How to Cite: Babeshina, L.G. (2025). Microscopic analysis of *Tilia cordata*. *Fundamental And Experimental Biology, 30,* 4(120), 110-117. https://doi.org/10.31489/2025FEB4/110-117

Research Article

https://doi.org/10.31489/2025FEB4/110-117

UDC 581.8 Received: 21.08.2025 | Accepted: 15.10.2025 | Published online: 31 December 2025

L.G. Babeshina

Synergy University, Moscow, Russian Federation
Corresponding author: lbabeshina@yandex.ru

Microscopic analysis of *Tilia cordata*

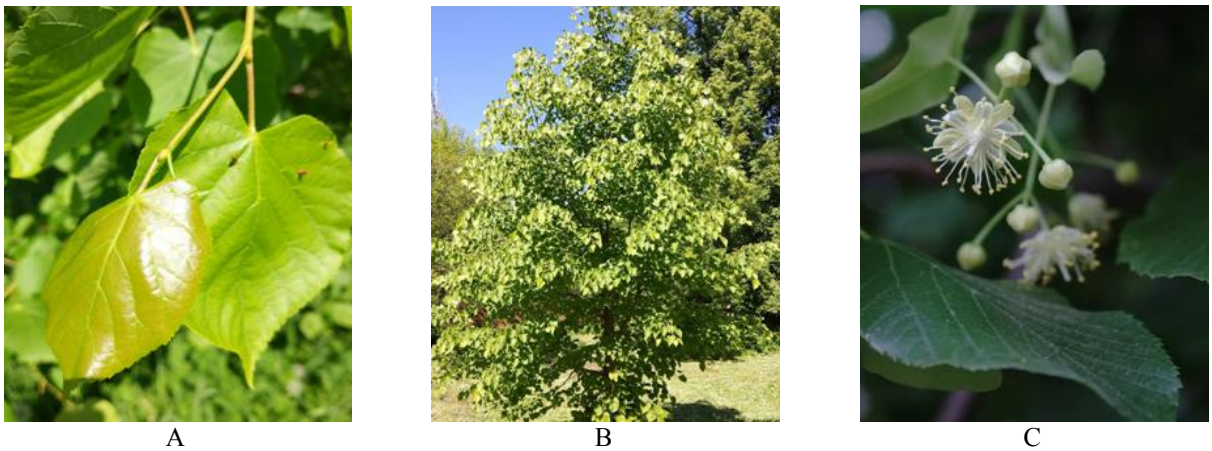
The raw material base for medicinal plant resources can be expanded both by introducing new plant species into practical use and by utilizing additional plant organs of species already in use. The flowers of *Tilia cordata* are a valuable type of medicinal raw material; however, their collection is constrained by a short flowering period. The leaves of this species also exhibit pharmacological activity, making them a promising independent source of medicinal plant material. This article presents the results of a microscopic analysis of *Tilia cordata* leaves. Anatomical examination was performed on dried leaves, with an assessment of the structural features observed in surface preparations and cross sections. The leaf was found to be hypostomatic, with the main epidermal cells having straight or wavy walls. On the abaxial surface, four types of trichomes were identified: glandular, forked, capitate, and stellate. A crystalline coating was observed along the leaf veins. In cross section, the leaf is dorsiventral, with a single-layered palisade mesophyll, a 2-3-layered spongy mesophyll, mucilage channels, and druses of calcium oxalate. Diagnostic microscopic features have been identified that may be used in the preparation of a pharmacopoeial monograph.

Keywords: *Tilia cordata*, plant raw material, microscopic analysis, leaf, micro-diagnostic signs

Introduction

The introduction of new types of plant medicinal raw materials is a pressing task for botany and pharmacy. Not all types of raw materials included in the Pharmacopoeia [1] are available in sufficient quantities to meet the needs of the pharmaceutical industry in the production of drugs.

One of the most sought-after types of raw materials is the flowers of the heart-shaped linden tree [1, 2]. In official medicine, heart-shaped linden flowers (*Tiliae flores)* are used as a diaphoretic, expectorant, and anti-inflammatory agent [3–6]. However, the flowering period of this plant species is short (late June to early July, about two weeks), which limits the time available for harvesting the flowers (Fig. 1). The plant itself has a limited habitat: the Caucasus, Karelia, Arkhangelsk Region, Komi Republic, Tomsk Region, and Khanty-Mansi Autonomous Okrug. Extensive areas are occupied by forests in the Urals [7].



