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ANATOMICAL AND PHYTOCHEMICAL STUDY LEAVES OF *PARTHENOCISSUS QUINQUEFOLIA* (L.) PLANCH

Topicality. *P. quinquefolia (L.) Planch. is often used in ornamental landscaping in urban cities. Over the past ten years, the species has naturalised in numerous natural and anthropogenic habitats, especially near megacities. In addition to its vertical growth form, it also has a ground growth form, and therefore often suppresses the growth of native species in the grass area, thereby transforming the coenosis into a new type. An analysis of the literature indicates the prospects for its use in medicine and pharmacy, as its compounds have a number of medicinal properties, including antioxidant, capillary-strengthening, detoxifying, anti-inflammatory, antiviral, and antidiabetic effects. Given the prospects of the species for use in medicine, as well as the availability of a sufficient resource base of P. quinquefolia, it can be considered a potential source of raw materials for pharmaceutical needs.*

The aim of the work was *to conduct morphological, anatomical and phytochemical studies of P. quinquefolia leaves to create a project of a monograph on the studied medicinal plant material.*

Materials and methods of the study. *The material for the study was the leaves of P. quinquefolia, which were harvested in the flowering and fruiting stages of the growing season. The microscopic diagnostic study was carried out according to the generally accepted methods of the SPhU. The obtained micro preparations were examined in aqueous medium and aqueous glycerol solutions of different concentrations under a ULAB microscope (×40, ×100, ×1000) equipped with a Canon EOS 550 digital microphotographic camera. The identification of biologically active substances of P. quinquefolia was carried out using conventional qualitative and histochemical reactions.*

Research results and their discussion. *According to the results of morphological and anatomical analysis of P. quinquefolia leaves, it was found that this species has a number of species-specific features that will allow to distinguish it from other species of the genus. According to the results of qualitative reactions, the presence of polysaccharides, saponins, tannins, anthocyanins, hydroxycinnamic acids and flavonoids in the leaves of P. quinquefolia was established.*

Conclusions. *The main morphological characters of P. quinquefolia leaves were determined. The main diagnostic anatomical features of P. quinquefolia leaves were determined: the presence of two types of calcium oxalate crystals (druses, raphides); cells-idioblast; simple 6-8 cell trichomes; lenticels of petioles and stomatas of anomocytic type. The main biologically active substances of P. quinquefolia leaves were determined: polysaccharides, flavonones, flavones, condensed tannins, hydroxycinnamic acids and saponins. Further detailed phytochemical study of this species will allow to develop a project monograph on the raw materials of P. quinquefolia.*

Key words: *P. quinquefolia, leaves, epidermis, identification.*

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АНАТОМІЧНЕ ТА ФІТОХІМІЧНЕ ДОСЛІДЖЕННЯ ЛИСТКІВ *PARTHENOCISSUS QUINQUEFOLIA* (L.) PLANCH

Актуальність. *P. quinquefolia (L.) Planch. часто використовується у декоративному озелененні різних міст. За останні десять років вид натуралізувався у численних природних та антропогенних біотопах, особливо поряд із мегаполісами. Він має окрім вертикальної форми росту ще й наземну, а тому часто пригнічує ріст аборигенних видів у трав'яному покриві, тим самим перетворюючи ценоз на новий тип. Аналіз літератури вказує на перспективність використання його в медицині та фармації, оскільки сполуки, що входять до його складу, проявляють низку лікувальних властивостей, серед яких: антиоксидантна, капілярозміцнююча, детоксикуюча, протизапальна, противірусна, антидіабетична. Ураховуючи перспективність виду щодо застосування у медицині, а також наявність достатньої ресурсної бази P. quinquefolia, його можна розглядати як потенційне джерело сировини для потреб фармації.*

Мета дослідження – *провести морфолого-анатомічне та фітохімічне дослідження листків P. quinquefolia для створення проєкту монографії на досліджувану лікарську рослинну сировину.*

Матеріал і методи. *Матеріалом для дослідження були листя P. quinquefolia, які заготовляли у фазі цвітіння і плодоношення. Мікроскопічне діагностичне дослідження проводили за загальноприйнятими методиками ДФУ. Отримані мікропрепарати досліджували у водному середовищі та водних розчинах гліцерину різної концентрації під мікроскопом фірми ULAB (×40, ×100, ×1000), який обладнаний цифровою мікрофотокамерою Canon EOS 550. Ідентифікацію біологічно активних речовин P. quinquefolia проводили за допомогою загальноприйнятих якісних та гістохімічних реакцій.*

