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LOCALISATION OF BIOACTIVE COMPOUNDS IN THE LEAVES OF *Hedera helix* L. (Araliaceae) – A MEDICINAL AND COSMETIC PLANT

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ABSTRACT

Hedera helix (common ivy) is an attractive climbing and groundcover plant. Its leaves containing various bioactive compounds are used in medicine and cosmetology. The aim of this study was to present the functional leaf anatomy and histochemical analyses localising various secondary metabolites in ivy leaf blade and petioles. Light and fluorescence microscopy and 8 histochemical tests and 3 fluorescence assays were used. The leaf tissues contained phenolic compounds, phenolic acids, flavonoids, pectinous/mucilagous substances, acid lipids, essential oil, terpenoids, and steroid-containing terpenes. They are mainly responsible for the biological and pharmacological activity of *H. helix* leaves. Most of these secondary metabolites were present in the epidermis, mesophyll, phloem, and epithelial cells. In turn, they were not found in the xylem and sclerenchyma tissues.

Key words: common ivy, secondary metabolites, histochemical assays, anatomy of leaf, fluorescence microscopy

INTRODUCTION

The common ivy *Hedera helix* L. (Araliaceae) is a climber with dark evergreen leaves. It occurs naturally in Europe and Asia Minor [Szweykowska and Szweykowski 2003]. At present, it is regarded as an invasive plant in some states of the USA [Maddox et al. 2018]. In previous years, it was a protected plant in Poland [Seneta and Dolatowski 2007]. The common ivy has been cultivated since ancient times. It can be a houseplant used as an element of interior decoration. In landscape architecture, it is planted as a groundcover species or a climber. It can also be used as a green element in flower arrangements [Seneta and Dolatowski 2007, Korszun and Bykowska 2011, Maddox et. al 2018].

Ivy leaves are heterophyllous. The usually 3–5 lobed leaves growing on juvenile shoots are tomentose at first and then glabrous. The strongly shiny leaves growing on flower shoots are ovate or ovoid-lanceolate. In natural habitats, the species blooms rarely and usually after many years. Juvenile plants with shoots reaching 20 m in length are the most common forms [Seneta and Dolatowski 2007]. Plants of this species may live for 450 years and the leaf lifespan is 2.5 years [Rostafiński and Siedl 1962, Nowiński 1977].

Hedera helix is a medicinal and cosmetic plant [Góra and Lis 2005, Jabłońska-Trypuć and Czerpak 2008, Kohlmünzer 2016, Kruttschnitt et al. 2020]. Its flowers appearing in September and October provide bees with valuable reward [Lipiński 2010]. All organs of the plant contain toxic substances, and its fruits are the most poisonous parts. Ivy saponins are toxic to humans, livestock, and pets (cattle, cats, dogs) [Gaillard et al. 2003, Maddox et al. 2018].

Ivy leaf (*Folium Hederae helicis*) is a medicinal raw material. It contains triterpenes, saponins,

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flavonoids, coumarins, polyacetylenes, phenolic acids, tannins, anthocyanins, sterols, alkaloids, amino acids, essential oil, resins, carbohydrates, vitamins C and E as well as provitamin A [Lutsenko et al. 2010, Lamer-Zarawska et al. 2012, Kohlmünzer 2016, Zeng et al. 2018]. The yellow-red ivy resin (*Gummiresina Hederae*) is obtained mainly from fruits and is used for production of incense and in dentistry [Nowiński 1977, Strzelecka and Kowalski 2000].

The special properties of ivy leaf extracts facilitating the penetration of cosmetics into the skin are used in the production of creams and lotions [Van Wyk and Wink 2004]. Ivy leaf extracts exhibit multivarious pharmaceutical and biological activities, i.e. anti-inflammatory, antifungal, anti-oxidative, anti-mutagenic, anti-viral, and anti-diabetic effects [Fazio et al. 2009, Lutsenko et al. 2010, Zeng et al. 2018]. Due to the presence of saponins, the extracts exert an expectorant and relaxant effect [Strzelecka and Kowalski 2000].

The presence of secretory canals in vegetative organs has been found to be a characteristic trait of representatives of the family Araliaceae. Secretory canals are regarded as an important diagnostic feature of this family [Metcalfe and Chalk 1972, Kolalite et al. 2003]. The anatomical features of ivy include the presence of secretory canals in all organs and stellate hairs on stems and leaves. Ivy leaves are hypostomatic and bifacial. The secretory canals are located primarily in leaf midribs close to vascular bundles [Săvulescu and Luchian 2009, Ostroumova and Oskolski 2010]. Some leaf mesophyll cells contain calcium oxalate crystals [Konyar et al. 2014].

Many compounds present in plant tissues e.g. phenolic acids, and flavonoids are important metabolites exhibiting multiple biological activities [Kelayeh et al. 2019]. These compounds are also present in *H. helix* leaves [Lutsenko et al. 2010, Lamer-Zarawska et al. 2013]. The distribution of active biological metabolites has practical and pharmacognostic importance in medicinal plants and can be used for chemosystematics. We have found no data on the location of secondary metabolites in the aerial organs of this species. Our study is an attempt to fill the gap in this knowledge.

The aim of the study was to present the anatomical features and histochemical characteristics of the *Hedera helix* leaf blade and petiole, which are a medicinal and cosmetics raw material. We localized the main classes of metabolites in leaf tissues using fluorescence and light microscopy as well as histochemical assays.

MATERIALS AND METHODS

The research material, *Hedera helix* L. leaves, was taken from the collection of plants at the UMCS Botanical Garden in Lublin, SE Poland (51°15'44"N, 22°30'48"E) in June 2018. The botanical identification was made by comparison with authentic samples deposited in the Department of Botany and Plant Physiology, University of Life Sciences in Lublin. Fragments of leaves were sampled from the middle part of one-year-old shoots. To compare their general anatomy, the leaves were collected from plants growing in sunlit and shaded sites. Since the histochemical tests did not reveal any differences in the distribution of biological active compounds in the tissues of leaves collected in both sites, we presented only the results of plants growing in the sunlit sites. Five leaves were used in each test. Cross-sections and the central part of the leaf blades, the midrib, and petioles were made with a razor blade using the free-hand sectioning technique.

