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LOCALISATION OF ACTIVE SUBSTANCES IN *Hedera helix* LEAVES AND *Linaria vulgaris* SHOOTS USED IN PHARMACEUTICAL AND COSMETIC INDUSTRY

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ABSTRACT

Although *Hedera helix* and *Linaria vulgaris* are considered toxic plants, their extracts containing many bioactive compounds are commonly used in the pharmaceutical and cosmetics industries. Plant secretory structures are considered the primary site of synthesis and accumulation of many valuable metabolites. The presence of secretory structures and the location of active substances in H. *helix* leaf petioles and *L. vulgaris* inflorescence shoots were detected using light microscopy and histochemical and fluorescence tests. The analyses showed schizogenic secretory ducts in the *H. helix* petioles and capitate glandular trichomes on the surface of the *L. vulgaris* shoots. The secretion and secretory cells of the *H. helix* ducts and the *L. vulgaris* trichomes contained acidic and neutral lipids, sesquiterpenes, polyphenols, tannins, flavonoids, phenolic acids, neutral and acidic polysaccharides, proteins and alkaloids. Data on the secretory structures and taxonomic and morpho-anatomical traits of the analysed raw materials may help evaluate their quality (falsification).

Key words: medicinal and cosmetics plants, secretory structures, lipidic and phenolic compounds, alkaloids and polysaccharides, falsification of raw material

INTRODUCTION

Progress and development have led to the widespread use of synthetic compounds in food, drugs and cosmetic products that have been shown to cause some lifestyle diseases, e.g. atherosclerosis, heart diseases, diabetes and cancer. The growing public awareness has contributed to changes in consumer preferences for body and healthcare products and increased interest in medicines and cosmetics based on natural substances [Jabłońska 2019]. Alkaloids, azulenes, balsams, essential oils, glycosides, mucilages, pectins, resins and tannins are the most important groups of bioactive substances present in pharmaceutical and cosmetic plant raw materials [Puvača 2018]. Plants species from which bioactive compounds are extracted are often found in the natural environment, but some have toxic properties, e.g. *Hedera helix* L. (Araliaceae) and *Linaria vulgaris* Mill. (Plantaginaceae) [Hua et al. 2002, Gaillard et al. 2003, Cheriet et al. 2015].

Hedera helix is a perennial evergreen climber occurring naturally in the forests of Europe and Asia Minor and is regarded as an invasive species in many regions outside its natural occurrence range [Metcalfe 2005]. The species is also cultivated in green areas as an indoor and ground cover. *Hedera helix* produces up to 20 m long creeping (sterile) and climbing (flowering) shoots. The evergreen, leathery, heterophyllous leaves with long petioles are arranged alternately on the stem. The leaves on the sterile shoots are 3–5 lobed and heart-shaped at the base, whereas those growing on the climbing shoots are ovate or ovate-lanceolate

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and have entire margins. Leaves of its creeping shoots have been used as a pharmaceutical and cosmetic raw material since ancient Greece [Bezruk et al. 2020a]. Raw *Hederae Folium* is extremely rich in triterpene saponins, flavonoids, phenolic acids, polyacetylenes, essential oil, sterols, sesquiterpenes, and coumarins [Lutsenko et al. 2010, Bezruk et al. 2020b, Rehman et al. 2022, Shokry et al. 2022] and contains some amounts of emetine alkaloids [Alkattan et al. 2021].

Drugs and cosmetics containing *H. helix* extracts exert strong anti-inflammatory, antioxidant and antimicrobial effects [Pop et al. 2017, Schulte-Michels et al. 2019]. They are dedicated to sensitive skin and are used to treat acne, psoriasis and seborrhoeic dermatitis. They also relieve symptoms of skin irritation, pruritus and genital mycoses [Lutsenko et al. 2010, Ibezim, 2012, Dumitriu et al. 2013]. *Hedera helix* extract positively affects hair growth and is commonly used in foaming agents and body lotions [EMA 2017, Góral and Wojciechowski 2020]. It also exhibits conditioning, soothing and toning properties and astringent activity that supports the tightening of skin pores [INCI Beauty 2022]. *Hedera helix* extract is one of the most effective ingredients of anti-cellulite cosmetics and products to protect stretch mark-prone skin [Hexsel and Soirefmann 2011]. In addition to the antioxidant, anti-inflammatory and antibacterial properties of *H. helix* leaf extracts, their analgesic, antispasmodic, expectorant and spasmolytic properties are essential for the pharmaceutical industry [Terlecka et al. 2020, Baharara et al. 2021]. Extracts of this raw material are ingredients of syrups and lozenges used in upper respiratory tract infections [Barnes et al. 2020]. They also have anticancer properties [Akhlaghi et al. 2022].

Linaria vulgaris is native to Europe and Asia but is considered an invasive species in the USA and Canada [Sing and Peterson 2011]. This perennial plant is found on grasslands, roadsides, pastures, meadows and fallow lands. It is also a weed of cultivated fields [Saner et al. 1995]. *Linaria vulgaris* has a 20–90 cm high erect stem with glandular trichomes in the inflorescence part. Its small oblong-lanceolate dense leaves are arranged alternately. The inflorescence is a raceme composed of nectariferous pale yellow snapdragon-like flowers. Its flowering shoots (*Herba Linariae*) are medicinal and cosmetic raw materials [Chiej 2009]. It contains iridoids, alkaloids, flavonoids, sterols, phenolic and mucilage compounds, organic acids and vitamins [Sokolowska-Wozniak et al. 2003, Vrchovská et al. 2008, Cheriet et al. 2015, Kuang et al. 2018, Sokornova and Matveeva 2021].

In folk medicine, *L. vulgaris* is known mainly for its mild laxative properties [Lewkowicz Mosiej 2012]. Its extract, used internally, reduces digestion problems and urinary disorders and exerts choleretic effects. Applied externally, it effectively treats haemorrhoids, venous skin ulcers and festering wounds [Gruenwald et al. 2000, Senderski 2016] as well as cough and asthma [Hua et al. 2002]. The extract also protects against hyperlipidaemia and hepatic steatosis, facilitating the removal of toxins from the organism [Berim and Gang 2016, Kuang et al. 2018]. Additionally, it exerts expectorant, strongly diaphoretic, sedative, somniferous, slightly analgesic and diuretic effects [Vrchovská et al. 2008]. Due to the content of alkaloids, the extract may have intoxicating activity, and its higher doses may cause nausea and diarrhoea [Fijałkowski and Chojnacka-Fijałkowska 2009]. Used as a cosmetic ingredient, *L. vulgaris* extract exhibits antioxidant activity, and therefore it retards skin ageing, softens the skin and enhances skin elasticity [Vrchovská et al. 2008]. Due to its anti-inflammatory, antimicrobial and anti-allergic properties, the extract is used to soothe skin itching, heal irritations and wounds and prevent infections [Kelemen and Scedo 2003, Senderski 2016]. The flower and herb of the species are used for the preparation of ointments, infusions and decoctions for such skin problems as psoriasis and acne and in formulations against conjunctivitis and dark under-eye circles [Fijałkowski and Chojancka-Fijałkowska 2009]. Diagnosing the morphological and anatomical traits of medicinal and cosmetic raw materials is crucial for assessing their quality, as the evident increase in the demand for herbal medicines and cosmetics often leads to massive plant harvesting from their habitats, which is inconsistent with the guidelines for obtaining plant material. The plant material may also be incorrectly identified and falsified, thus not meeting quality standards [Sultana et al. 2011, Minarchenko et al. 2021].