A B C

Figure 1. Leaves (A), tree (B), and flowers (C) of *Tilia cordata*

Recently, the leaves of *Tilia cordata* Mill. (family Malvaceae) have attracted the attention of scientists. Experiments have shown that total extracts obtained from *Tilia cordata* leaves exhibit a wide range of bio-

logical activity, including general strengthening, anti-inflammatory, wound-healing, and antimicrobial effects [8–10].

In this regard, *Tilia cordata* leaves are of scientific and practical interest for detailed study, and their introduction as medicinal raw materials will allow for the partial replacement of the scarce flowers of this species.

Based on the above, the aim of this study was to examine the anatomical structure of *Tilia cordata* leaves and identify the diagnostic features of the raw material.

Experimental

The objects of the study were *Tilia cordata* leaves (Fig. 2) collected at different times of the growing season (early May to late September 2023) in the forest park area of the State Humanitarian-Technical University (Orekhovo-Zuyevo).

Figure 2. Dried samples of *Tilia cordata* leaves

The raw material was soaked in hot water and fixed in a mixture of 50 % aqueous glycerin, distilled water, and 90 % ethyl alcohol [11]. Copper salts were added to the fixative to preserve the color of the cells. Surface preparations and cross sections were made manually using a razor. For cross sections, fragments of leaf plates along the midrib were selected.

Cross sections were stained with a 5 % alcohol solution of Lugol's iodine for a clearer picture.

The studies were conducted using a MICMED-6 microscope with a UCMOS05100 digital camera at magnifications of 10x4, 10x10, and 10x40. The micro-preparations were described in accordance with the recommendations of R. Crang [12], P.J. Rudall [13], and the State Pharmacopoeia of the Russian Federation [14].

Results and Discussion

The leaf *of Tilia cordata* is simple, on a long petiole, with a heart-shaped or broadly ovate base, a pointed tip, and a finely serrated margin [15, 16]. The upper side of the leaf blade is dark green and smooth, while the lower side is light green, almost grayish, with characteristic colored tufts of trichomes along the veins. The leaves are 3–10 cm long and 4–12 cm wide.

A leaf fragment taken from the upper side of the leaf (Fig. 3) shows the absence of stomata, but prismatic crystals are visible through the epidermis. The main epidermal cells have straight anticlinal walls or wavy walls.

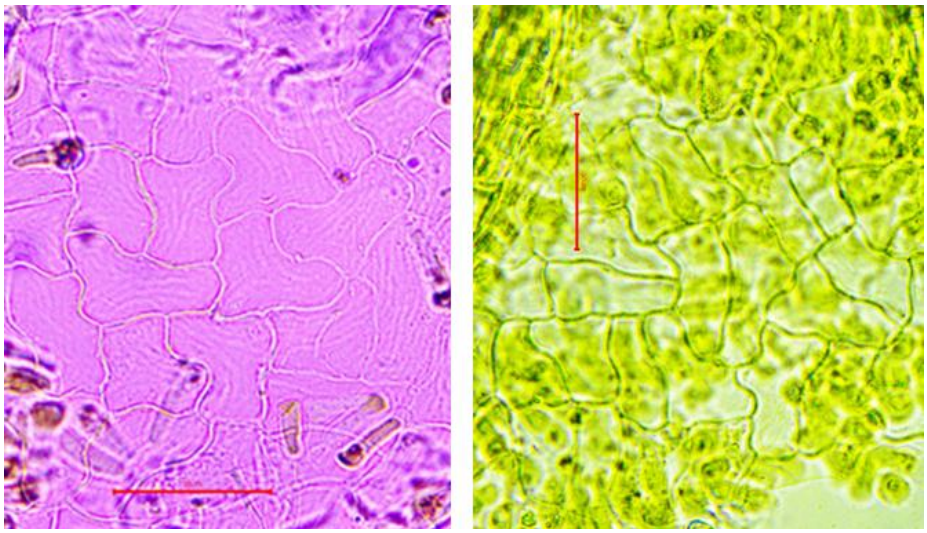


Figure 3. Upper epidermis of a *Tilia cordata* leaf

A superficial preparation of the underside of the leaf revealed the presence of stomatal cells of the anomocytic type (Fig. 4). The stomata have an average size of 14.6 × 23.5 μm and are surrounded by 3–4–5 epidermal cells. Thus, the leaf *of Tilia cordata* is defined as hypostomate, since the stomata are located on the lower surface of the leaf blade.