Результати дослідження. *За результатами морфолого-анатомічного аналізу листків P. quinquefolia встановлено, що даний представник має низку видоспецифічних ознак, які дадуть змогу виокремити його серед інших представників роду. За результатами проведених якісних реакцій установлено наявність у листках P. quinquefolia полісахаридів, сапонінів, дубильних речовин, антоціанів, гідроксикоричних кислот та флавоноїдів.*

Висновок. *Установлено основні морфологічні ознаки листків P. quinquefolia. Визначено головні діагностичні анатомічні ознаки листків P. quinquefolia: наявність двох типів кристалів оксалату кальцію (друзи, рафіди); клітин-ідіобластів; простих 6–8-клітинних трихом; сочевичок черешка та аномоцитного продихового апарату. Визначено основні біологічно активні речовини листків P. quinquefolia: полісахариди, флавонони, флавони, конденсовані дубильні речовини, гідроксикоричні кислоти та сапоніни. Подальше детальне фітохімічне вивчення цього виду дасть змогу розробити проєкт монографії на сировину P. quinquefolia.*

Ключові слова: *P. quinquefolia, листя, епідерма, ідентифікація.*

Introduction. Topicality. Biological pollution of the environment due to invasion and rooting of adventive species in communities of natural and restorative native vegetation has been recognised as one of the most pressing environmental problems not only in Ukraine but also worldwide (Davis, 2003; Blossey, 1999). P. quinquefolia Planch. is a kenophyte, agriophyte, ergasiophyte native to North America (Burda, 2015). There are three species of the genus *Parthenocissus* in Ukraine: *P. inserta* Fritsch., *P. tricuspidata* Planch., and *P. quinquefolia* (L.) Planch. (Mosyakin, 1999). The latter is often used in decorative landscaping of our cities. Over the past ten years, the species has naturalised in numerous natural and anthropogenic habitats, especially near megacities and in disturbed areas (Burda, 2015). In addition to its upright growth form, it also has a ground growth form, and therefore often suppresses the growth of native species in the grass cover, thus transforming the coenosis into a new type. One way to control this species, which is alien to our flora, is to use it in medicine

and pharmacy, as it has a variety of medicinal properties. In particular, β-amyrilhexadecanoate, extracted from *P. quinquefolia* leaves, acts as a thrombin inhibitor (Yang J, 2010). Chinese scientists have isolated resveratrol trans-dehydrodimer, 3,4,5-trihydroxy-benzoic acid, cifostemin A and B, palidol from this representative by means of chromatographic method, quercetin-3-O-alpha-L-rhamnoside, myricetin-3-O-alpha-L-rhamnoside, which exhibited antioxidant, capillary strengthening, detoxifying anti-inflammatory, antiviral and antidiabetic effects (Yang J, 2010; Chistohodova, 2002; Baur, 2006). The infusion of the leaves had astringent, diuretic, anti-diarrhoea, choleretic, analgesic, antioxidant and antiparasitic effects (Moerman, 1998). Since P. quinquefolia is a perspective species for its use in medicine and has a sufficient resource base, our goal was to study the morphological and anatomical features of the leaves as a potential raw material for pharmaceutical needs.

The aim of the work. The aim of this work was to study the morphological, anatomical and phytochemical characteristics of *P. quinquefolia* leaves, which will be used to create a project of a monograph on the research raw material.

Materials and methods of the study. The material for the research was the leaves of *P. quinquefolia*. The leaves were harvested in two phases: flowering (green, summer leaves) and fruiting (crimson, autumn leaves). Sections of the leaf blade were used for microscopic diagnostic analysis. Both fresh and dried raw materials were used for the preparation of micro preparations. The microscopic diagnostic study was carried out according to the generally accepted methods of the SPhU (SPhU, 2015). To better separate the epidermis of the leaves, they were pre-boiled in a 5% sodium hydroxide solution for 2 min. To lighten the objects, they were boiled in an aqueous solution of chloral hydrate (4:1). The resulting micro preparations were examined in water and glycerol solutions of different concentrations under a ULAB microscope (×40, ×100, ×1000) equipped with a Canon EOS 550 digital microphotographic camera. To increase the objectivity of the results, 10 microdissections were examined for each object. The stomatal index was calculated according to the standard method described in the State Pharmacopoeia of Ukraine (SPhU, 2015). The identification of biologically active substances of *P. quinquefolia* was carried out using conventional qualitative and histochemical reactions (SPhU 2004, Kovalov, 2014).

For histochemical reactions, fresh and pre-soaked dried raw materials were used. For easier separation of the epidermis of leaf petioles, they were boiled in a 5% sodium hydroxide solution for 5 min before preparation of microdissections. To obtain illuminated objects, they were boiled in an aqueous solution of chloral hydrate (4:1). To perform qualitative reactions for the detection of polysaccharides, saponins, tannins, catechins, anthocyanins, hydroxycinnamic acids, flavonoids, and phenolic compounds, aqueous and aqueous-alcoholic extracts from green (summer) and purple (autumn) leaves were prepared according to generally accepted methods (Dolia, 2003; Kovalov, 2014).