While the medicinal and cosmetic properties of *H. helix* and *L. vulgaris* extracts have been investigated in detail, the anatomical traits of the raw material and the *planta* distribution of active compounds are unknown. Therefore, this study focused on analysing

the microstructural traits and the location of active substances in *H. helix* leaves and *L. vulgaris* inflorescence shoots, used as raw materials in the pharmaceutical industry and cosmetic formulations. The analyses were carried out with the use of stereoscopic and bright-field light microscopy as well as histochemical and fluorescence assays, which are effective methods for preliminary evaluation of the occurrence of valuable bioactive substances and assessment of the medicinal and cosmetic potential of plant raw material [e.g. Tajik et al. 2019, Konarska and Łotocka 2020]. Moreover, the taxonomic value of some morphological and anatomical traits, which may help evaluate the quality (falsification) of medicinal raw materials, was considered.

MATERIAL AND METHODS

Plant material. On August 10, 2021, older lobate *Hedera helix* leaves (n = 50) were collected randomly from several specimens growing in Akademicka Street in Lublin, Poland (51°15'44"N, 22°30'48"E). Flowering *Linaria vulgaris* shoots (n = 20) of plants growing in a massive group at Dobrzańskiego Street in Lublin, Poland (as above) were collected randomly on August 20, 2021. The herbarium specimens used in this study have been deposited in the Department of Botany and Plant Physiology at the University of Life Sciences in Lublin (Akademicka 15, Lublin, Poland).

Microscopy and histochemical and fluorescence tests. The study was conducted using light and fluorescence microscopy and histochemical assays detecting the presence of secretory structures and biologically active compounds based on the specific chromogenic reactions or (auto)fluorescence. All images were saved in the .tiff format with a resolution of 300 dpi and compression LZW (Lempel-Ziv-Welch). CorelDRAW x3 software was used to prepare the images for publication, and Corel PHOTO-PAINT x3 was the integral image processing tool used to enhance the clarity of the images.

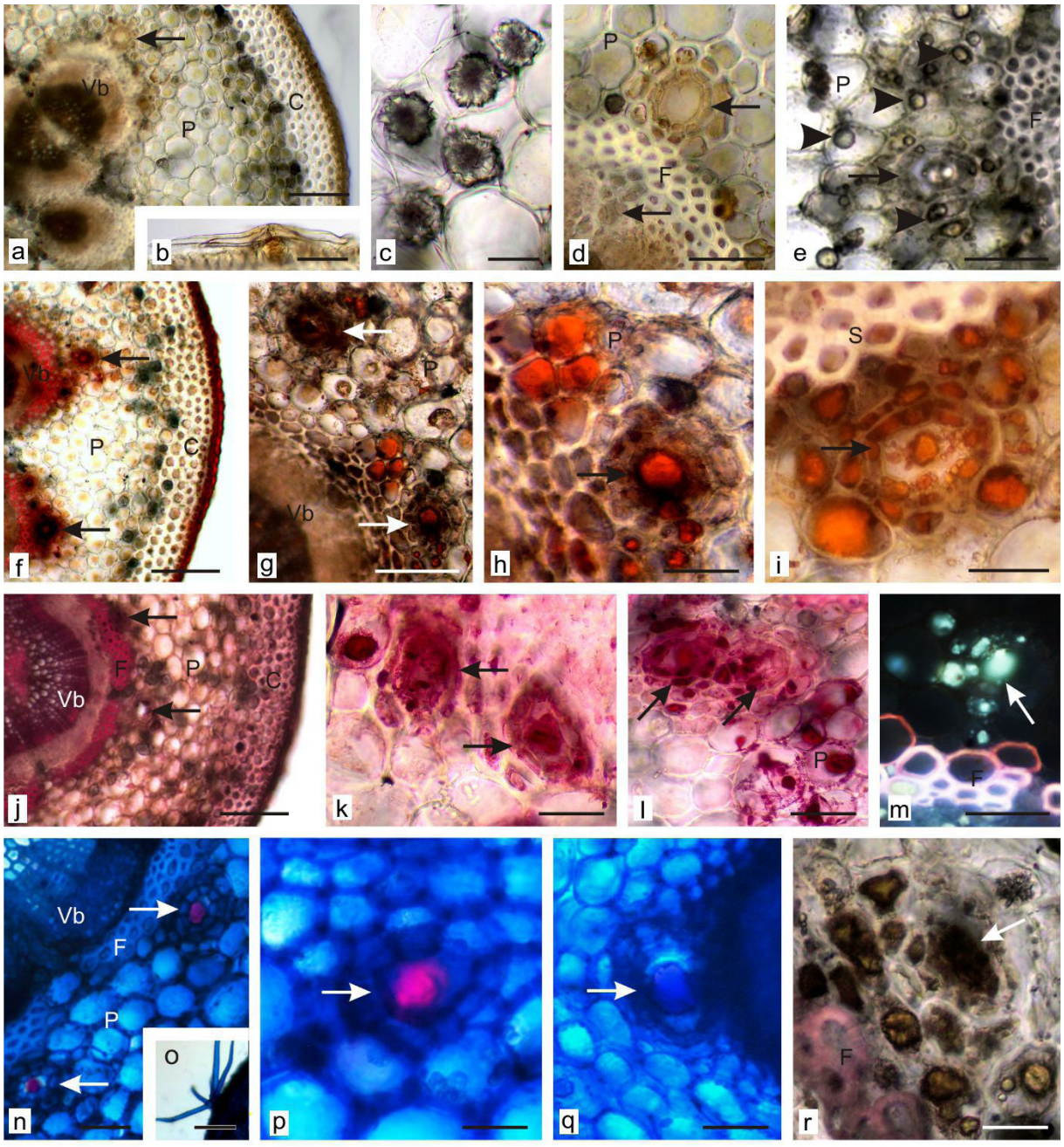
Hand-cut sections of fresh *H. helix* petioles (at their mid-length) and *L. vulgaris* stems (below the inflorescence) were viewed in a 50% aqueous glycerol solution (as a control) and after application of various histochemical assays and fluorochromes using a Nikon SE 102 light microscope or a Nikon 90i fluorescence microscope, both equipped with a digital camera (Nikon Fi1) and NIS-Elements Br 2 software. The analyses of the control preparations (n = 10) revealed the location of secretory structures, the number of secretory ducts, and the number of secretory epithelial cells. The inner diameter of the ducts' lumen (n = 20) in *H. helix* and the height of the secretory trichomes (n = 20) in *L. vulgaris* were measured.

The following histochemical assays were used: Sudan IV or Neutral red for total lipids [Clark 1981, Pearse 1985], the Nile blue for neutral and acidic lipids [Jensen 1962], concentrated sulphuric acid for sesquiterpenes [Geissmann and Griffin 1971], aqueous solution of Toluidine blue O (pH 5.6) [Gutmann 1995] or ferric trichloride [Gahan 1984] for polyphenols, potassium dichromate [Gabe 1968] or vanillin-hydrochloric acid [Gardner 1975] for tannins, Ruthenium red for acidic polysaccharides (pectins and mucilages) [Johansen 1940, Jensen 1962], Lugol's solution [Johansen 1940] or Wagner's reagent [Furr and Mahlberg 1981] for alkaloids, proteins and starch.