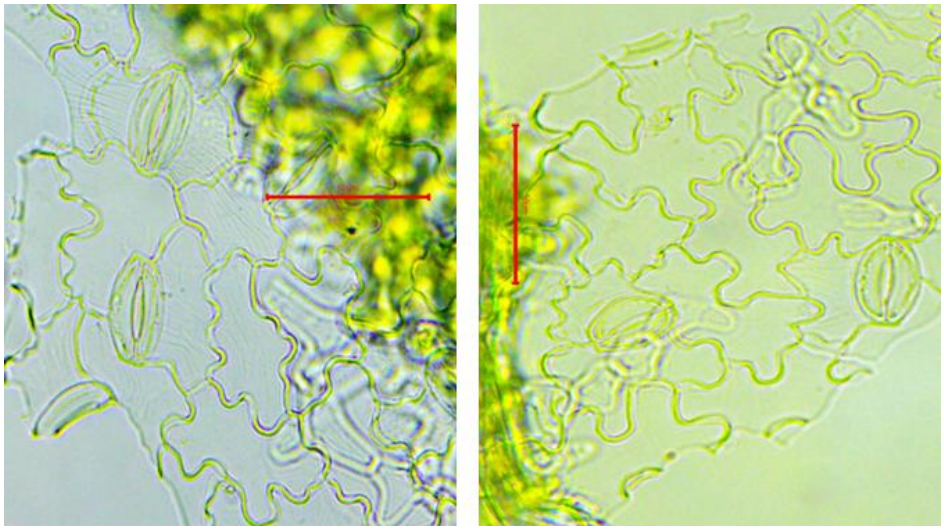


Figure 4. Lower epidermis of *Tilia cordata* leaf

There are different types of trichomes [17]. For example, glandular trichomes are confined to the sinuses of secondary or tertiary veins (Fig. 5). They are usually bulbous or bottle-shaped.

Along the main veins are forked trichomes (Fig. 6), which have a short stalk and are pressed against the surface of the axis. In the axils of the veins, there are capitate multicellular (Fig. 7) and stellate trichomes (Fig. 8). The latter vary in the number of branches, from 4 to 8.

