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RESEARCH ARTICLE

Phytochemical profile of *Liquidambar styraciflua* L. leaves in conditions of Kyiv city

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Abstract

For the first time in Ukraine, phytochemical studies of the raw material of *Liquidambar styraciflua* L. were conducted. In particular, the content of the main groups of biologically active compounds in the leaves of these plants was analyzed, including flavonoids (catechins, leucoanthocyanins, anthocyanins), vitamins (ascorbic acid), pigments (carotenoids, chlorophylls), polysaccharides, and tannins.

It was found that flavonoid compounds in the leaves of *L. styraciflua* contain 356.13 mg % of catechins, 151.86 mg % of anthocyanins, and 872.93 mg % of leucoanthocyanins. This composition of secondary metabolites is supplemented by ascorbic acid (51.43 mg %), polysaccharides (3.50 %), and a significant amount of tannins (5.07 %). There is also a relatively high content of lipophilic compounds – carotenoids (49.01 mg %) and chlorophylls (220.60 mg %). The results of the studies advocate the prospective application of *L. styraciflua* plants as a source of flavonoid compounds, vitamins, and other biologically active substances. The results of this study can be helpful for identifying the *Liquidambar* species and searching for new promising phytochemical sources for the pharmaceutical, food, and cosmetic industries.

Keywords: liquidambar, flavonoid compounds, vitamins, pigments, polysaccharides, tannins

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Introduction

The undeniable role of botanical gardens is preserving and enriching the biological diversity of cultural and natural plant resources, particularly medicinal ones. The purposeful preservation and enrichment of the gene pool of medicinal plants is an urgent and promising task for today. Recently, the development of comprehensive research on plants with medicinal properties has been considered appropriate in the system of botanical gardens of Ukraine. Among such plants, the introduced *Liquidambar* species are promising. Currently, in Ukraine, these plants are used exclusively for decorative purposes, thanks to the attractive color of the leaves – emerald in spring and bright red or burgundy in autumn, their habit, and their variety of forms. In many countries these plants have both decorative and medicinal values.

There is a growing understanding of the role of biologically active compounds in plant objects, which is of interest in various aspects of research. Along with morphological and anatomical traits, phytochemical data are crucial for plant taxonomy and for understanding the functions performed by biologically active compounds in plants. Data on the chemical composition of plants is essential for their application in the pharmaceutical, food, and cosmetic industries.

The involvement of medicinal plants in botanical gardens makes them available for versatile and comprehensive study and allows us to identify individual features and resolve issues related to preserving biodiversity and their rational use.

The genus *Liquidambar* (Altingiaceae) includes 15 species of plants with edicinal properties (POWO, 2024). Globally, liquidambar plants are used as medicinal raw materials for the pharmaceutical industry (Lebeda, 2009; El-Readi et al., 2013; Mancarz et al., 2019; Minarchenko et al., 2019). They are also applied in perfumery, as a source of high-quality wood, and for the production of insecticidal preparations (Kim et al., 2008; Lingbeck et al., 2015). They are sometimes applied in phytomelioration, bioenergy production, and ornamental horticulture (Kohno & Kuznetsov, 2005; Smilyanets & Svitylko, 2021).

An important feature of the plant is the production of a resin (storax), which has been used for centuries to treat skin problems, pneumonia, and ulcers. Storax has recently been shown to be a potent antimicrobial agent against drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* Rosenbach. The storax is most commonly obtained from *L. styraciflua* and *L. orientalis* Mill. The storax of *L. orientalis* is rich in free and bound cinnamic acid, which can comprise 30–47% of the total content of balsamic acids. The resinous part of storax consists of storesinol, an amorphous white substance that is found both in a free state and in compounds with cinnamic acid (Minarchenko et al., 2019). The essential oil includes about 60% triterpene acids, known as cytoresins (Teker & Kolancılar, 2020).

The leaves, bark, and seeds of liquidambar also contain vital biologically active compounds, namely shikimic acid, which is a precursor in the production of oseltamivir phosphate, the active ingredient in the antiviral drug Tamiflu, which is active against most influenza viruses. Extracts from different parts of the liquidambar can produce antioxidant, anti-inflammatory, and chemopreventive agents. In particular, preparations from the liquidambar resin have hypotensive effects, and the seeds have anticonvulsant effects, indicating prospects for use in treating epilepsy (Lingbeck et al., 2015).