Fluorescence microscopy was used for the localisation of essential oils, flavonoids and phenolic acids after the application of various fluorochromes: Neutral red for essential oils [Kirk 1970], aluminium chloride or magnesium acetate for flavonoids [Charrière-Ladreix 1976] and UV-induced autofluorescence for phenolic acids [Talamond et al. 2015]. Fluorescence was observed using a Cy5 filter set (excitation radiation of 590–650 nm and a barrier filter wavelength 663–738 nm), a FITC filter set (excitation radiation of 465–495 nm and a barrier filter wavelength 515–555 nm) and a TRITC filter set (excitation radiation of 525–565 nm and barrier filter wavelength 555–600 nm). The histochemical and fluorescence methods were applied following standard control procedures suggested by various authors. Each histochemical assay was repeated 6 times to eliminate incorrect interpretation. Similar positive results were obtained 3–5 times.

RESULTS

***Hedera helix* petiole.** The transverse sections showed a uniseriate epidermis, several layers of angular collenchyma, parenchyma layers, and fibre-sheathed collateral vascular bundles in the structure of the *H. helix* petioles (Fig. 1a). The parenchyma external



a–e – unstained control; f–i – Sudan IV, j-l – Neutral red; m – Neutral red in UV-excited fluorescence; n–q – Nile blue, r – conc. sulphuric acid

a – fragment of a cross-section with a secretory duct (arrow), b – stellate non-glandular trichome, c – druses in parenchyma cells, d, e – small and large secretory ducts; lipid droplets (arrow-heads) are visible in the secretory duct and adjacent cells, f–l – total lipids present in the fibre-sheathed vascular bundles, secretory ducts, epithelial cells and adjacent cells, m – intensive fluorescence of essential oils in the secretory duct and adjacent cells, n, p – essential oils present in secretory ducts, o, q – acidic lipids in the non-glandular trichome (o) and secretory duct (q), r – sesquiterpenes present in the secretory duct, epithelial cells and adjacent cells. Arrows – secretory ducts, C – collenchyma, F – fibres, Vb – vascular bundles, P – parenchyma. Scale bars: f, j – 300 µm; a, o – 200 µm; b, g – 100 µm; c–e, m, n – 50 µm; h, i, k, l, p, r – 30 µm

Fig. 1. Anatomy and histochemistry of the *Hedera helix* petiole

to the vascular bundles usually had 7–9 larger and 3–5 smaller schizogenic secretory ducts lined by 4–9 small secretory epithelial cells (Fig. 1d, 1e). Sometimes, the additional layer of closely arranged parenchyma cells is adjacent to the epithelial cells from the outside (external to them), and then the sheath is built around the ducts (Figs 1b, 1i, 1n; 2b, 2c; 3b). The diameter of the ducts' lumen ranged from 26.1 µm to 43.5 µm (mean: 37.2 µm). Additionally, idioblasts with large druses were present in petiolar parenchyma (Fig. 1c). Multicellular branched non-glandular trichomes was also visible on the surface of the petioles (Fig. 1b, 1o).

As shown by the histochemical assays, large droplets of total lipids stained orange or red after the application of Sudan IV (Fig. 1f–1i) or Neutral red (Fig. 1j–1l), respectively, were detected in the secretory ducts, epithelial cells and parenchyma cells adjacent to the ducts. Essential oils emitted intense fluorescence after incubation in Neutral red fluorochrome (Fig. 1m). The reaction with Nile blue revealed the acidic and neutral lipids in the secretion (Fig. 1n, 1p, 1q). Acidic lipids were also detected in the non-glandular trichomes (Fig. 1o). Moreover, sesquiterpenes – stained yellow after the application of concentrated sulphuric acid – were visible in the secretion, epithelial cells and surrounding parenchyma cells (Fig. 1r).

The outer cell walls of the epidermis with cuticle, collenchyma and parenchyma cells, fibre-sheathed vascular bundles, and the duct secretion in the *H. helix* petioles exhibited the presence of phenolic compounds stained blue or navy blue in the reaction with Toluidine blue O (Fig. 2a–2c) and brown after the use of ferric chloride (Fig. 2d–2f). The application of potassium dichromate or vanillin-hydrochloric acid revealed the presence of tannins in the collenchyma cells (Fig. 2g) and in the duct secretion, epithelial cells and parenchyma cells located near the secretory ducts (Fig. 2h). In turn, flavonoids contained in the parenchyma and vascular bundle cells exhibited intense fluorescence in the presence of the aluminium chloride and magnesium acetate fluorochromes (Fig. 2i–2k). Phenolic acids in the duct secretion and parenchyma cells adjacent to the ducts exhibited strong red autofluorescence under UV excitation (Fig. 2l).

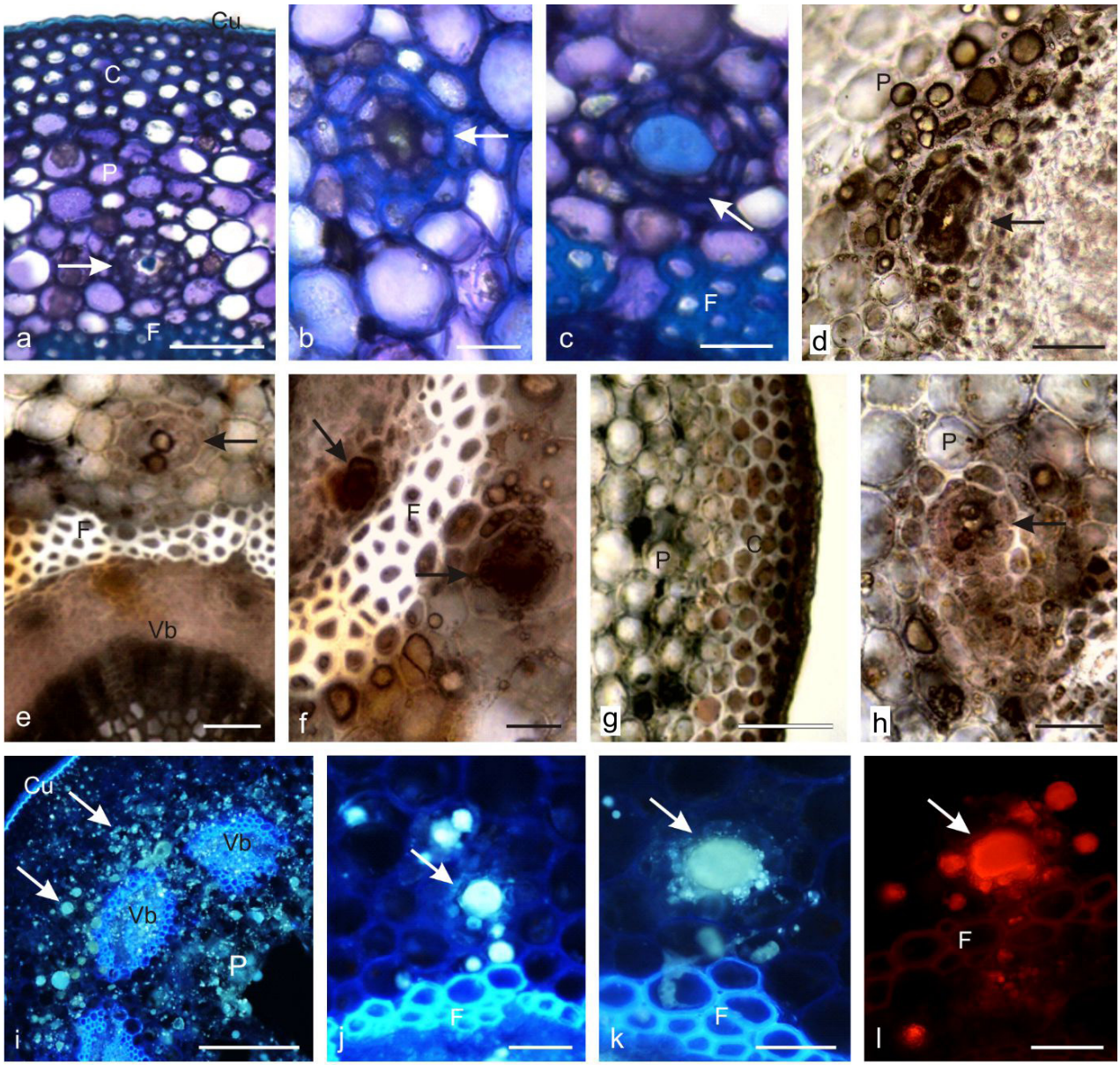
Acidic polysaccharides, stained red in the reaction with the Ruthenium red reagent, were detected in all petiole tissues' secretion and cell walls except fibre-sheathed vascular bundles (Fig. 3a, 3b).