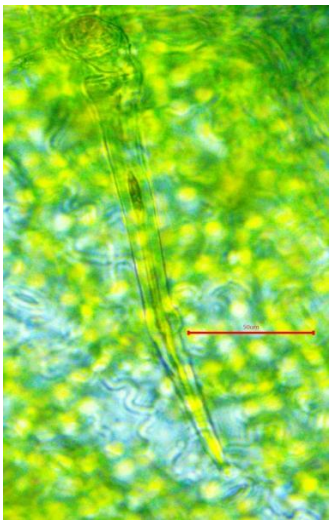


Figure 5. Glandular trichomes on the leaves of *Tilia cordata*

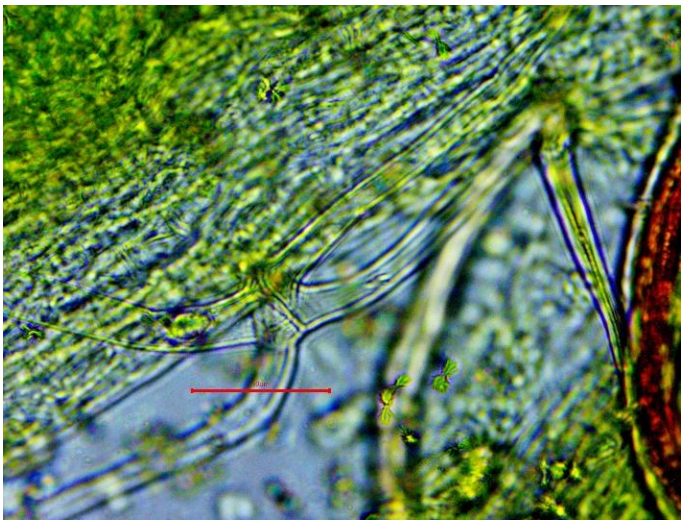


Figure 6. Forked trichomes on the leaves of *Tilia cordata*

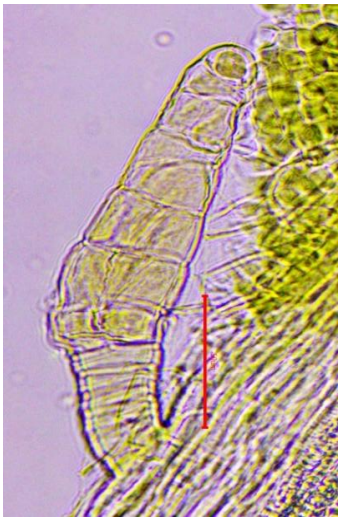


Figure 7. Capitate trichomes on the leaves of *Tilia cordata*

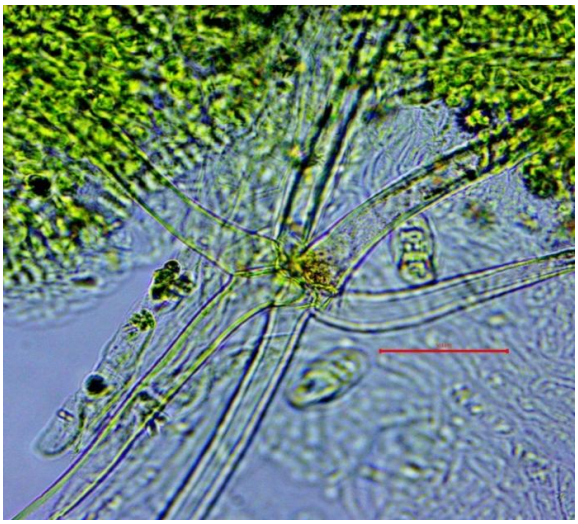


Figure 8. Stellate trichomes on the leaves of *Tilia cordata*

Crystalline inclusions are visible along the veins of the leaves on both sides (Fig. 9). The crystals are prismatic in shape.

The leaf cross-section (Fig. 10) is identified as dorsoventral, containing palisade parenchyma on one side and spongy parenchyma on the other. The palisade mesophyll is single-layered, while the spongy mesophyll contains 2-3 layers.

On the cross section of the midrib (Fig. 10a) and lateral fragments (Fig. 10b), a single-layered epidermis is located around the perimeter. Its cells are elongated, almost rectangular. In the area of the midrib, there are 1-2 layers of collenchyma, and the parenchyma and sclerenchyma consist of 3-4 layers. In the midrib, mucous channels containing calcium oxalate crystals are visible. The conducting bundle is collateral, closed, and broadly ovoid in shape.