The autofluorescence of phenolic compounds in fresh leaf tissues and stain reactions with magnesium acetate and antimony trichloride [Ascensão et al. 1999] were observed using a Cy5 filter set (excitation light 590–650 nm and a barrier filter wavelength 663–738 nm). The analyses were carried out with the use of a Nikon 90i fluorescence microscope equipped with a digital camera (Nikon Fi1) and NIS-Elements Br 2 software.

We performed 8 histochemical assays using fresh sections of leaf blades and 6 tests for petioles. The leaf blade, which has been analysed in great detail in the study, was the main object of the study, whereas the petiole was analyzed as an additional element to compare the location of the selected metabolites. The main classes of metabolites in tissues were detected using the following assays: magnesium acetate for flavonoids [Charrière-Ladreix 1976], Sudan IV for lipids [Brundrett et al. 1991], Nile Blue for neutral and acid lipids [Jensen 1962], NADI reagent for terpenoids [David and Carde 1964], antimony trichloride for steroid-containing terpenes [Mace et al. 1974], ferric trichloride for total phenolic compounds [Gahan

1984], potassium dichromate for tannins [Gabe 1968], ruthenium red for pectins and mucilaginous substances [Johansen 1940], neutral red for essential oils and mucilaginous substances [Conn 1977, Lulai and Morgan 1992], and Toluidine blue O (pH 4) for phenolic compounds, tannins and pectins [Baker 1966, O'Brien et al. 1981]. The staining effects were compared with control samples. We detected the presence of oxalate crystals based on the dissolution reaction in 10% HCl [Broda 1971]. The study was conducted using an Eclipse 400 (Nikon) light microscope coupled to a Coolpix 4500 (Nikon) camera.

RESULTS

Leaf blade

Anatomical traits. Unilayered **epidermis** covered the adaxial and abaxial surface of the *H. helix* leaf blade (Fig. 1A, B). This tissue consisted of cells that were flattened in cross section and had a thick outer cell wall with a cuticle layer (Fig. 1B). Stellate hairs were present on the leaf blades and petioles (Fig. 1C). **Collenchyma** reinforced only the midrib zone and formed several layers under the adaxial and abaxial epidermis (Fig. 1A, B). **Chlorenchyma** was observed in the midrib (Fig. 1A, B). Idioblasts containing **calcium oxalate druses**, whose presence was confirmed by the dissolution reaction, were located under the epidermis and collenchyma in the midrib and surrounded the main vascular bundle. Idioblasts formed a continuous layer in some areas, but were dispersed in others (Fig. 1A). In turn, in both palisade and spongy parenchyma of the leaf blade, they were arranged singly or in groups (Fig. 1D). The leaf **mesophyll** varied in the palisade parenchyma zone, depending on the light conditions during plant growth. In cross section, the palisade parenchyma in leaves collected from the sunlit sites was composed of two layers of cylindrical, elongated cells (Fig. 1E), whereas the parenchyma of leaves originating from shaded locations usually had three layers of much shorter, sometimes square-shaped cells (Fig. 1F). Large collateral **vascular bundles** surrounded by several sclerenchyma layers were located centrally in the leaf midribs. In the parenchyma, secretory canals were visible around the xylem and phloem or within the sclerenchyma on the cross section (Fig. 1A, G, H) and on the longitudinal section (Fig. 1I). Orange **secretion** was visible in the lumen of the canals and in the epithelial cells, which formed a single layer surrounding the canals (Fig. 1G). A substance with a similar colour as that of the secretion contained in the canals and epithelial cells was observed in the parenchyma cells as well (Fig. 1G).

Histochemical traits. The fluorescence microscope studies and histochemical assays facilitated detection of the following substances in the *H. helix* leaf blades and petioles: phenolic compounds, phenolic acids, flavonoids, tannins, pectinous/mucilagous substances, lipids, essential oil, terpenoids, and steroid-containing terpenes (Tab. 1).

Epidermis. In the cells of this tissue, we found phenolic acids (autofluorescence; Fig. 2A), flavonoids (magnesium acetate, UV; Fig. 2B), phenolic compounds (ferric trichloride; Fig. 2D, Toluidine blue O; Fig. 2F–H), tannins (potassium dichromate; Fig. 2E), lipids (Nile blue; Fig. 3A), essential oil (Sudan IV, neutral red; Fig. 3C–F), and terpenoids (Nadi Reagent; not shown). The outer epidermis cell walls with the cuticle layer were stained intense orange upon treatment with Sudan IV, which indicates their lipid nature (Fig. 3B). A high concentration of phenolic acids (light blue autofluorescence) and flavonoids (light yellow secondary fluorescence) was observed in the cuticle (Fig. 2A, B). After treatment of the preparations with ruthenium red, intense red staining was visible in the epidermis cells, which indicates high content of pectinous substances or mucilages (Fig. 4A, B).

Collenchyma. The protoplasts of collenchyma cells contained phenolic compounds (Fig. 2G, H), phenolic acids, and acid lipids (Tab. 1).