In the reaction with Wagner's reagent or Lugol's solution, proteins present in the cuticle, fibre-sheathed vascular bundles and parenchyma cells stained yellow (Figs. 3c-e, 3h, 3i), while brown-stained alkaloids were detected in the epidermis and collenchyma cells and duct secretions (Fig. 3c, f-i).

***Linaria vulgaris* inflorescence stem.** The *L. vulgaris* inflorescence stem was composed of the uniseriate epidermis with thickened outer and inner walls, several layers of chlorenchyma, uniseriate endodermis, a continuous ring of the secondary vascular tissues with xylem fibres and parenchymatous pith (Fig. 4a). On the stem surface were observed capitate glandular trichomes composed of a 2-4-celled stalk and an oval 2-4-celled head with an average length of 83 µm (ranging from 70 to 94 µm) – Figure 4b, 4c.

The histochemical Sudan IV and Neutral red assays revealed the presence of total lipids in the cuticle, phloem and xylem elements, trichome heads and stalks (Fig. 4d–4i, 4k), and some epidermal and chlorenchyma cells (Fig. 4j). Fluorescence microscopy revealed bright green fluorescent essential oils in the trichome stalk cells after the application of the Neutral red fluorochrome (Fig. 4l, 4m). Sesquiterpenes in the trichome heads and stalks stained yellow with concentrated sulphuric acid (Fig. 4n, 4o). After the application of Nile blue, acidic lipids were present in all *L. vulgaris* stem cell layers and in the secretory trichomes stained blue to navy blue (Fig. 4p–4t).

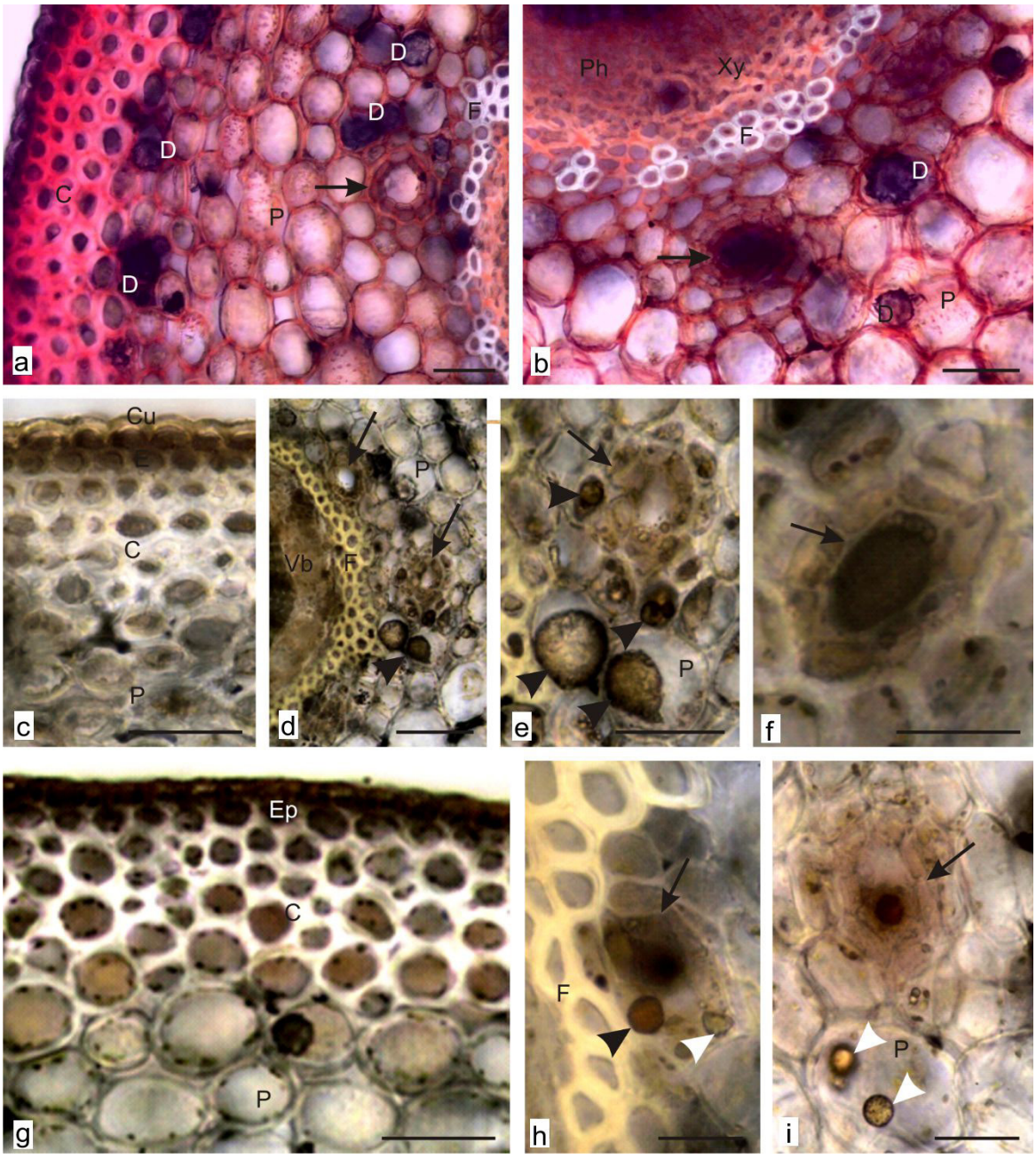
The cuticle, fibres' cell walls, trichome heads and stalks stained navy blue or turquoise after the application of Toluidine blue O, indicating the presence of phenolics (Fig. 5a–5g), while polyphenols contained in trichome cells stained brown in the reaction with iron chloride (Fig. 5h, 5i). Tannins were detected using potassium dichromate or vanillin-hydrochloric acid in the cells of trichome heads and stalks, cuticles, and vascular tissue elements, especially fibres (Fig. 5j–5m). Flavonoids in the cuticle, epidermis, trichome heads and stalks, and duct secretions emitted intense fluorescence light in the reaction with aluminium chloride or magnesium acetate fluorochromes (Fig. 5n–5q). In turn, intense autofluorescence of phenolic acids was visible in trichome heads (Fig. 5r).