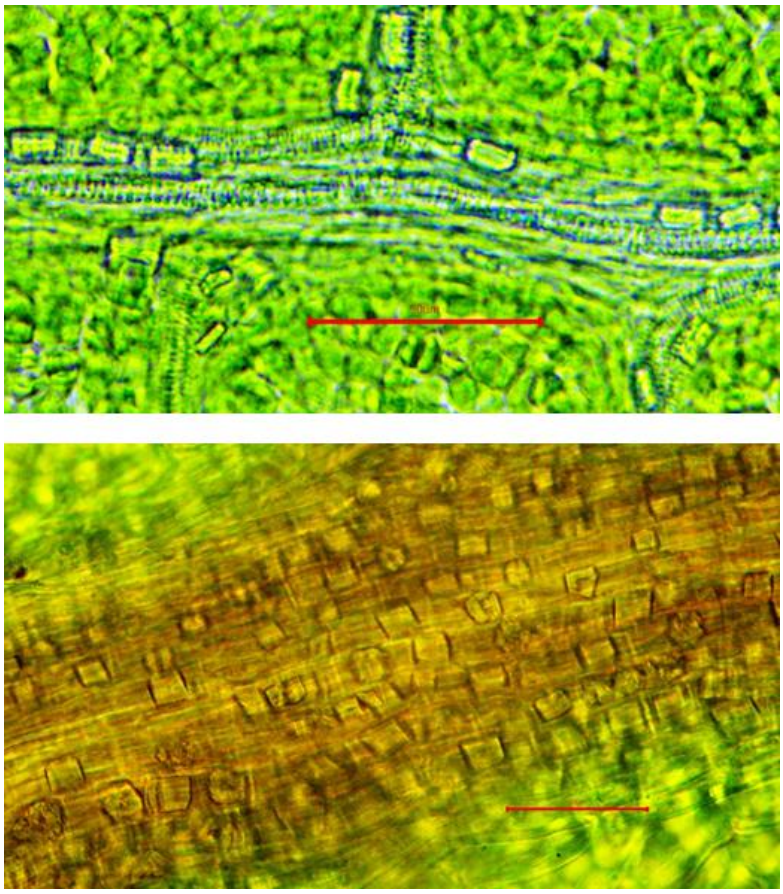


Figure 9. Crystalline coating along the veins of a *Tilia cordata* leaf

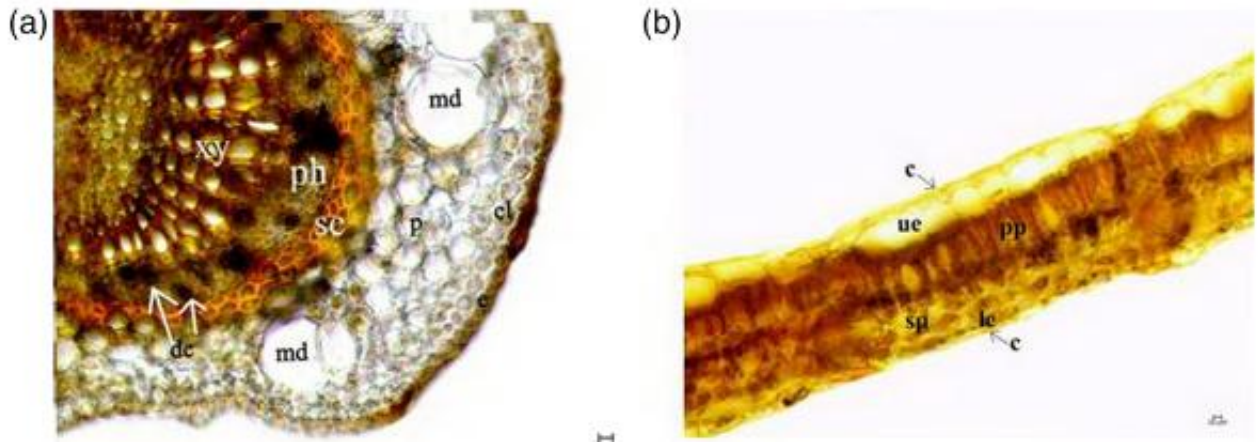


Figure 10. Cross section of a *Tilia cordata* leaf
a — midrib; b — lateral fragment; c — cuticle; cl — collenchyma; dc — calcium oxalate druses; le — lower epidermis; md — mucous canal; p — cortical parenchyma; ph — phloem; pp — palisade mesophyll; sc — sclerenchyma; sp — spongy mesophyll; ue — upper epidermis; xy — xylem

The structure of a true *Tilia cordata* leaf has similar features to the leaves of other species [18, 19]. However, there are distinctive features, consisting of the peculiarities of the structure of the epidermis cells, the types of hairs, and the presence of mucous channels and crystal druses in the cross section.

The identification of structural features can serve as diagnostic criteria for the identification of plant raw materials at the microscopic level.

Conclusion

Thus, a study of the anatomical structure of the *Tilia cordata* leaf was carried out, including analysis of surface preparations and cross sections. The structural features of the leaf epidermis, the type of leaf based on the arrangement of veins, and the presence of four types of trichomes were established.

For the transverse section, a dorsoventral type of leaf plate, the presence of a single-layer palisade and multi-layer spongy tissue, the presence of mucous channels and calcium oxalate druses were identified. The bundle is collateral, closed type, surrounded by 2-3 layers of sclerenchyma.

The diagnostic features identified can help in determining the species and identifying medicinal plant raw materials.

Acknowledgements

The author would like to thank the staff of the State University of Humanities and Technology for their help in collecting raw materials.

Conflict of Interest

The author declares no conflict of interest.

Author contribution

Babeshina L.G. — investigation, visualization, manuscript writing.

References

1 Атлас лекарственных растений России. — М., 2021. — 646 с.

2 Карематов И.Д. Липа сердцевидная / И.Д. Карематов, А.Т. Абдувохидов // Биология и интегративная медицина. — 2017. — № 8. — С. 134–141.