Research results and their discussion. The morphological and anatomical analysis of *P. quinquefolia* leaves demonstrated that this representative has a number of species-specific features that allow to distinguish it from other representatives of the genus.

The leaves of *P. quinquefolia* are palmately compound, five-lobed, elliptical with an acuminate apex and a wedge-shaped base, unevenly narrowed, large-toothed along the edge (fig. 1).

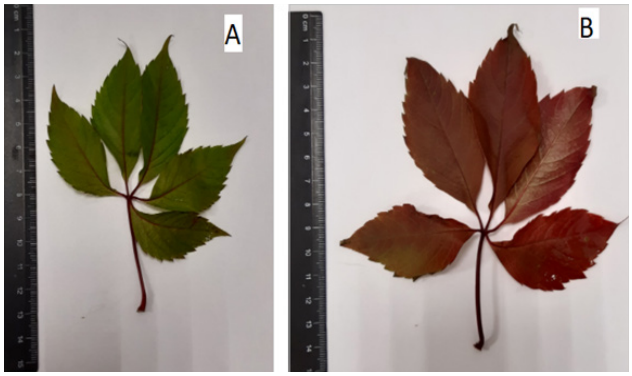

Fig. 1. Leaves of *P. quinquefolia*: A – green (summer), B – crimson (autumn)

On the dorsal side, pubescence is found along the central and lateral veins, and on the ventral side along the edge of the leaf blade and along the central vein (fig. 2).

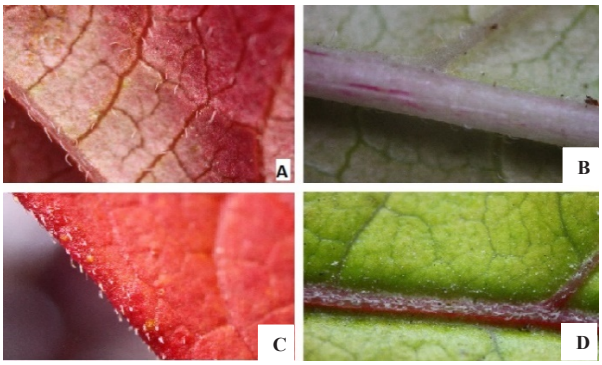

Fig. 2. Fragment of the leaf blade of *P. quinquefolia* x 40: A, B – dorsal side; C, D – ventral side

In the epidermal cells of the petiole and around the fibrovascular bundles, druses are arranged in orderly rows (fig. 3).

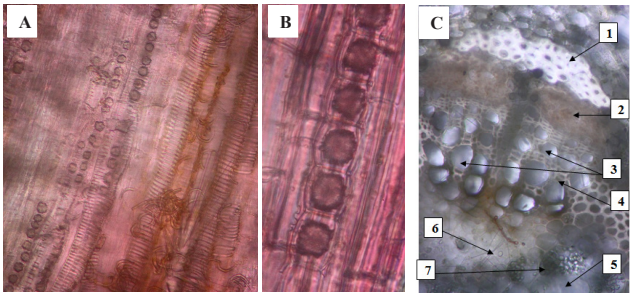

Fig. 3. Fragment of epidermis and cross-section of the central part of petiole *P. quinquefolia*: A – x400, B – x1000: C – open collateral vascular bundle x400: 1 – phloem, 2 – cambium, 3 – xylem, 4 – mechanical tissue, 5 – main parenchyma, 6 – raphides, 7 – druses

In the cells of the main parenchyma of both petiole and leaf blade, idioblasts with raphides that are pointed at one end and blunt at the other are often found; stomata are of anomocytic type; trichomes, which occur mainly along the leaf veins on the abaxial side, are simple 6-8-celled with slightly warty epidermis (fig. 4).

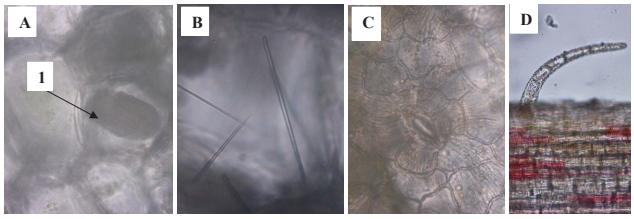

Fig. 4. Fragment of the leaf blade and epidermis of *P. quinquefolia* petiole: A, B – cells of the main parenchyma with needle-shaped calcium oxalate crystals x1000: 1 – cell-idioblast; C – anomocytic stomatal apparatus x1000; D – fragment of abaxial epidermis with trichome x400

The leaves of *P. quinquefolia* are of hypostomatal type with numerous anomocytic stomata only on the lower epidermis (fig. 5). The stomatal index was 11.3 ± 4 %, indicating an average degree of transpiration of the leaf blade surface.