a-c – Toluidine blue O, d–f – ferric chloride, g, h – potassium dichromate, i, j – aluminium chloride under UV, k – magnesium acetate under UV, l – autofluorescence of phenolic acids

a–c – polyphenols present in the cuticle, collenchyma, fibres' cell walls, and secretory ducts; d–f – polyphenols present in the secretory ducts and adjacent parenchyma cells; g, h – tannins in the collenchyma cells, secretory ducts, and adjacent cells; i–k – flavonoids present in the collenchyma cells, parenchyma, vascular bundles, and secretory ducts; l – phenolic acids present in the secretory ducts and adjacent parenchyma cells. Arrows – secretory ducts, C – collenchyma, F – fibres, Vb – vascular bundles, P – parenchyma. Scale bars: i – 200 µm; a, g – 100 µm; c-f, h, j – 50 µm; b-d, f, k, l – 30 µm

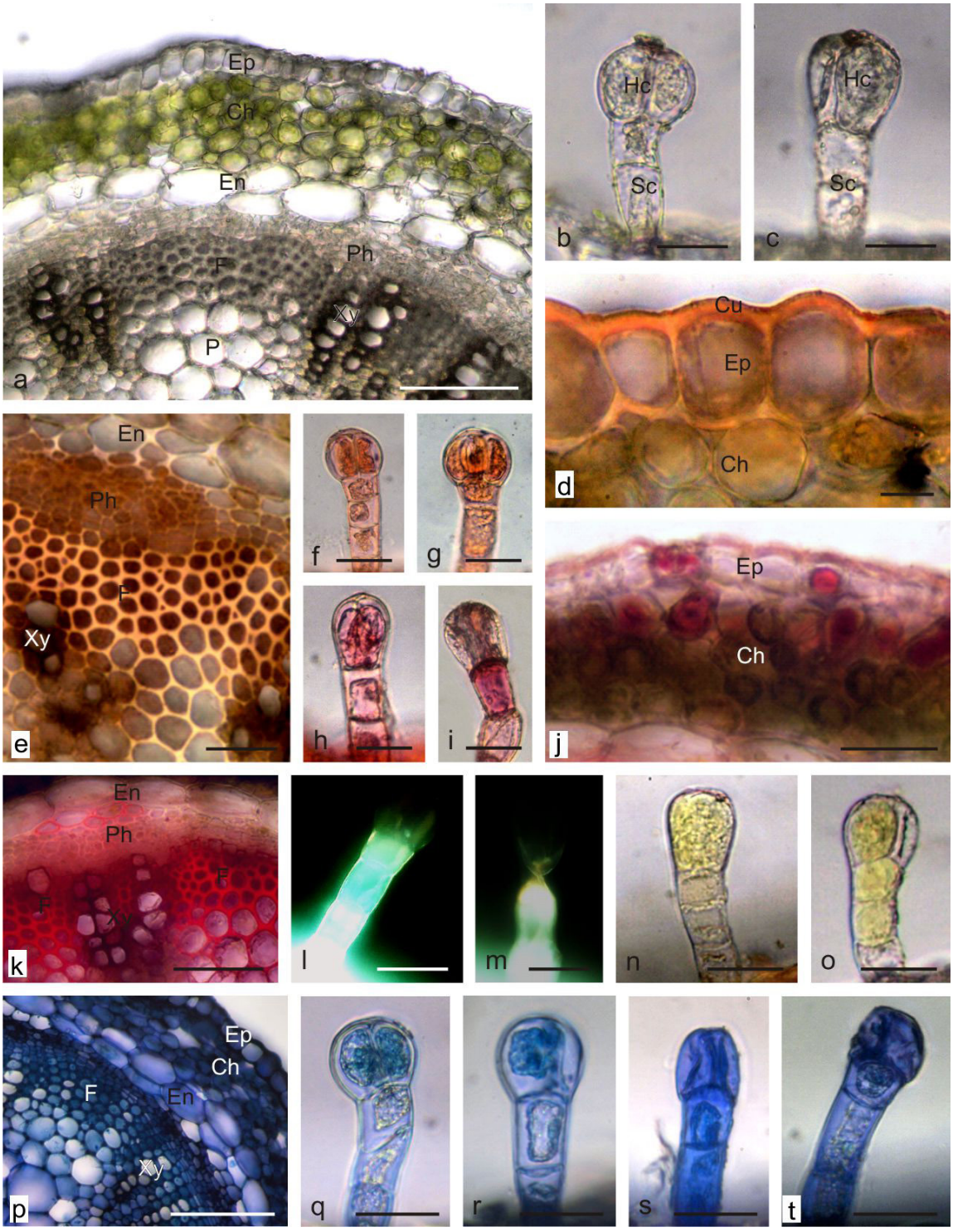
Fig. 2. Histochemistry and fluorescence in the *Hedera helix* petiole cross-section – detection of phenolic compounds



a, b – Ruthenium red, c–f – Wagner's reagent, g–i – Lugol's solution

a, b – acidic polysaccharides present in the secretory ducts and walls of all cell layers except for fibres cells, c–f – proteins stained yellow present in the cuticle, fibres, and parenchyma cells (arrow-heads); alkaloids stained brown present in the epidermal cells and duct secretion, g–i – alkaloids stained brown visible in the epidermis, collenchyma, duct secretion, and adjacent cells (black arrow-heads); proteins stained yellow present in the parenchyma cells (white arrow-heads). Arrows – secretory ducts, Cu – cuticle, E – epidermis, C – collenchyma, F – fibres, Vb – vascular bundles, Ph – phloem, P – parenchyma, D – druses. Scale bars: a, c, d, g – 100 µm; b, e – 50 µm; f, h, i – 30 µm