3 Zhou Y.-J. Comprehensive review of Tilia L.: phytochemical profiles, edible value, therapeutic potentials, and ecological significance / Y.-J. Zhou, Ren Qiang, Y.-B. Shen // Food & Medicine Homology. — 2025. — Vol. 2(2). — 9420035. https://doi.org/10.26599/FMH.2025.9420035

4 Kruk A. Tiliae flos metabolites ad their beneficial influence on human gut microbiota biodiversity ex vivo / A. Kruk, S. Granica, D. Popowski, N. Malinowska, J.P. Piwowarska // Journal of Ethnopharmacology. — 2022. — Vol. 294. — P. 1–12. https://doi.org/10.1016/j.jep.2022.115355

5 Попов Д.М. Сравнительное качественное и количественное определение углеводов в цветках и листьях липы сердцевидной / Д.М. Попов, Н.В, Зарубина // Разработка и регистрация лекарственных средств. — 2013. — № 3. — С. 50–53.

6 Медведева Т.М. Препараты на основе экстрактов липы: получение и фармакологическая активность / Т.М. Медведева, В.В. Сорокин, И.Е. Каухова, В.Ц. Болотова // Фармация. — 2011. — № 7. — С. 34–36.

7 Власова Н.В. Семейство Tiliaceae — Липовые / Н.В. Власова // Флора Сибири. — Т. 10: Geraniaceae — Cornaceae. — Новосибирск: Наука, 1996. — С. 65-66.

8 Ali S.A. Antibacterial and antioxidant activity of flavonoid, glycoside and alkaloid extracts of *Tilia Cordata* / S.A. Ali, H.S. Al-Atbi, F. Moein, B.M. Ali // International Journal of Health Sciences. — 2022. — Vol. 6(S3). — P. 3976–3983. https://doi.org/10.53730/ijhs.v6nS3.6664

9 Qaralleh H. Antibacterial and Antibiofilm Activities of a Traditional Herbal Formula against Respiratory Infection Causing Bacteria / H. Qaralleh, M. Al-Limoun, A. Khlaifat, K. Khleifat, N. Al-Tawarah, K. Alsharafa, H. Abu-Harirah // Tropical Journal of Natural Product Research. — 2020. — Vol. 4(9). — P. 527–534.

10 Wissam Z. Extracting and studying the antioxidant capacity of polyphenols in dry linden leaves (*Tilia cordata*) / Z. Wissam, A.A. Nour, J. Bushra, N. Zein, D. Saleh // Journal of Pharmacognosy and Phytochemistry. — 2017. — Vol. 6(3). — P. 258–262.

11 Ruzin S.E. Plant microtechnique and microscopy / S.E. Ruzin. — Oxford: Oxford University Press, 1999. — 415 p.

12 Crang R. Plant Anatomy. A concept-based approach to the structure of seed plants / R Crang, Sh. Lyons-Sobaski, R. Wise. — Springer Nature, 2018. — 250 p.

13 Rudall P.J. Anatomy of flowering plants. An introduction to structure and development / P.J. Rudall. — Cambridge: Cambridge University Press, 2006. — 315 p.

14 Государственная Фармакопея Российской Федерации. XIV издание. — М., 2018. — 650 с.

15 Исайкина Н.В. Изучение анатомо-морфологического строения цветков липы / Н.В, Исайкина, В. Андреева // Здоровье и образование. — 2015. — Т. 17, № 6. — С. 1–6.

16 Gibadulina I.I. Anatomical and Morphological Features of the Leaves of *Tilia Cordata* Mill. As an Indicator of the Adaptive Capabilities of the Species to the Conditions of the Urban Environment / I.I. Gibadulina, M.V. Larionov, N.N. Maslennikova // IOP Conference Series: Earth and Environmental Science. — 2022. — Vol. 988(3). — Article ID 032082.

17 Ramirez-Diaz M. Leaf architecture and anatomy of eight species of Tilia (Malvaceae) / M. Ramirez-Diaz, J. Gutierrez, T. Terrazas // Acta Botanica Mexacana. — 2024. — Vol. 131. — Article ID e2332. https://doi.org/10.21829/abm131.2024.2332

18 Strelnikov B.G. Anatomy and micromorphology of *Tilia* species from Turkey and its taxonomic implication / B.G. Strelnikov, B. Gurdal // Microscopy Research and Technique. — 2024. — Vol. 87(11). — P. 2625–2635. https://doi.org/10.1002/jemt.24634

19 Popoviciu D.R. Biometric and morphologic observation on *Tilia cordata* Mill. (Tiliaceae) leaves / D.R. Popoviciu // Analele Univ Craiova. — 2019. — P. 150–158.