The balsam of *L. styraciflua* is listed in the US Pharmacopeia and is used in official medicine as an antiseptic treatment in the form of inhalations, an expectorant, a diuretic, and an analgesic preparation (USP, 2010; Svitylko, 2023). The pharmacological properties of *L. styraciflua* fruits are actively studied. In particular, the aqueous extract of the fruits containing aqueous (polysaccharide aqueous extract) and ethanolic (sucrose aqueous extract) fractions was analyzed. The ethanolic fraction appeared to be unselectively toxic to cells. While the aqueous fraction was found promising for cancer treatment due to its high toxicity to cancer cells and low toxicity to other cells (Pozzobon et al., 2023).

In Ukraine, two Liquidambar species (i.e., *L. formosana* Hance and *L. styraciflua* L.) are currently cultivated. Among them, *L. styraciflua* is the most common (Kokhno et al. 2002) and is applied as a decorative plant. *Liquidambar styraciflua* is native to the eastern United States, as far north as Connecticut, south to Florida, and west to Texas. It can also be found in mountainous regions of Mexico and Central America (POWO, 2024).

It was found that *L. styraciflua* is a promising culture for broader application in various industries in Ukraine, primarily forestry and horticulture. It was also noted that the chemical composition of Liquidambar species includes tannins, ellagic acid, proanthocyanidins (derivatives of cyanidin and delphinidin), flavonols (glycosides of quercetin and myricetin), and iridoids (Horbenko et al., 2022). Horbenko et al. (2022) also pointed out that the phytochemical composition of *L. styraciflua* should be further investigated, and plantation cultivation of this species in

Table 1. Air temperature indicators in Kyiv, °C (CGO, 2024).

	Month												Average annual
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2024	−2.6	+2.9	+4.8	+12.8	+16.3	+21.5	+24.3	+23.1	+20.6	+10.9	+2.7	0.0	+11.4
Long-term average (1991–2020)	−3.2	−2.3	+2.5	+10.0	+15.8	+19.5	+21.3	+20.4	+14.9	+8.6	+2.6	−1.9	+9.0

Table 2. Precipitation indicators in Kyiv, mm (CGO, 2024).

	Month												Total annual
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2024	48	48	55	78	15	135	52	24	21	63	51	52	642
Long-term average (1991–2020)	37	39	40	42	65	74	68	56	58	46	46	47	618

Ukraine for purposes of the pharmaceutical industry should be considered.

The lack of information about the phytochemical profile of *L. styraciflua* determines the urgent need for detailed analysis. The search for plants with a diverse phytochemical potential of biologically active compounds, mainly secondary metabolites, which inhibit the formation of free radicals and toxic metabolic products, contribute to increasing its adaptive potential and nonspecific resistance, and determine the pharmacological effect of plants on the human body for further use in the pharmaceutical and food industries, remains relevant.

Phytochemical studies of raw material from *L. styraciflua* have not been conducted in Ukraine yet. In this regard, we investigated the main groups of biologically active compounds of secondary metabolites: flavonoids (catechins, leucoanthocyanins, and anthocyanins), vitamins (ascorbic acid), pigments (carotenoids and chlorophylls), polysaccharides, and tannins in the leaves of this species.

Material and methods

The M.M. Gryshko National Botanical Garden (NBG) is located in the north part of Ukraine, on the right bank of the Dnipro River within Kyiv city, where high hills are cut by valleys and provide favorable conditions for many introduced plants. The total area of NBG is ca. 130 ha, and the latitude ranges from 100 to 190 m a.s.l. The climate of Kyiv is temperate continental. The average annual temperature is +7.4 °C. The average temperatures in Kyiv in the hottest month (July) are +20.5 °C, and in the coldest month (January) are -3.5 °C. The highest recorded temperature was +39.9 °C, and the lowest was -32.2 °C. The annual precipitation is about 550–650 mm, and the average total solar radiation is 97–100 kcal/cm² per year (Vakulenko et al., 2019).

During the research period, in 2024, the temperature in Kyiv was relatively high. The average annual temperature was 11.4 °C, exceeding the long-term average of 2.4 °C. All months also showed higher temperatures compared to respective long-term average ones (Table 1). The average annual precipitation during the study period also exceeded the long-term average, but precipitations were unevenly distributed by months (Table 2).