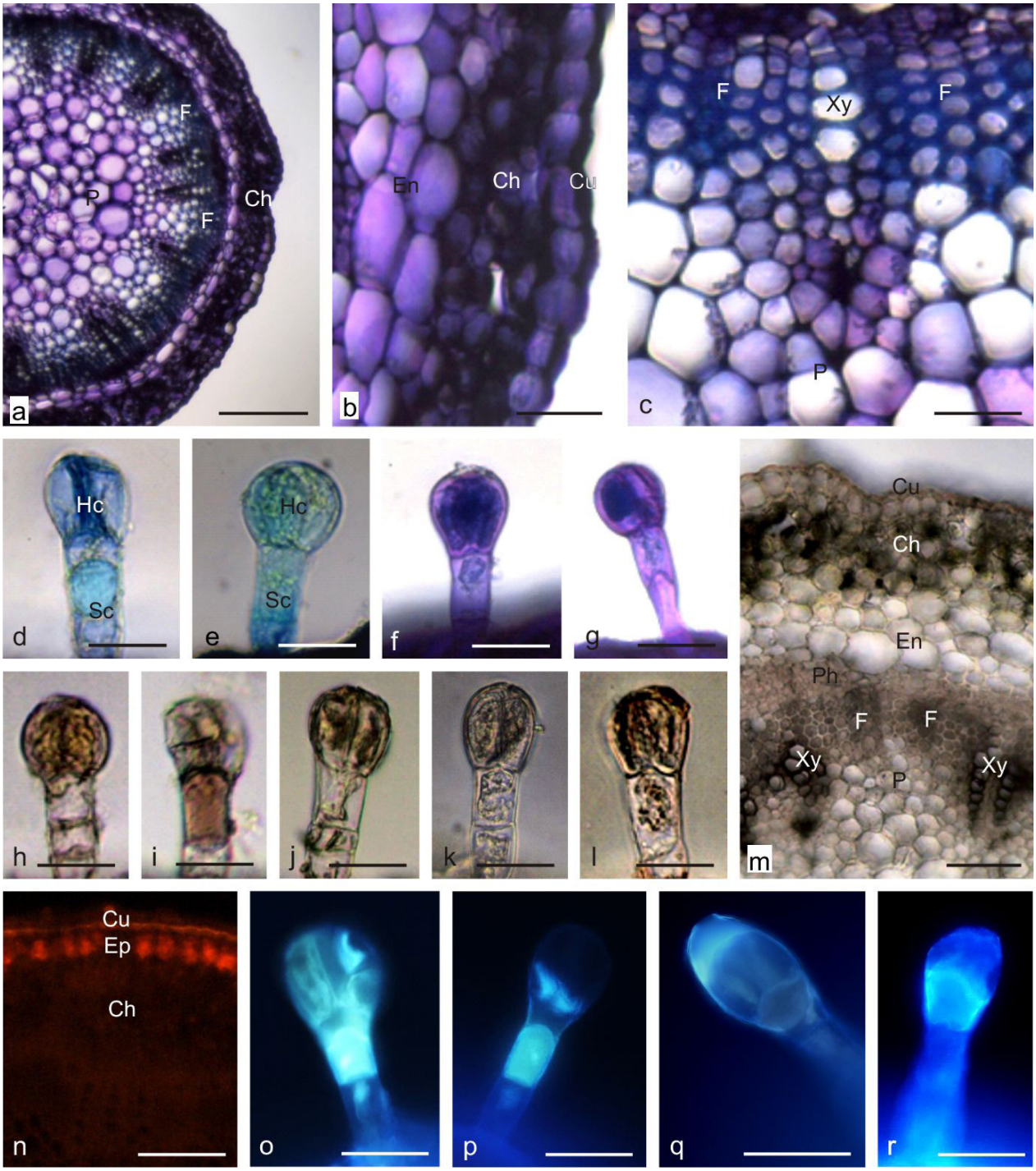
Fig. 3. Fresh cross-sections of the *Hedera helix* petiole subjected to histochemical tests



a–c – non-treated control, d–g – Sudan IV, h–k – Neutral red, j, m – Neutral red under UV excitation, n, o – conc. sulphuric acid, p–t – Nile blue

a – fragment of the cross-section, b, c – glandular trichomes, d–k – total lipids in the cuticle, phloem and xylem elements, and some epidermis and chlorenchyma cells, l, m – intensive yellow-light fluorescence of essential oils visible in the stalk cells, n, o – sesquiterpenes present in the trichome head and stalk cells, p–t – acidic lipids present in the glandular trichomes and all cell layers. Cu – cuticle, Ep – epidermis, Ch – chlorenchyma, En – endodermis, Ph – phloem, Xy – xylem, F – fibres, P – parenchyma, Hc – head cells, Sc – stalk cells. Scale bars: a, p – 100 µm; j, k – 50 µm; b, c, e-i, m-o, q-t – 30 µm; d – 10 µm

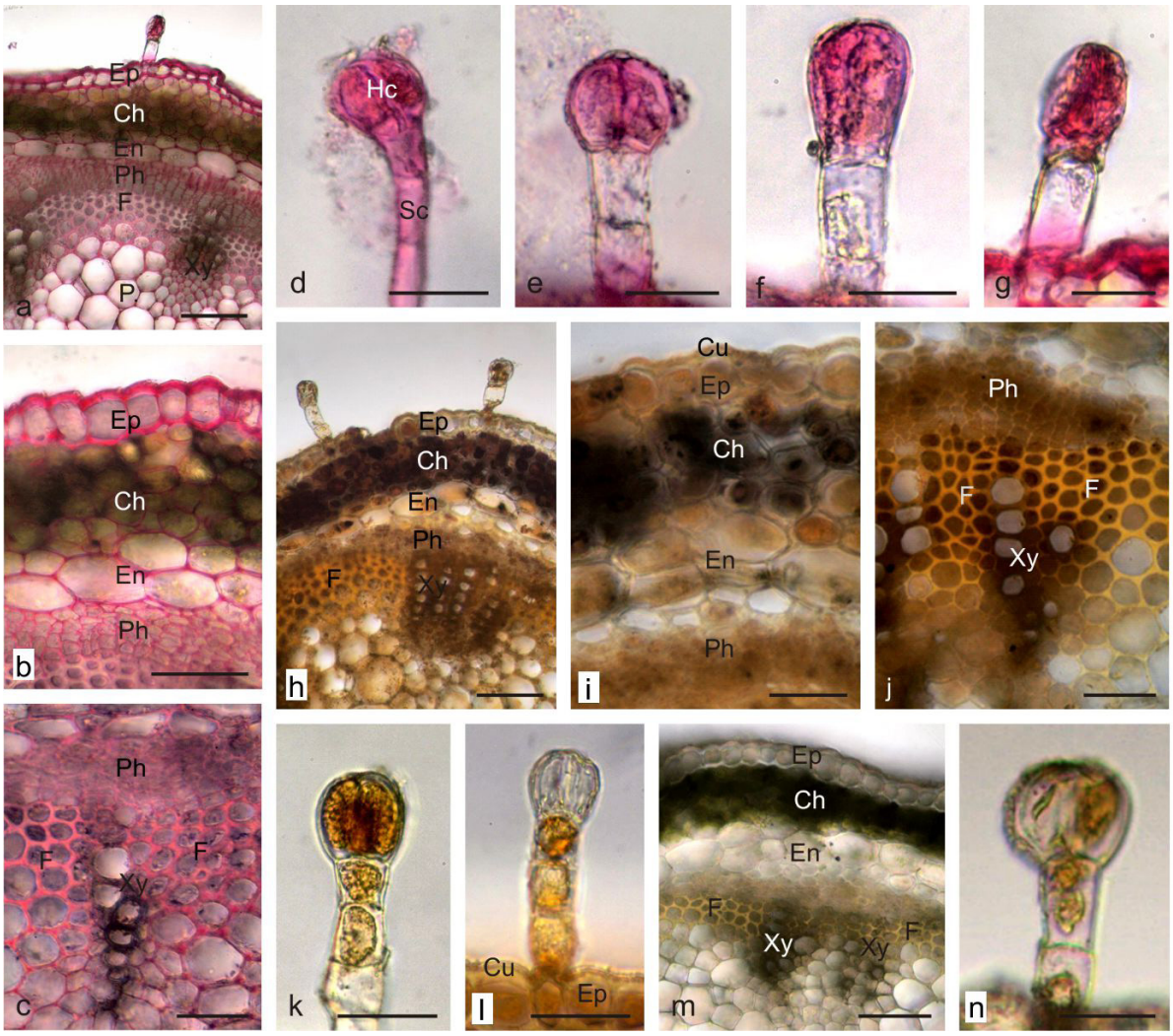
Fig. 4. Anatomy and histochemistry of the *Linaria vulgaris* inflorescence stem



