q. robur*) dense layers of fibers, which in cross section are round, oval, triangular, rhombic or irregular in shape (Fig. 2: A.2,3, B.1,2). In cross sections the thickness of the ring of mechanical tissues from different sides in *Q. rubra* is almost the same; in *Q. robur* it is mostly thicker on the abaxial side. Fibers of both species are larger at the base of the petiole and almost do not differ in diameter (in *Q. rubra* – 19.31 ± 5.52; in *Q. robur* – 18.38 ± 5.15) and wall thickness (in Q. rubra – 4.87 ± 0.78; in *Q. robur* – 4.12 ± 1.17). In both species crystalline inclusions occur in the sclerenchyma sheath.

The average thickness of the phloem layers in the studied species does not vary significantly in different parts of the anatomical cross sections of petioles. In *Q. rubra* the ring of phloem tissue on cross sections is more or less the same thickness, in *Q. robur* it is thinner on the adaxial side. Phloem elements with thin walls; in shape from round, oval, triangular, rhombic to irregular (Fig. 2: A.2, B.2).

The xylem vessels in different parts of petioles in both species have a radial arrangement, they are surrounded by libriform fibers and ray parenchyma cells (Fig. 2: A.2,4, B.2,4). Vascular bundles in the abaxial block of conducting tissues have the greatest thickness. The average thickness of the xylem in petioles in both species decreases from the base to the apex; in all parts of petioles it reaches higher values in *Q. rubra*. The vessel diameter is also larger in *Q. rubra*.

The pith is more developed in the petioles of *Q. rubra*. Parenchyma cells of the pith are thin-walled, round or irregular in shape (Fig. 2: B.2). The sizes of pith cells in petioles of both species are larger at their bases and do not significantly decrease toward the distal part. In all parts of petioles the parenchyma cells of the pith are larger in *Q. rubra*; the largest cells are identified in the pith of the their base (23.06 ± 4.94 – in *Q. rubra*; 22.88 ± 9.25 – in *Q. robur*). In both species the pith crystals are smaller compared to the cortex. The bases of petioles of *Q. rubra* (25.37 ± 5.64) compared to *Q. robur* (19.44 ± 5.27) are characterized by the largest number of crystals and their largest sizes.

In the histochemical reaction with phloroglucinol/HCl collenchyma of the primary cortex, sclerenchyma, and xylem acquired a crimson-red colour in all cross sections of petioles of both species (Fig. 1: II, Fig. 2: A.4, B.4). Cuticles of the epidermis in all cross sections of petioles of both species acquired an orange-red colour in the histochemical reaction with Sudan III (Fig. 2: B.5). According to the histochemical reaction with iron (III) chloride, the highest concentration of phenolic compounds was determined in the parenchyma of the xylem and phloem of the petiole (Fig. 2: B.7). Amyloplasts were detected by reaction with Lugol's solution in the parenchyma of the primary cortex, especially in the inner layers adjacent to the sheath of mechanical tissues (Fig. 2: B.6), cells of the pith parenchyma and pith rays.

Conclusions. According to the obtained results of the study of the anatomical cross sections of *Q. rubra* and *Q. robur* petioles in the distal, medial and proximal parts diagnostically significant features which can be used for species identification were determined. Petioles of *Q. rubra* in the proximal part differ significantly in: the U-shaped contour of the cross section and a distinct notch on the adaxial side; a greater number of vascular bundles in the adaxial and abaxial blocks of conducting tissues; the presence of the central block of the secondary vascular bundles. The differences in the medial part of *Q. rubra* petioles are the complete fusion of vascular bundles in the adaxial and abaxial blocks, a clear separation of the well-developed xylem of the central block from the xylem of the adaxial block by parenchymal tissue. In the distal part petioles of both species acquire a similar structural plan, but may differ in the degree of fusion of vascular bundles in all blocks and the degree of development of the central block of conducting tissues. Petioles of Q. rubra differ in the number of crystalline inclusions. Druses and prismatic crystals are characteristic of petioles of Q. rubra; in Q. robur, drusen are more common, prismatic crystals are rare.

Most of the dimensions of the anatomical structure of the leaves were found to be quite variable in both species, which reduces their diagnostic value. The contours of petioles, the relationship in the development of basic, mechanical and conducting tissues are more or less stable. A greater variability of the structure of the conducting system of the proximal and distal parts of the petioles, and a greater stability of its structure in the medial part, were also noted. In addition, in the proximal and distal parts the general structure of *Q. rubra* and *Q. robur* petioles turned out to be the most similar, and the best species differences were identified precisely in the structure of the medial part. Clear species differences in the structure of the medial part of *Q. rubra* and *Q. robur* petioles can be used as diagnostic during a pharmacognostic analysis to determine the identity of the cut raw material.

The round petiole contours, greater proportion of sclerenchyma, greater xylem development, and vessel diameter characteristic of *Q. rubra* leaves are considered as specific adaptations of long petiole leaves with a large leaf blade to better providing mechanical support and water supply (Filartiga et al., 2022). Smaller leaves and shorter petioles of *Q. robur* have a less developed sclerenchyma layer, and smaller vessel diameter and xylem thickness. On the other hand, the anatomical structure of *Q. robur* petioles shows more adaptation to the conditions of growth in places with limited water resources, that is, features of xeromorphism, in particular, a greater thickness of the epidermis, cuticles, collenchyma, and the development of pubescence.

According to histochemical reactions, the presence of wax-like substances, lignified tissues, tannins and starch was confirmed in petioles of both species.

Thus, the analysis of features of petiolar anatomy in pharmacognostic studies can become a convenient tool for determining the identity of species and standardization of medicinal plant raw materials, and will also be informative for taxonomy of the genus and for clarification of anatomical adaptations of leaves.

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