Kyiv is located on the border of two soil zones. Sandy soils of fluvioglacial origin prevail toward the north part of Kyiv, while loesses occur in the south. The soils of the NBG are dark gray, podzolized, and slightly washed out. Hygroscopic humidity corresponds to this type of soil. The pH indicator of the soil is close to neutral, although acidity increases when the horizon is deepened below 55 cm. Such pH does not limit the use of these soils, which are generally favorable for most plants. Humus and nitrogen values are typically not

Table 3. Chemical composition of soils in the M.M. Gryshko National Botanical Garden, according to Bedrykivska, regarding absolutely dry matter (Smilyanets, 1993).

Horizon, cm	Hygroscopic humidity, %	pH	Total humus, %	Total nitrogen, %	K_2O, mg/100 g	Hydrolytic acidity, mgEq/100g	Sum of absorbed bases, mgEq/100g	Absorption capacity, mgEq/100g	Base saturation, %
0–25	1.7	6.6	1.02	0.06	16.27	0.15	47.95	48.1	99.69
25–55	1.68	6.6	0.6	0.03	6.51	0.15	19.29	19.44	99.23
55–100	1.76	5.4	–	–	6.52	0.75	7.48	8.23	90.89

high, but with the deepening of the horizon, their contents decrease rapidly. Soil porosity is low, but with the lowest soil moisture capacity, it is relatively favorable for most plants (Table 3).

Liquidambar styraciflua is a deciduous tree up to 45 m tall and up to 1.5 m in trunk diameter. It has a wide-pyramidal crown, a deeply furrowed dark gray trunk bark, and corky outgrowths on the branches. Young shoots are pubescent. The attractive five- to seven-lobed leaves are 10–16 cm long, with dark green adaxial and lighter abaxial lamina surfaces. In autumn, the leaves turn dark crimson. The lamina is oblong-triangular, pointed, finely toothed along the edge, and pubescent in the corners of the prominent veins. The petiole is up to 12 cm long. The flowers are inconspicuous and apetalous. Staminal flowers are aggregated in terminal racemes, pistillate – in solitary spherical heads on thin pedicels. The composite fruit consists of fused brown, shiny, pointed capsules containing 1–2 seeds per capsule. The fruit is spherical, up to 3 cm in diameter. The seeds are tiny and have short wings.

Liquidambar styraciflua blooms in May and bears fruit in October. The natural range of this species covers Eastern North America – Florida, Ohio, Indiana, Oklahoma, and Mexico. In Ukraine, it is cultivated in parks and botanical gardens of Lviv, Uzhgorod, Chernivtsi, Kyiv, Simferopol, and Yalta. These are relatively frost-resistant, light-requiring, and moisture-loving plants adapted to urban conditions. *Liquidambar styraciflua* plants are propagated by seeds, cuttings, and ofsprings. It is one of the most decorative species for landscaping (Kokhno et al., 2002).

The biochemical composition of the leaves was studied using *L. styraciflua* plants growing in the Laboratory of Medical Botany collection plot of the NBG (Fig. 1). Samples were collected in the phase of active leaf growth in July. No agronomic practices (fertilizers application, treatment with plant protection products, irrigation, pruning, etc.) were applied.

Quantifying tannins was performed using the permanganatometry, which makes it possible to determine tannins and other oxidative polyphenols (Karpiuk et al., 2022). Flavonoid compounds (catechins and leucoanthocyanins) were determined in plant raw material using absorption spectrophotometry (SPhU, 2014). In particular, the quantitative content of anthocyanins was determined spectrophotometrically at a wavelength of 528 nm (SPhU, 2020). Optical density was measured on a Specol-1500 spectrophotometer (Germany). Polysaccharides were extracted from the raw material using water heating for 30 min and precipitated with three times the volume of 96 % ethanol. After that, the precipitate was filtered, dried, and weighed. The content of polysaccharides was calculated using absolutely dry raw material by gravimetric method (SPhU, 2021). The ascorbic acid content was evaluated by spectrophotometric method at a wavelength of 520 nm (SPhU, 2014).

The content of photosynthetic pigments was determined according to generally accepted methods (Musiyenko et al., 2001) on a Specol-1500 spectrophotometer (Germany). 96 % ethanol was used to isolate pigments. Extraction was carried out with a pre-cooled solvent. To calculate the concentrations of chlorophylls *a*, *b*, and carotenoids in each sample, their optical density was determined. For chlorophyll *a*, the maximum absorption in 96% ethanol was observed at 665 nm wavelength, and for chlorophyll *b* – at 649 nm

Figure 1. The common view (A) and the leaves (B, C) of *Liquidambar styraciflua* tree growing at the M.M. Gryshko National Botanical Garden.

wavelength. Carotenoids were determined at a wavelength of 441 nm.