Parenchyma. Regardless of the parenchyma type in the leaves, the same secondary metabolites were detected in both palisade and spongy parenchyma cells (Tab. 1). The presence of phenolic acids in the parenchyma cells was indicated by the light blue autofluorescence of these metabolites (Fig. 2A). The presence of flavonoids was indicated by the light yellow secondary fluorescence of these compounds (Fig. 2B). Phenolic compounds stained black in the ferric trichloride reaction (Fig. 2D), brown in the potassium dichromate treatment (Fig. 2E), and blue in Toluidine blue O reaction (Fig. 2F). Blue-stained acid lipids were observed after the treatment of the preparations with the Nile blue reagent (Fig. 3A), whereas orange-stained

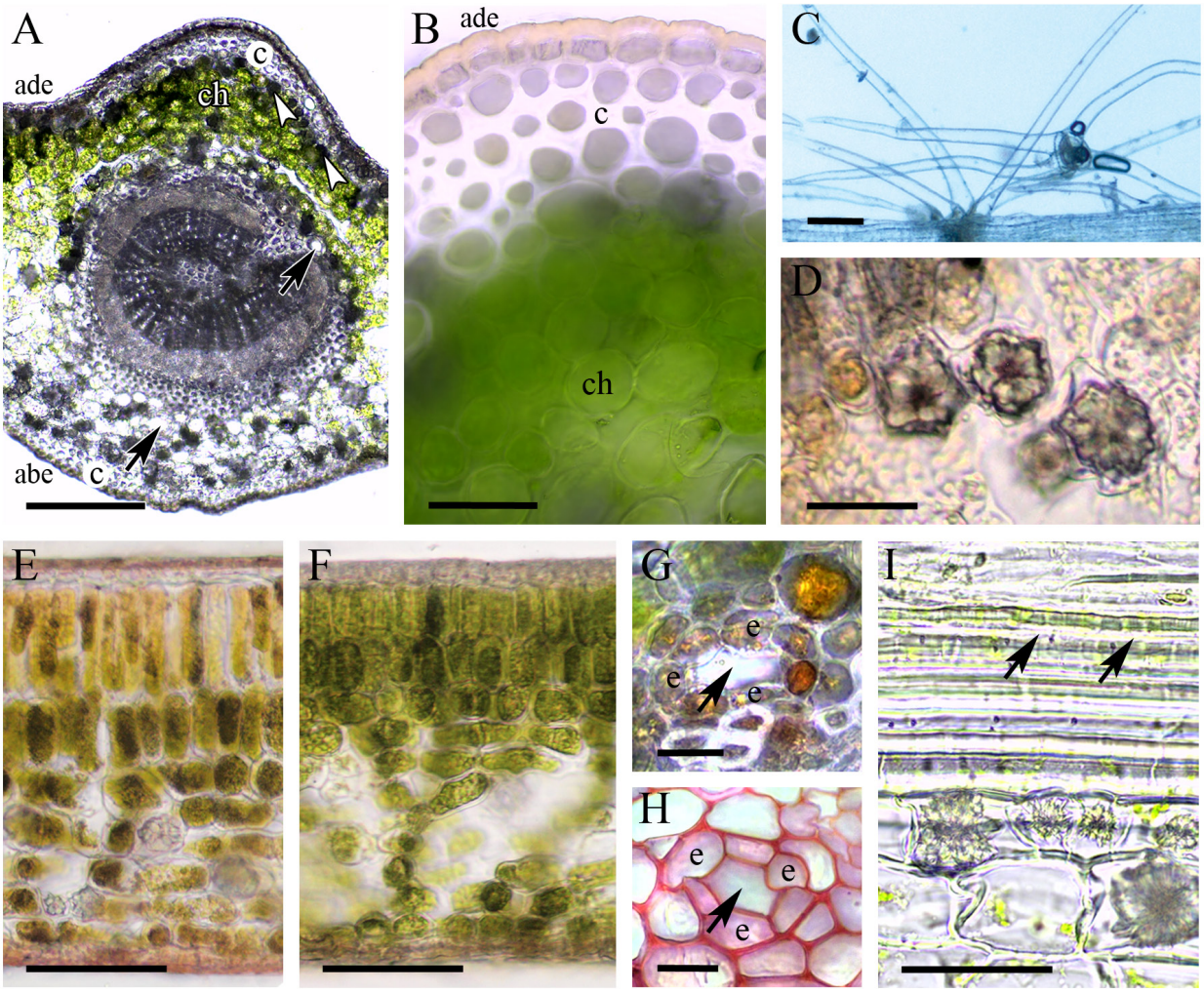

Fig. 1. Cross-sections of *Hedera helix* leaves and their fragments.

A. Cross-section of the leaf midrib with visible secretory canals (arrows) and numerous idioblasts containing calcium oxalate crystals (arrowheads). Control preparation. ade – adaxial epidermis, abe – abaxial epidermis, c – collenchyma, ch – chlorenchyma
B. Fragment of a cross-section of the midrib (control) with visible adaxial epidermis (ade), collenchyma (c), and chlorenchyma (ch)
C. Stellate trichomes on the petiole surface. Nile Blue staining
D. Druses visible in spongy parenchyma
E. Cross section of a leaf blade with two palisade parenchyma layers (sunlit site)
F. Fragment of a cross section of a leaf blade with three palisade parenchyma layers (shaded site)
G, H. Enlarged secretory canals (arrows) surrounded by epithelial cells (e)
I. Fragment of a longitudinal leaf section with visible secretory canal (arrows)
Scale bars = 200 µm (A), 100 µm (C, E, F), 50 µm (B, D, I), 20 µm (H, G)

lipids were observed after the application of Sudan IV (Fig. 3C). Steroid-containing terpenes, which stained yellow after the antimony trichloride (UV) treatment, were detected in some parenchyma cells located mainly at the vascular bundles (Fig. 3G, H). After the application of ruthenium red, the cell walls of parenchyma tissues mainly stained red (Fig. 4A–F).

Phloem. All types of metabolites were detected in the phloem cells (Tab. 1). Noteworthy is the particularly intense staining of this tissue after the treatment

with the Nile blue reagent (Fig. 3A) and Sudan IV (Fig. 3C), which indicates the presence of substantial amounts of lipids and essential oil stained with neutral red (Fig. 3D).

Epithelial cells. The black colour after treatment with ferric trichloride (Fig. 2D) and the dark blue colour after treatment with Toluidine blue O (Fig. 2H) observed in the epithelial cells around the secretory canals indicated the content of phenolic compounds. The treatment of the preparations with potassium dichromate revealed the presence of tannins in these cells (Fig. 2E). The secretory epithel contained intensely blue acid lipids – Nile Blue (Fig. 3A). Moreover, phenolic acids, flavonoids, lipids, terpenoids and steroid-containing terpenes were present in the cells of this secretory tissue (Tab. 1).