a–g – toluidine blue O; h, i – ferric chloride; j, k, m – potassium dichromate; l – vanillin-hydrochloric acid; n, o – aluminium chloride under UV-excitation; p, q – magnesium acetate under UV-excitation; r – autofluorescence of phenolic acids

a-c – polyphenols identified in the cuticle and xylem fibres; d–g – polyphenols present in the trichome head and stalk cells; h–i – polyphenols present in the trichome cells, j–m – tannins present in the trichome head and stalk cells (j–l), cuticle, vascular tissue, and fibres (m); n–q – intensive fluorescence of flavonoids in the cuticle and epidermis (n) and in the trichome head, stalk, and secretion (o–q); r – intensive autofluorescence of phenolic acids in the trichome head. Cu – cuticle, Ep – epidermis, Ch – chlorenchyma, En – endodermis, Ph – phloem, Xy – xylem, F – fibres, P – parenchyma, Hc – head cells, Sc – stalk cells. Scale bars: a – 200 µm; b, c, m, n – 50 µm; d-l, o-r – 30 µm

Fig. 5. Fresh cross-sections across the *Linaria vulgaris* inflorescence stem subjected to histochemical tests



a–g – ruthenium red; h–l – Wagner's reagent; m, n – Lugol's solution

a–g – acidic polysaccharides present in the walls of all cell layers (a–c) and in the trichome head and stalk cells (d–g); h–j – alkaloids present in the epidermis and vascular elements; k – alkaloids in the trichome head cells and proteins in the trichome stalk cells; l–n – proteins in the fibres (m) and glandular trichomes (l, n); h, i, m – starch present in the chlorenchyma cells. Cu – cuticle, Ep – epidermis, Ch – chlorenchyma, En – endodermis, Ph – phloem, Xy – xylem, F – fibres, P – parenchyma, Hc – head cells, Sc – stalk cells. Scale bars: a, h – 100 µm; b, m – 50 µm; c-g, i-l, n – 30 µm

Fig. 6. Fresh cross-sections across the Linaria vulgaris inflorescence stem subjected to histochemical tests

Acidic polysaccharides were detected using Ruthenium red in all stem cell walls and trichome heads (Fig. 6a–5g).

Alkaloids present in the epidermis, vascular elements and secretory trichomes (Figs. 6h-61, 6n), as well as proteins contained in xylem fibres (Fig. 6m), were identified using Lugol's solution or Wagner's reagent. Additionally, both reagents stained the chlorenchyma almost black, confirming starch's presence (Fig. 6h, 6i, 6m).

DISCUSSION

Plant secretory structures are regarded as the main sites of the synthesis and accumulation of biologically active substances used in the pharmaceutical and cos-

metic industry [e.g. Haratym et al. 2020, Sulborska et al. 2020].

External to the vascular bundles in the petioles of *H. helix*, there were 7–9 larger and 3–5 smaller schizogenic secretory ducts, usually surrounded by 4–9 secretory epithelial cells. It supports the data by Săvulescu and Luchian [2009], who showed that these cells had thin walls, prominent nuclei, and dense cytoplasm. As shown by Amini et al. [2019], *H. helix* petioles have 9 to 12 secretory ducts, whereas their number in *H. pastuchovii* petioles is lower, i.e. from 6 to 8. The presence of secretory ducts is a general characteristic of other araliacean species [Plunkett et al. 2018]. The present study showed the presence of non-glandular stellate trichomes on the *H. helix* petiole surface and druses in many petiole cells. Similarly, other authors observed numerous oxalate crystals in the leaf cells in this taxon [Săvulescu and Luchian 2009, Amini et al. 2019]. The presence of non-glandular stellate trichomes on the leaf surface of this species was also described by Kamińska et al. [2018]. The authors found that the compounds contained therein may cause skin reactions – lesions. As Hart and Henwood [2006] and Wen [2011] demonstrated, the indumentum in the araliaceans often comprises simple or multicellular stellate trichomes. As suggested by Amini et al. [2019] and Lestari and Elya [2019], the presence of secretory ducts, calcium oxalate crystals, and non-glandular trichomes has great diagnostic importance in *Hedera* spp. and the family Araliaceae. These traits may be helpful for the evaluation of the quality (falsification) of medicinal raw materials and in taxonomy [Konarska and Domaciuk 2018, Haratym et al. 2020].

The approx. 80 µm long multicellular capitate glandular trichomes on the surface of the *Linaria vulgaris* inflorescence stems were composed of several stalk cells and a 2–4-celled head. Similar spherical glandular trichomes with a similar length were observed by Jachuła et al. [2018] on the pedicel and sepals of this species. Capitate glandular trichomes with varying distribution, density, height and number of trichome stalk and head cells have also been identified on stems, leaves, bracts, inflorescences, flowers and capsules in other *Linaria* species, e.g. *L. aeruginea*, *L. arabiniana*, *L. depauperata* and *L. supina* [Segarra and Mateu 2001]. As suggested by these authors, the morphological traits of glandular trichomes are essential diagnostic and taxonomic traits of the *Linaria* spp.

With the use of the histochemical assays, several groups of bioactive substances, i.e. lipid compounds, phenolic compounds, polysaccharides, proteins, and alkaloids, were identified in the secretion, secretory epithelial cells of *H. helix* ducts, and secretory trichomes of *L. vulgaris*. Neutral lipids, essential oil, and sesquiterpenes were present in the secretory structures of both species. The presence of essential oil in *H. helix* leaves was also confirmed by histochemical studies conducted by Kamińska et al. [2018], while Jachuła et al. [2018] demonstrated essential oil content in *L. vulgaris* trichomes. As reported by Gruenwald et al. [2000] and Wichtl [2004], essential oil extracted from *H. helix* leaves contained germacrene B, β-elemene, γ-elemene, methyl ethyl ketone, methyl isobutyl ketone, trans-2-hexenal, trans-2-hexanol, germacrene D, β-caryophyllene, sabinene, α-, β-pinene, limonene and furfural. No information on the composition of *L. vulgaris* essential oil is available in the literature. In turn, essential oils extracted from the herb of other *Linaria* species were found to contain 2-methoxy-4-vinylphenol, α-terpinene and 3,5-dimethylphenyl isocyanate [Nafis et al. 2021]. Sesquiterpenes, which were identified in the *H. helix* petioles and *L. vulgaris* stems, are standard essential oils components exhibiting anti-inflammatory, antiparasitic, anti-carcinogenic, anti-diabetic, anti-proliferative, and antimicrobial properties [Chadwick et al. 2013, Bartikova et al. 2014]. The present study also showed the presence of acidic lipids in the *H. helix* ducts and the *L. vulgaris* secretory trichomes. Unsaturated fatty acids, i.e. petroselinic, oleic and vaccenic acids, representing the group of acidic lipids, were identified in *H. helix* extracts by Lognay et al. [1987], whereas linoleic acid was detected in *L. vulgaris* extracts by Ucciani et al. [1994].