The obtained data were statistically processed in the Microsoft Excel software environment.

Results and discussion

It was found that the leaves of *L. styraciflua* contain a complex of biologically active compounds, represented by secondary metabolites, such as flavonoid compounds (catechins, leucoanthocyanins, and anthocyanins), tannins, polysaccharides, vitamins (ascorbic acid), pigments (carotenoids and chlorophyll).

Flavonoid compounds are an essential constant component of many plants. They are localized in fruits, leaves, bark, and wood but are distributed unevenly. In particular, they are primarily accumulated in reproductive and young plant organs, which indicates their active participation in metabolism. Flavonoids include a significant group of compounds with antioxidant properties that participate in redox processes. Considering antioxidant activity, catechins and leucoanthocyanidins belong to the most reduced flavonoid compounds. Flavonoids, along with capillary-strengthening properties, exhibit cardiotonic, antisclerotic, antitumor, radioprotective, insulin-like, and interferon-like effects and also affect the composition of the blood (Shtrigol & Tovchyga, 2010).

The content of catechins in the leaves of studied *L. styraciflua* was 356.13 ± 7.38 mg% (Table 4). This is a relatively high value, allowing to consider extracts of *L. styraciflua* leaves as a potential therapeutic source of agents with high anti-inflammatory properties and synergistic action with antibiotics against bacteria. Thus, the aqueous-alcoholic extract of leaves with tetracycline, due to phenolic compounds, is effective against gram-positive bacteria

Table 4. Content of flavonoid compounds, polysaccharides, and tannins in the leaves of *Liquidambar styraciflua* at the M.M. Gryshko National Botanical Garden.

Flavonoid compounds, mg%			Polysaccharides, %	Tannins, %
Catechins	Anthocyanins	Leucoanthocyanins		
356.13±7.38	151.86±3.87	872.93±10.99	3.50±0.31	5.07±0.27

Table 5. Biologically active compounds of vitamin origin in the leaves of *Liquidambar styraciflua* at the M.M. Gryshko National Botanical Garden.

Ascorbic acid, mg%	Carotenoids, mg%	Chlorophylls *a* + *b*, mg%
51.43±1.65	49.01±1.57	220.60±4.60

Enterococcus faecalis (Andrewes & Horder) Schleifer & Kilpper-Bälz (Mancarz et al., 2016).

The leaves of *L. styraciflua* contain anthocyanins (151.86±3.87 mg%), the plant glycosides of phenolic origin (Table 4). They reduce the level of blood cholesterol, inhibit lipid oxidation processes, and exhibit cardioprotective, antioxidant, and anti-inflammatory activities (Kobzar, 2007).

A significant content of leucoanthocyanins (872.93±10.99 mg%) was also noted (Table 4). This class of phytochemical compounds has wide applications. In the food industry, anthocyanins are used primarily as a natural dye, and in the pharmaceutical industry, they are applied to produce drugs with antioxidant properties. Anthocyanins also have bactericidal and solar protective properties. The high content of catechins, anthocyanins, and leucoanthocyanins in the leaves of *L. styraciflua* may be a prospective source of these compounds for the listed industries.

Polysaccharides are complex carbohydrates that can be hydrolyzed into monosaccharides. The content of polysaccharides in the studied leaves of *L. styraciflua* was 3.50±0.31 mg% (Table 4). Polysaccharides exhibit a wide range of pharmacological activity, which depends on their composition and structure. They play a significant role in biochemical processes and are widely used in various branches of science and industry as biologically active and auxiliary substances (Smoilovska et al., 2023). The high content of polysaccharides in the leaves of *L. styraciflua* positively characterizes this plant as a raw material for creating biologically active additives.

Plants containing tannins are used as astringent, anti-inflammatory, hemostatic, and bactericidal. They are used in diseases of the gastrointestinal tract and poisoning with metal salts and plant poisons. A significant amount of tannins (5.07±0.27 %) was found in the leaves of *L. styraciflua* plants (Table 4), which is also promising for the creation of drugs.