Л.Г. Бабешина

Tilia cordata микроскопиялық талдауы

Дәрілік шикізаттың шикізат базасын кеңейтуді өсімдіктердің жаңа түрлерін іс жүзінде пайдалануға енгізу, сондай-ақ бұрын іс жүзінде пайдалануға енгізілген жаңа түрлерді қолдану есебінен жүргізуге болады. *Tilia cordata* гүлдері шикізаттың сұранысқа ие түрі, алайда оларды жинау қысқа гүлдену кезеңімен шектеледі. Осы түрдің жапырақтары да фармакологиялық белсенділікті көрсетеді, бұл оларды дербес дәрілік өсімдік шикізаты ретінде перспективалы етеді. Мақалада *Tilia cordata* жапырақтарын микроскопиялық талдау нәтижелері келтірілген. Кептірілген жапырақтардың анатомиялық талдауы жүргізілді, оның ішінде беткі препараттар мен көлденең қималардың құрылымдық ерекшеліктерін талдау жүргізілді. Жапырақтың гипостоматикалық типке жататыны анықталды, эпидермистің негізгі жасушалары түзу немесе толқынды қабырғаларға ие; төменгі жағында трихомалардың 4 түрі бар, олар: безді, айырлы, бас тәрізді және жұлдыз тәрізді. Жапырақ тамырларының бойында кристалды жабын бар. Көлденең қимада жапырақтың дорсовентральды құрылым түрі бар: палисадты мезофилл бір қабатты, кеуек тәрізді — 2-3 қабатты, шырышты арналар мен кальций оксалат кристалдарының друздары бар. Фармакопеялық мақаланы дайындау үшін қолдануға болатын микроскопиялық деңгейде диагностикалық белгілер анықталды.

Кілт сөздер: *Tilia cordata*, өсімдік шикізаты, микроскопиялық талдау, жапырақ, микро диагностикалық белгілер.

Л.Г. Бабешина

Микроскопический анализ *Tilia cordata*

Расширение сырьевой базы лекарственного сырья можно производить как за счет введения в практическое использование новых видов растений, так и за счет применения новых органов, ранее включенных в практическое использование. Цветки *Tilia cordata* являются востребованным видом сырья, однако их сбор ограничен коротким периодом цветения. Листья данного вида также проявляют фармакологическую активность, что делает их перспективными в качестве самостоятельного лекарственного растительного сырья. В настоящей статье приводятся результаты микроскопического анализа листьев *Tilia cordata*. Анатомирование проведено для высушенных листьев с анализом особенностей строения поверхностных препаратов и поперечных срезов. Установлено, что лист относится к гипостоматическому типу, основные клетки эпидермиса имеют прямые или волнистые стенки; с нижней стороны присутствуют 4 типа трихом: железистые, вильчатые, головчатые и звездчатые. Вдоль жилок листьев присутствует кристаллоносная обкладка. На поперечном срезе лист имеет дорзовентральный тип строения: палисадный мезофилл однослойный, губчатый — 2-3-слойный, присутствуют слизистые каналы и друзы кристаллов оксалата кальция. Установлены диагностические признаки на микроскопическом уровне, которые могут использоваться для подготовки фармакопейной статьи.

Ключевые слова: *Tilia cordata*, растительное сырье, микроскопический анализ, лист, микро диагностические признаки

References

1 (2021). *Atlas lekarstvennykh rastenii Rossii* [Atlas of Medicinal Plants of Russia]. Moscow [in Russian].

2 Karomatov, I.D., & Abduvokhidov, A.T. (2017). Lipa serdtsevidnaia [Heart-shaped linden]. *Biologiia i integrativnaia meditsina — Biology and Integrative Medicine,* 8, 134–141 [in Russian].

3 Zhou, Y.-J., Qiang, Ren, & Shen, Y.-B. (2025). Comprehensive review of Tilia L.: phytochemical profiles, edible value, therapeutic potentials, and ecological significance. *Food & Medicine Homology, 2*(2), 9420035. https://doi.org/10.26599/FMH.2025.9420035

4 Kruk, A., Granica, S., Popowski, D., Malinowska, N., & Piwowarska, J.P. (2022). Tiliae flos metabolites ad their beneficial influence on human gut microbiota biodiversity ex vivo. *Journal of Ethnopharmacology, 294*, 1–12. https://doi.org/10.1016/j.jep.2022.115355

5 Popov, D.M., & Zarubina, N.V. (2013). Sravnitelnoe kachestvennoe i kolichestvennoe opredelenie uglevodov v tsetkakh i listiakh lipy serdtsevidnoi [Comparative qualitative and quantitative determination of carbohydrates in the flowers and leaves of heart-shaped linden]. *Rasrabotka i registratsiia lekarstvennykh sredstv — Development and registration of medicinal products,* 3, 50–53 [in Russian].