Secretion. The secretory product contained in the secretory canals of the control preparations was orange. After treatment with magnesium acetate (UV), the secretion stained grey-beige with little yellow droplets indicating the presence of flavonoids (Fig. 2C). In the presence of Sudan IV and Neutral Red, the lipid- and essential oil-rich secretion stained dark orange (Fig. 3C). The presence of large amounts

Table 1. Location of biological active compounds in the tissues of *Hedera helix* leaves

			Leaf blade						Petiole					
Test	Compounds	Colour observed	Epidermis	Collenchyma	Mesophyll	Phloem	Epithel	Secretory product	Epidermis	Collenchyma	Parenchyma	Phloem	Epithel	Secretory product
Autofluorescence (UV)	phenolic acids	light blue	+	+	+	+	+	+	+	+	+	+	+	+
Magnesium acetate (UV)	flavonoids	yellow--greenish	++	–	+	+	+	+	++	+	+	+	+	++
Ferric trichloride	total phenolic compounds	black	+	+	+	+	+	+	++	+	+	+	++	++
Potassium dichromate	phenolic compounds, tannins	brown	+	–	+	+	+	+	*	*	*	*	*	*
Toluidine blue O	phenolic compounds	blue	++	+	++	++	++	+	*	*	*	*	*	*
	pectins	violet	+	–	–	++	+	+	*	*	*	*	*	*
Ruthenium red	pectins/ mucilagous substances	red	++	+	+	+	+	–	*	*	*	*	*	*
Sudan IV	total lipids, resin, essential oil	orange	+	–	+	++	+	+	+	+	+	+	+	+
Nile Blue	acidic lipids	blue	+	+	+	++	++	+	+	+	+	++	+	+
Neutral red	essential oil, mucilagous substances	red	+	+	–	+	++	++	*	*	*	*	*	*
Nadi Reagent	terpenoids	violet--blue	++	+	+	+	++	++	++	+	+	+	+	++
Antimony trichloride (UV)	steroid-containing terpenes	yellow	–	–	+	+	+	–	*	*	*	*	*	*

* not detemined

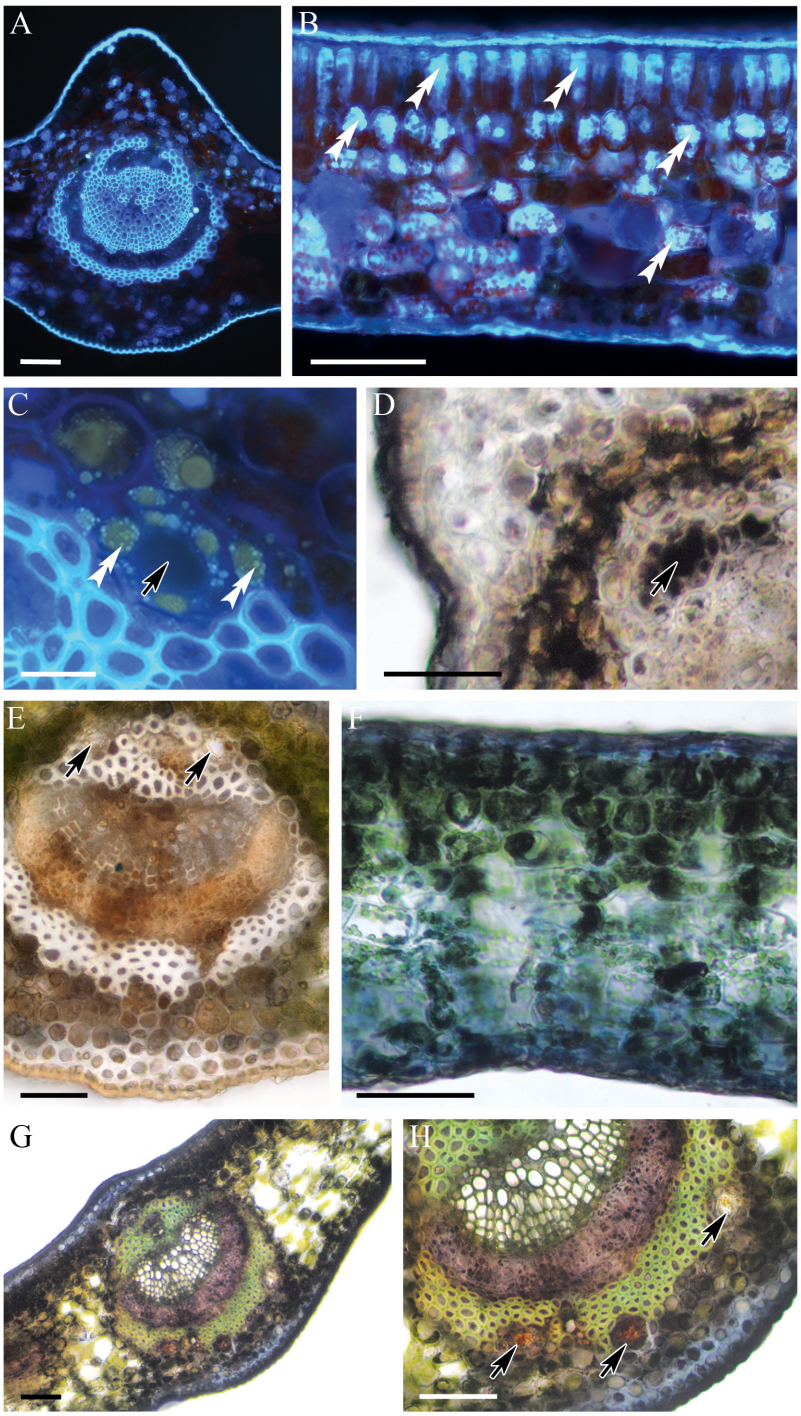

Fig. 2. Effects of histochemical assays detecting phenolic compounds in cross sections of *Hedera helix* leaves