Vitamins are a vital component of the plant that maintains the activity of the antioxidant system and correlates with the content of free radical oxidation reactions, preventing the accumulation of toxic products in the body. Vitamins can be water-soluble (ascorbic acid) or fat-soluble (carotenoids and chlorophylls).

Ascorbic acid ensures the functional state of connective tissue and exhibits antioxidant, antiatherogenic, regenerative, anti-inflammatory, antiscorbutic, antiviral, and immunomodulatory activity (Camarena & Wang, 2016). The leaves of *L. styraciflua* contained 51.43±1.65 mg% of ascorbic acid (Table 5).

Lipophilic compounds (i.e., carotenoids and chlorophylls) are important for plants and humans. Carotenes are responsible for many essential functions: they contribute to the processes of vision, as well as other aspects of the vital activity: cell differentiation and proliferation, growth, and reproduction, participate in the process of hematopoiesis, and the functioning of the immune system (El-Agamey et al., 2004; Simonova, 2010). The leaves of *L. styraciflua* contained 49.01±1.57 mg% of carotenoids (Table 5).

As is known, chlorophyll in the human body supports vital functions and prevents the development of many diseases. It is an important element contributing to the vitality and productivity of plants (Yakovenko et al., 2021). Chlorophyll has a tonic effect, enhances basic metabolism, stimulates tissue regeneration, and has bactericidal properties. The chemical structure of chlorophyll is similar to hemoglobin, which explains its impact on the human circulatory system. It induces the production of leukocytes, erythrocytes, and hemoglobin. In the leaves of *L. styraciflua*, the content of chlorophylls (*a* + *b*) was quite significant – 220.60±4.60 mg% (Table 5). Considering that chlorophyll has outstanding biological significance, exhibiting bactericidal, detoxifying, antioxidant, and anticarcinogenic effects, promotes the restoration of damaged tissues, and can inhibit the growth of bacteria and other harmful microorganisms (Lysiuk et al., 2018), *L. styraciflua* can be considered as a promising medicinal plant.

Conclusions

In Ukraine, phytochemical studies of *L. styraciflua* leaves were conducted for the first time. It was shown that leaves of *L. styraciflua* contain a significant amount of biologically active compounds: catechins – 356.13 mg%; anthocyanins – 151.86 mg%; leucoanthocyanins – 872.93 mg%; polysaccharides – 3.50%; tannins – 5.07%; ascorbic acid – 51.43 mg%; carotenoids – 49.01 mg%, chlorophyll – 220.60 mg%.

The obtained results confirm the prospects of further phytochemical studies of various organs of *L. styraciflua*. Such studies are essential for expanding the range of phytochemicals with high prophylactic and treatment effects against human diseases. The phytochemical compounds of *L. styraciflua* can be applied against pathogeneses, in which the activation of free radical oxidation, particularly lipid peroxidation, plays a significant role. These plants are also a promising raw material source for the pharmaceutical, food, and cosmetic industries.

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Фітохімічний профіль листків *Liquidambar styraciflua* L. в умовах м. Києва

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Вперше в Україні проведено фітохімічні дослідження сировини *Liquidambar styraciflua* L. Зокрема, проаналізовано вміст основних груп біологічно активних сполук, зокрема: флавоноїдів (катехіни, лейкоантоціани, антоціани), вітамінів (аскорбінова кислота), пігментів (каротиноїди, хлорофіли), полісахаридів і дубильних речовин у листках цих рослин.

Установлено, що флавоноїдні сполуки, які локалізовано в листках *L. styraciflua* містять: 356,13 мг % катехінів, 151,86 мг % антоціанів, 872,93 мг % лейкоантоціанів. Цей склад вторинних метаболітів доповнюють: аскорбінова кислота (51,43 мг %), полісахариди (3,50 %), значний відсоток дубильних речовин (5,07 %). Також спостерігається відносно високий вміст ліпофільних сполук – каротиноїдів (49,01 мг %) та хлорофілів (220,60 мг %). Результати досліджень доводять перспективність використання рослин *L. styraciflua* як джерела флавоноїдних сполук, вітамінів та інших біологічно активних речовин. Результати цих досліджень можуть бути застосовані для ідентифікації видів роду *Liquidambar* та пошуку нових перспективних продуцентів для фармацевтичної, харчової, косметичної промисловості.

Ключові слова: ліквідамбар, флавоноїдні сполуки, вітаміни, пігменти, полісахариди, дубильні речовини