6 Medvedeva, T.M., Sorokin, V.V., Kaukhova, I.E., & Bolotova, V.T. (2011). Preparaty na osnove ekstraktov lipy: poluchenie i farmakologicheskaia aktivnost [Preparations based on linden extracts: production and pharmacological activity]. *Farmatsiia — Pharmacy*, 7, 34–36 [in Russian].

7 Vlasova, N.V. (1996). Familiia Tiliaceae — Lipoviie [Family Tiliaceae — Linden]. *Flora Sibiri — Flora of Siberia*, *10*, 65-66. Novosibirsk: Nauka [in Russian].

8 Ali, S. A., Al-Atbi, H. S., Moein, F., & Ali, B. M. (2022). Antibacterial and antioxidant activity of flavonoid, glycoside and alkaloid extracts of *Tilia Cordata. International Journal of Health Sciences, 6*(S3), 3976–3983. https://doi.org/10.53730/ijhs.v6nS3.6664

9 Qaralleh, H., Al-Limoun, M., Khlaifat, A., Khleifat, K., Al-Tawarah, N., Alsharafa, K., & Abu-Harirah, H. (2020). Antibacterial and Antibiofilm Activities of a Traditional Herbal Formula against Respiratory Infection Causing Bacteria. *Tropical Journal of Natural Product Research*, *4*(9), 527–534.

10 Wissam, Z., Nour, A.A., Bushra, J., Zein, N., & Saleh, D. (2017). Extracting and studying the antioxidant capacity of polyphenols in dry linden leaves (Tilia cordata). *Journal of Pharmacognosy and Phytochemistry, 6*(3), 258–262.

11 Ruzin, S.E. (1999). *Plant microtechnique and microscopy*. Oxford: Oxford University Press.

12 Crang, R., Lyons-Sobaski, Sh., & Wise, R. (2018). *Plant Anatomy. A concept-based approach to the structure of seed plants*. Springer Nature.

13 Rudall, P.J. (2006). *Anatomy of flowering plants. An introduction to structure and development*. Cambridge: Cambridge University Press.

14 (2018). *Gosudarsvennaia Farmakopeia Rossiiskoi Federatsii. XIV izdanie* [State Pharmacopoeia of the Russian Federation. XIV edition]. Moscow [in Russian].

15 Isaikina, N.V., & Andreeva, V.Yu. (2015). Izuchenie anatomo-morfologicheskogo stroeniia tsvetkov lipy [Study of the anatomical and morphological structure of linden flowers]. *Zdorovie i Obrazovanie — Health and Education, 17*(6), 1–6 [in Russian].

16 Gibadulina, I.I., Larionov M.V., & Maslennikova, N.N. (2022). Anatomical and Morphological Features of the Leaves of Tilia Cordata Mill. As an Indicator of the Adaptive Capabilities of the Species to the Conditions of the Urban Environment. *IOP Conference Series: Earth and Environmental Science, 988*(3), ID 032082.

17 Ramirez-Diaz, M., Gutierrez, J., & Terrazas, T. (2024). Leaf architecture and anatomy of eight species of Tilia (Malvaceae). *Acta Botanica Mexacana, 131,* e2332. https://doi.org/10.21829/abm131.2024.2332

18 Strelnikov, B.G., & Gurdal, B. (2024). Anatomy and micromorphology of Tilia species from Turkey and its taxonomic implication. *Microscopy Research and Technique*, *87*(11), 2625–2635. https://doi.org/10.1002/jemt.24634

19 Popoviciu, D.R. (2019). Biometric and morphologic observation on *Tilia cordata* Mill. (Tiliaceae) leaves. *Analele Univ Craiova*, 150–158.

Information about the author

Babeshina Larissa Gennadievna — Doctor of Biological Sciences, Professor of the Pharmacy Department, Synergy University, Moscow, Russian Federation; e-mail: lbabeshina@yandex.ru; https://orcid.org/0000-0002-2028-0316