A. Cross-section of the leaf midrib with visible blue autofluorescence (UV) of phenolic acids
B. Cross-section of a leaf blade treated with magnesium acetate (UV) – yellow fluorescence of flavonoids in cells (white double arrowheads)
C. Fragment of a cross section of the midrib with a visible secretory canal (arrow), in which the epithel cells contain fluorescent droplets of flavonoids – white double arrowheads (magnesium acetate, UV)
D. Fragment of a cross section of the midrib with black content of phenolic compounds (ferric trichloride). The arrow indicates the secretory canal
E. Fragment of the midrib after treatment with potassium dichromate with brown-stained tannins in the cells. The arrows indicate the secretory canals
F. Cross section of the leaf blade with blue-stained phenolic compounds in the tissues (Toluidine blue O staining)
G, H. Cross sections of the midrib after treatment with Toluidine blue O. Tissues containing phenolic compounds are stained blue and tissues containing pectin are stained purple. H – arrows indicating secretory canals
Scale bars = 100 µm (A, B, D–H), 25 µm (C)

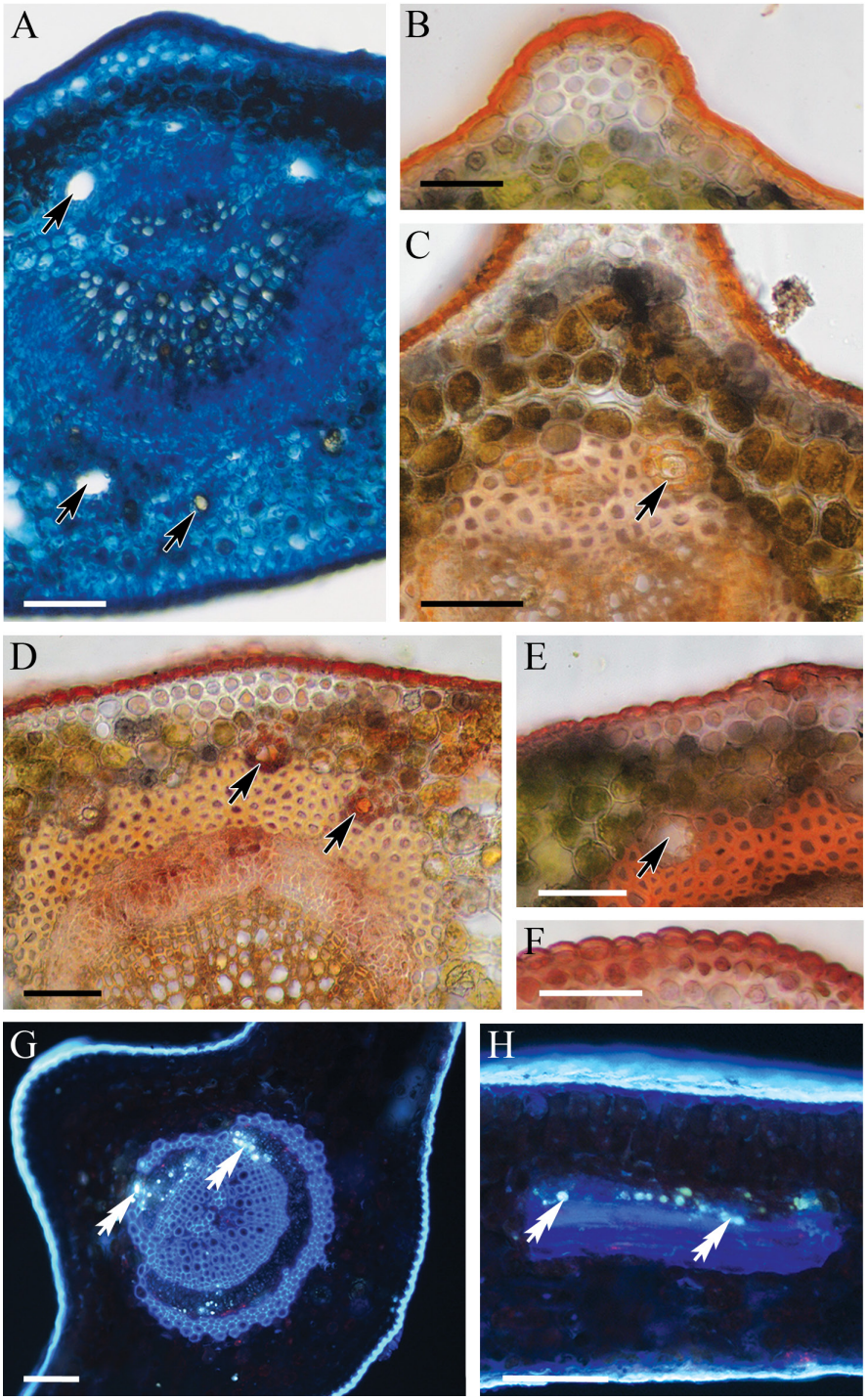

Fig. 3. Effects of histochemical assays detecting lipids, essential oil/resins, and steroid-containing terpenes on cross sections of *Hedera helix* leaves

A. Cross-section of the main leaf midrib after treatment with Nile Blue – cells containing acid lipids stained blue. The arrow indicates the secretion in the secretory canal
B, C. Fragments of the cross sections of the midrib after treatment with Sudan IV – lipids and essential oil/resin contained in the cells stained orange. B – the arrow indicates the secretory canal
D, E–F. Fragments of the midrib after treatment with neutral red – essential oil/mucilages stained red. C, D – The arrows indicate the secretory canals
G, H. Cross sections of leaves after treatment with antimony trichloride (UV) – visible fluorescence of steroid containing terpenes in tissues (double white arrows)
Scale bars = 100 µm (A–H)