The cells of the adaxial epidermis of the leaf are parenchymal, square or rectangular in shape with thickened membranes and simple straight pores in the cell membrane without stomata (fig. 5A). The cells of the abaxial epidermis are sinuous, irregular in shape with thin membranes and stomata (fig. 5B), and rectangular in shape along the veins (fig. 5C). Single, needle-shaped crystals are found in the cells of the lower and upper epidermis.

The results of the qualitative reactions revealed the presence of polysaccharides in the leaves of *P. quinquefolia* polysaccharides (with 96% ethanol), saponins (foaming reaction; with lead acetate), tannins (with 1% gelatin solution; with iron-ammonium alum; with lead acetate), anthocyanins (with sulfuric acid), hydroxycinnamic acids (with hydrochloric and sulfuric acids) and flavonoids (cyanidin reaction; with lead acetate; reaction with 1% vanillin solution).

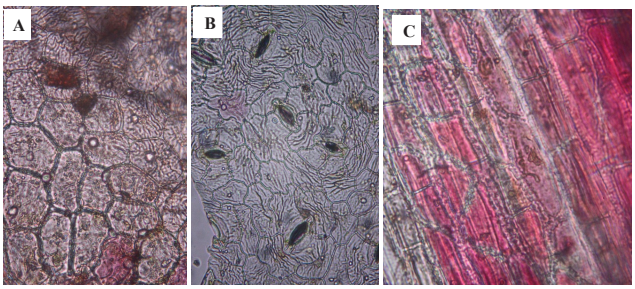

Fig. 5. Leaf epidermis of *P. quinquefolia* x1000: A – adaxial epidermis, B – abaxial epidermis, C – abaxial epidermis along the vein

To confirm the qualitative reactions for the detection of carbohydrates and tannins, a number of histochemical reactions were performed.

The presence of polysaccharides in the sample was confirmed by the Molisch reaction (Dolia, 2003). We observed the coloration of the cut cells in orange-red colour, which indicates the presence of polysaccharides in the sample (fig. 6 A, D).

The reaction with the addition of methylene blue to a transverse section of a wide part of the petiole confirmed the presence of mucilage in the cells (fig. 6 B, E).

A section of the petiole of the object under study with a 1% solution of iron (III) chloride resulted in the appearance of a dirty black-green colour of the cells, indicating the presence of tannins in the sample (fig. 6 C, F).

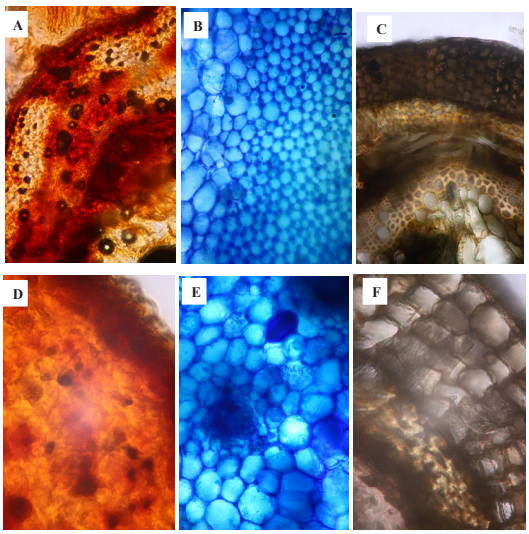

Fig. 6. Results of histochemical reactions: A – x400, D – x1000 detection of polysaccharides on the cross-section of the petiole; B – x400, E – x1000 identification of slime in the cells of the wide part of the petiole on the cross-section: 1 – cell-idioblast; C – x400, F – x1000 results of the reaction to detection of tannins on the cross-section of the petiole

Conclusions. It was found that the main morphological characters of the leaves of *P. quinquefolia* are the shape of the leaf blade, type of venation, pubescence and the presence of an expanded part at the base of the rachis.

It has been found that the main diagnostic anatomical features of *P. quinquefolia* leaves are the presence of two types of calcium oxalate crystals (druses, raphids); cells-idioblast; simple 6-8-celled trichomes; petiole lenticels and anomocytic stomatal apparatus.

It was determined that the leaves of *P. quinquefolia* contain the following biologically active substances: polysaccharides, flavonones, flavones, condensed tannins, hydroxycinnamic acids and saponins.

The leaves of *P. quinquefolia* can potentially be a source of medicinal plant material containing polysaccharides, tannins and anthocyanins. Further detailed phytochemical study of this species will allow to develop a project monograph on *P. quinquefolia* raw materials.

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