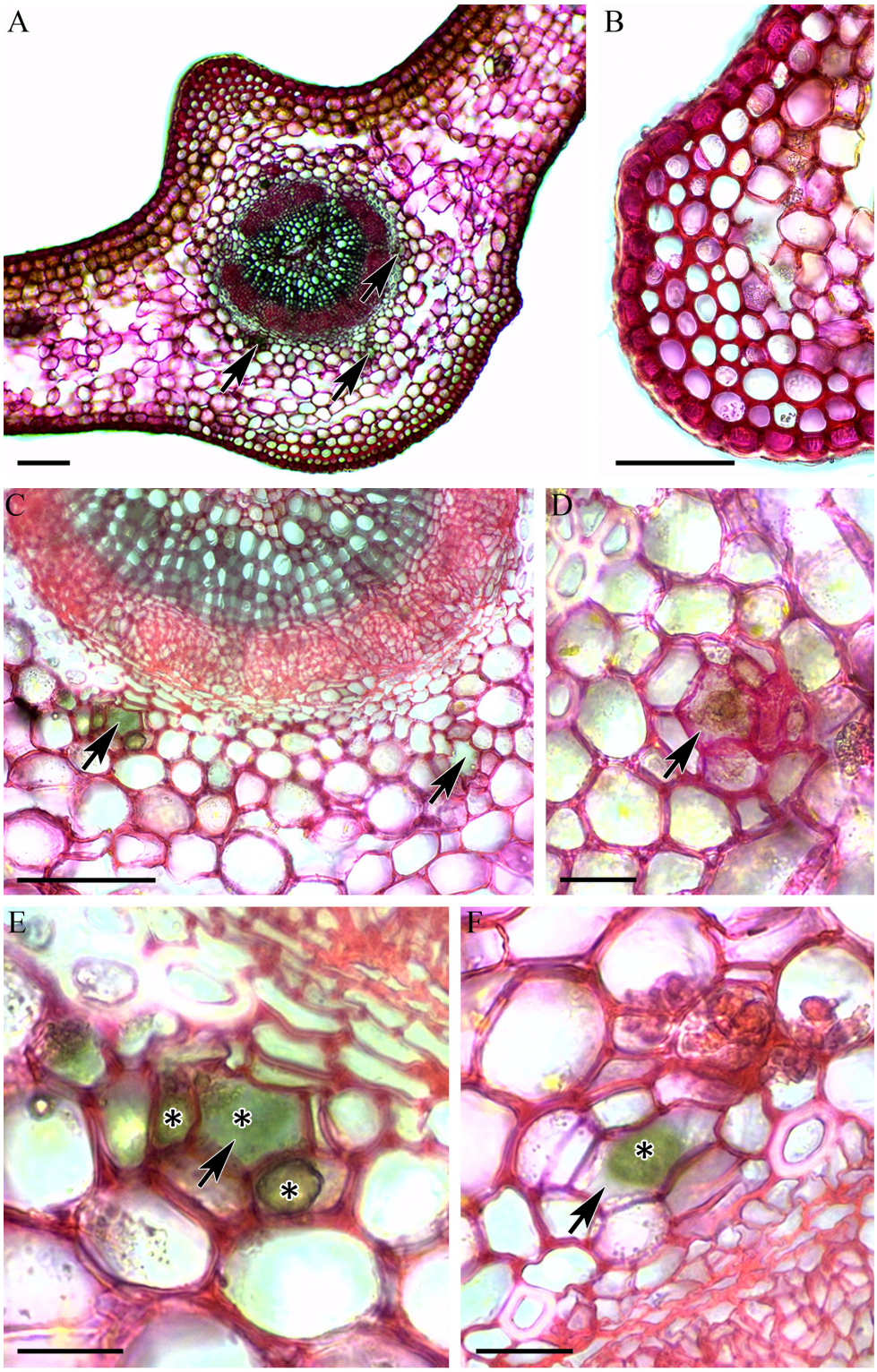

Fig. 4. Cross sections of *Hedera helix* leaves after treatment with ruthenium red

A–F. In most tissues, pectinous compounds contained in the cell walls stained red, with the exception of sclerenchyma and xylem (green staining). D–F – magnified secretory canals, stars indicate the secretory products
B. Content of pectinous compounds/mucilages in the epidermis is visible in cell protoplasts (red staining)
A, C–F. The arrows indicate the secretory canals
Scale bars = 100 µm (A–C), 50 µm (D–F)

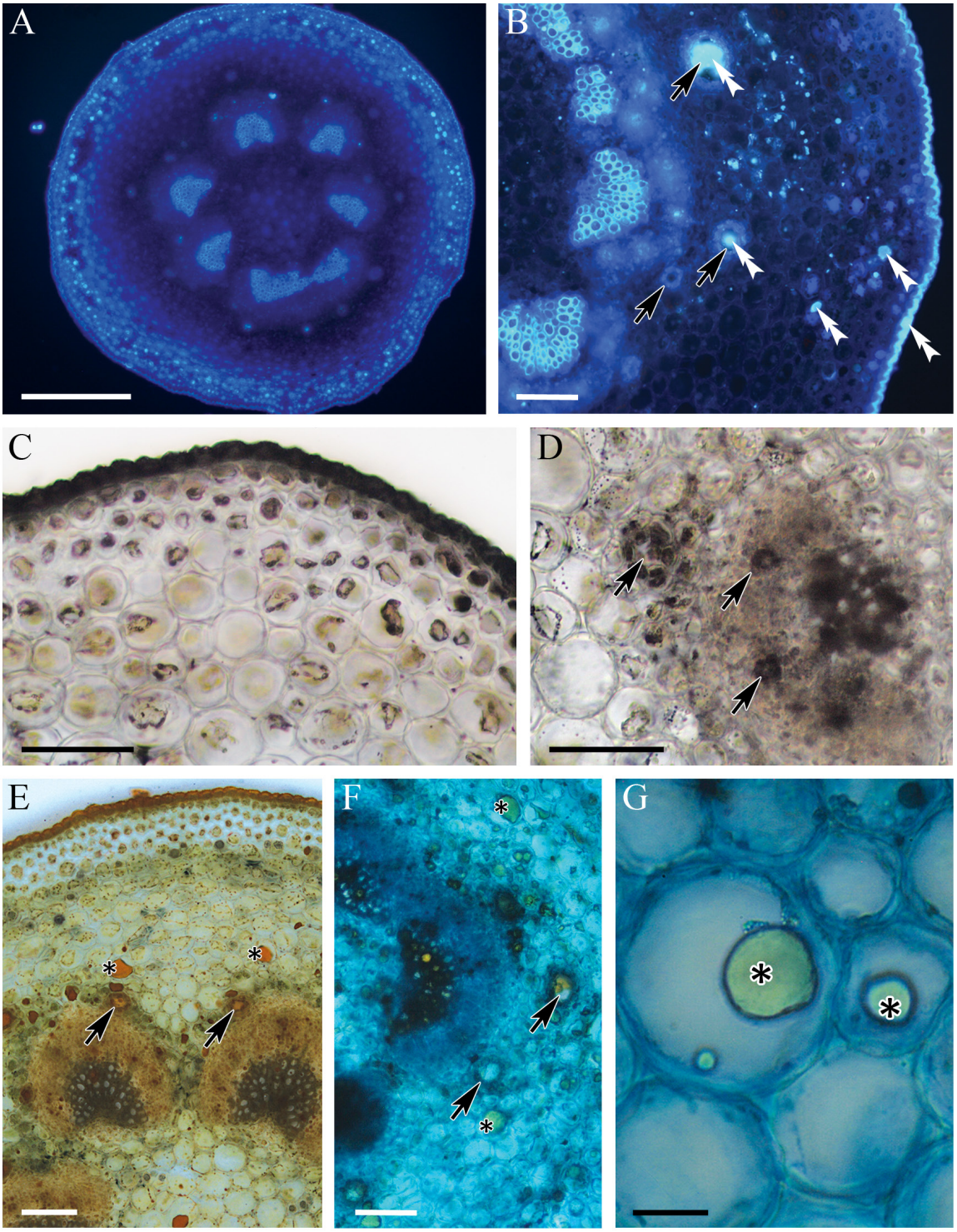

Fig. 5. Effects of histochemical assays in cross sections of the *Hedera helix* leaf petiole

A. Autofluorescence of phenolic acids (blue staining)
B. Secondary fluorescence of flavonoids (yellow staining – double white arrowheads) in petiole tissues after treatment with magnesium acetate
C, D. Black staining of phenolic compounds in leaf petiole tissues after treatment with ferric trichloride
E. Fragment of a cross section after treatment with Sudan IV – lipid resin/essential oil stained orange (stars)
F, G. Petiole tissues after treatment with Nile Blue – acid lipids stained blue; G – irregularly shaped translucent drops (stars) of the secretory product secreted through the secretory canal are visible in the parenchyma cells
B, D–F – The arrows indicate the secretory canals
Scale bars = 500 µm (A), 100 µm (B–F), 20 µm (G)

of terpenoids in the secretory product was indicated by a violet-blue colour after treatment with Nadi Reagent (not shown). The secretion present in the canals also contained phenolic acids, phenolic compounds, and acid lipids (Tab. 1). In the presence of ruthenium red the secretory product contained in the canals stained green (Fig. 4C, E, F).

Petiole

Anatomical traits. The unifacial petiole resembled a stem in cross section (Fig. 5A). As in the leaf blade, its **epidermis** cells had thick outer cell walls with a substantial cuticle layer (Fig. 5B). The **collenchyma** formed 2–3 subepidermal layers (Fig. 5C). The **parenchyma** consisted of different-sized cells both in the cortex zone and in the medulla. There were few idioblasts with druses mainly in the peripheral parenchyma. The open collateral **vascular bundles** formed an annular system in petiole cross section (Fig. 5A). There were numerous singly arranged secretory canals located near the phloem and sometimes associated with the phloem (Fig. 5B, D–F).

Histochemical traits. Phenolic acids showing light blue autofluorescence and flavonoids showing yellow secondary fluorescence were detected in the petiole **epidermis** cells (Fig. 5B). The epidermis cells also contained phenolic compounds (Fig. 5C), lipids, and acid lipids (Tab. 1). The presence of lipids, phenolic compounds, phenolic acids, and flavonoids was detected in the collenchyma cells (Tab. 1). The peripheral **parenchyma** cells contained phenolic acids (Fig. 5A), flavonoids (Fig. 5B), and phenolic compounds (Fig. 5C). Additionally, there were lipids (Fig. 5E) and acid lipids (Fig. 5F) in this tissue. The **phloem** cells exhibited the presence of phenolic acids, flavonoids (Fig. 5B), and phenolic compounds (Fig. 5D). They also contained lipids, acid lipids (Fig. 5F), and terpenoids (Tab. 1).

The same metabolites as in the phloem were detected in the **epithelial cells** surrounding the secretory canals and in the **secretion** contained in the lumen of canal, with greater amounts of flavonoids (Fig. 5B) and phenolic compounds (Fig. 5D, Tab. 1) in the secretion. In some parenchyma cells nearby canals we observed a lipid substance (Fig. 5E). Droplets of a similar substance were also visible in Nile Blue stained parenchyma cells (Fig. 5F, G).

DISCUSSION

One of the adaptations to ecological conditions in the evergreen *Hedera helix* leaves is the structure of their epidermis cells, which have thick periclinal outer walls covered by a considerably thick cuticle layer. The cuticle showed strong light blue autofluorescence, which indicated the presence of phenolic acids. Moreover, the histochemical assays revealed the presence of flavonoids. Investigations conducted by other authors have shown that the cuticle of the outer walls of epidermis cells mainly contains phenolic acids and flavonoids bound to the cuticular matrix [Karabourniotis et al. 2014].

Additionally, phenolic acids and flavonoids were detected in the protoplasts of *H. helix* leaf epidermis cells. As reported by other authors, these compounds are located in the vacuoles and walls of epidermal cells [Cerovic et al. 2002, Dmitruk et al. 2019, Sulborska et al. 2020]. The presence of phenolic compounds is closely related to the function of the epidermis as a protective barrier for other tissues. Previous studies have shown that flavonoids protect cells against UV radiation and intense visible radiation. Phenolic acids provide defence against pathogens and herbivores [Solovchenko and Merzlyak 2003, Karabourniotis et al. 2005, 2014].

Another adaptation to winter conditions may be the content of pectins/mucilages in epidermis cells. Accumulation of mucilagous substances has been found in *Galanthus nivalis* and *Leucojum aestivum*, i.e. plants adapted to low temperatures [Ščepánková and Hudák 2004]. The epidermis in the *H. helix* leaves can be regarded as a tissue with special metabolism. It was shown to contain resin/essential oil and pectinous material. As shown by the literature, pectins are the basic components of gums [Rioux et al. 1998, Zheng et al. 2017]. Gum resin is therefore present in the epidermis of this species. This substance is a resinous product and a mixture of resin with gum or mucilagous substances [Parimal et al. 2011]. Gum resin is mainly extracted from *H. helix* fruits [Nowiński 1977, Strzelecka and Kowalski 2000]. The present study has demonstrated its presence also in the epidermis of the *H. helix* leaves.

In this study, the palisade parenchyma in the leaves of the analysed *H. helix* plants growing in different

lighting conditions exhibited considerable structural variability. The cells of this parenchyma in leaves from the sunlit locations forming only two layers were elongated in cross sections. In turn, the palisade parenchyma cells in leaves collected from the shady sites were almost square and formed three layers. Similar differences in the palisade parenchyma of *H. helix* leaf cells was observed in plants grown in a roof garden (elongated cells) and in a typical ground garden (short cells) [Petra et al. 2020]. Other authors reported only the presence of short (square) palisade parenchyma cells forming a 2-seriate layer [Săvulescu and Luchian 2009] or a 2–3 seriate layer in *H. helix* leaves [Ostroumova and Oskolski 2010].

Numerous idioblasts with druses were located between mesophyll cells, in the subepidermal layer of the midrib, and around vascular bundles. Despite the common occurrence of calcium oxalate crystals in tissues of higher plants, their function is still not fully understood. It is assumed that they (i) play an important role in regulation of the calcium concentration and maintenance of proper ionic balance, (ii) support the processes of detoxification of heavy metals, (iii) neutralise other toxic substances in plants by deposition thereof in vacuoles, (iv) serve a protective function by deterring herbivores through irritation of mucous membranes, and (v) facilitate toxin penetration through damaged skin of herbivores in the case of toxic plants [Salinas et al. 2001, Volk et al. 2002, Nakata et al. 2003, Konyar et al. 2014]. It can be assumed that the presence of large amounts of druses in the evergreen tissues of *Hedera helix* leaves that survive for over two years is highly important. In addition to the above-mentioned functions, these crystals may contribute to mechanical reinforcement of leaves.

Our study showed the presence of phenolic acids and flavonoids in many *H. helix* mesophyll cells, likewise in epidermis cells. As reported by other authors, phenolic acids are most often contained in parenchyma cell walls, contributing to the mechanical strengthening and providing a UV screen. In turn, the vacuole, chloroplasts, and nucleus are indicated as the location of flavonoids in mesophyll cells. In parenchyma cells, flavonoids are involved in antioxidant protection and prooxidant action [Ferreres et al. 2011, Karabourniotis et al. 2014].

Phenolic acids and flavonoids in the phloem cells and in the secretory canal epithel were detected as well. Additionally, the phloem cells contained terpenoids, and some cells had essential oil, steroid-containing terpenes, and acid lipids as well. The orange secretion, presumably resin, present in the secretory canal lumen was a mixture of acid lipids, phenolic compounds, essential oil, and terpenoids. As shown by literature reports, phenolic compounds, fatty acids derivatives, and terpenes are the main components of volatile organic compounds as well as essential oils and resins [Caissard et al. 2004]. Terpenes serve different functions in plants: they are herbivore deterrents [Chwil et al. 2016] and pollinator attractants [Evert 2006, Chizzola 2013]. Moreover, they exhibit antioxidant, antiviral, antibacterial, and antifungal activity [Abdellatif et al. 2014, Tantry et al. 2014].

The lipid secretion in observed in the lumen of the canals and in the epithelial cells was similar to the content of some parenchyma cells located near the secretory canals. Other authors also observed lipid droplets in leaf parenchyma cells [Kromer et al. 2016, Zhai et al. 2017; Bezerra et al. 2018]. The substance present in the canals and in the parenchyma cells can probably be used by the plant in interactions with the environment [Bezerra et al. 2018].

The secretion produced in the *H. helix* leaf canals can be regarded as a resinous substance due to its physical properties. In microscopic slides, it was visible as a homogenous translucent and amorphous material with irregular shapes inside and outside the secretory canals. In contrast, essential oil contained in plant tissues most often forms droplets and circular bodies [Zhai et al. 2017, Dmitruk et al. 2019]. The results of the present study confirm the data on ivy resin reported by other authors [Nowiński 1977, Strzelecka and Kowalski 2000].

CONCLUSIONS

1. The histochemical assays demonstrated the presence of various biologically active compounds in the tissues of *Hedera helix* leaf blade and petioles.

2. They were found to contain phenolic acids, flavonoids, pectinous/mucilagous compounds, acid lipids, essential oil, resin, terpenoids, and steroid-containing terpenes.

3. These secondary metabolites are accumulated in most tissues except the xylem and sclerenchyma.

4. The compounds are largely responsible for the biological and pharmacological activity of the medicinal raw material from *H. helix* leaves.

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