The fluorescence and histochemical tests confirmed the presence of phenolic compounds (flavonoids, tannins, and phenolic acids) in the cells and secretory ducts of *H. helix* petioles and in the tissues and the secretory trichomes of *L. vulgaris* stems. As suggested by Wichtl [2004] and Bezruk et al. [2020b], quercetin and kaempferol, i.e. substances responsible for the anti-arthritic, antioxidant, and antimicrobial activity, are the essential flavonoids of *H. helix* extracts [Pop et al. 2017, Shokry et al. 2022]. In turn, *L. vulgaris*

extracts have been found to contain linarin, neo-linarin, pectolin, and isolinarin A and B, which have mainly anti-inflammatory and antipyretic properties [El'kina et al. 2014, Kuang et al. 2018]. As demonstrated by other researchers, many *Linaria* species are a rich source of valuable flavonoids [e.g. Hanfer et al. 2019, Ahmed-Chaouch et al. 2021]. Intensive autofluorescence of phenolic acids was observed in the secretory structures of both examined taxa. Wichtl [2004] reported the presence of caffeic, dihydroxy-benzoic, chlorogenic, neo-chlorogenic, dihydroxybenzoic, protocatechuic, and p-coumaric acids in *H. helix* extracts and protocatechuic, gallic, ρ-hydroxybenzoic, vanillic, salicylic, caffeic, ρ-coumaric, ferulic, homoprotocatechuic, ohydroxyphenylacetic, gluco-syringic, and ρ-methoxybenzoic acids in *L. vulgaris* herb [Hua et al. 2000, Sokolowska-Wozniak et al. 2003]. As Wang et al. [2019] show, phenolic acids exhibit strong antibacterial, antioxidant, and anti-inflammatory properties. Tannins were another group of phenolic compounds detected in the *H. helix* and *L. vulgaris* raw material. These metabolites were located in the peripheral layers, and secretory ducts of the *H. helix* petioles and in the secretory trichomes and vascular bundles of the *L. vulgaris* stems. The presence of tannins in the epidermis of *H. helix* leaves was also confirmed by histochemical studies [Kamińska et al. 2018] and phytochemical analyses [Suica-Bunghez et al. 2020]. Like other phenolic compounds, tannins have many pharmaceutical and cosmetic applications due to their antibacterial, antiviral, antioxidant, antitumour, and anti-oncogenic activities [Pizzi 2019 and references therein].

The use of Ruthenium red helped indicate mucilages from the group of acidic polysaccharides in the *H. helix* duct secretions and the *L. vulgaris* trichome heads. The presence of mucilage in *H. helix* leaves was also confirmed by Huang et al. [2016], who demonstrated that pectic rhamnogalacturonan-I polysaccharide domains held together by a nanospherical arabinogalactan protein molecule were its significant components. *H. helix* mucilage nanoparticles have protective activity against UV radiation; hence, they can be used as components of photoprotective products and cosmetic fillers or as nanocarriers in drug delivery systems. Plant mucilages are also used as cosmetics stabilisers and pharmaceutical agents in therapies for various metabolism-related diseases. They have natural antimicrobial activity and can also be a matrix for introducing other antimicrobial agents in manufacturing food, cosmetics and healthcare materials [Dybka-Stępień et al. 2021].

The presence of alkaloids in the *H. helix* and *L. vulgaris* raw material was confirmed by the histochemical assays based on the use of Wagner's reagent and Lugol's solution. Nitrogen-supplying amino acids, which may be provided by protein decomposition in the organs of both plants, are precursors required for the biosynthesis of alkaloids. The presence of the alkaloid emetine in some *H. helix* varieties was confirmed by Mahran et al. [1975] and Alkattan et al. [2021]. Emetine exerts an expectorant, emetic, and protozoicidal effect; however, its high doses accumulate in the body and may cause damage to the stomach or liver and even cardiac arrest [Gaillard et al. 2003, ChemIDplus 2022]. Other researchers detected the presence of two quinazoline alkaloids with spasmolytic and peristaltic activity, i.e. linarinic acid and peganine (vasicine) in *L. vulgaris* herb [Hua et al. 2002, Ma et al. 2008]. Peganine also exerts a cardiac-depressant effect and uterine-stimulant activity [Rajani et al. 2008]. In a previous study, quinazoline alkaloids were also demonstrated to have potential anti-inflammatory and anti-allergic activities [Chandrika et al. 2008]. Sing and Peterson [2011] and Hanfer et al. [2019] reported high content of tricyclic quinazoline alkaloids in *Linaria tingitana* and *L. dalmatica* organs.

CONCLUSIONS

The secretory structures in the *H. helix* petioles and the *L. vulgaris* inflorescence shoots were the sites of accumulating many valuable bioactive substances with cosmetic and medicinal potential. The secretions of the *H. helix* secretory ducts and the *L. vulgaris* trichomes contained acidic and neutral lipids, essential oil, sesquiterpenes, polyphenols, flavonoids, phenolic acids, tannins, neutral and acidic polysaccharides, proteins, and alkaloids. Some metabolites were present not only in the secretory structures but also in other layers of cells in the *H. helix* petioles, and *L. vulgaris* stems. The presence of secretory ducts, glandular and non-glandular trichomes, oxalate crystals and other

anatomical traits of the cosmetic and medicinal raw material of both species have taxonomic value for the identification of the analysed genera and families and may be helpful in the evaluation of the quality (falsification) of these raw materials.

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REFERENCES

Ahmed-Chaouch, M., Cheriet, T., Beretta, G., Sarri, D., Bensouici, C., Ouelbani, R., Mancini, I., Sekhara, I., Seghiri, R. (2021). Chemical composition, in vitro antioxidant, anticholinesterase and antibacterial activities of *Linaria scariosa* Desf. Nat. Prod. Res., 35(10), 1722–1726. https://doi.org/10.1080/14786419.2019.1629913

Akhlaghi, M., Taebpour, M., Sharafaldini, M., Javani, O., Haghiralsadat, B.F., Orojalian, F., Shadani, S., Tofighi, D. (2022). Fabrication, characterization and evaluation of anti-cancer and antibacterial properties of nanosystems containing *Hedera helix* aqueous extracts. Nanomed. J., 9(1), 43–56. https://doi.org/10.22038/NMJ.2022.62364.1647

Alkattan, A., Alameer, R., Alsalameen, E., Almaary, M., Alkhairat, M., Alkhalifah, A., Alghanim, F., Radwan, N. (2021). Safety of English ivy (*Hedera helix*) leaf extract during pregnancy: retrospective cohort study. DARU J. Pharm. Sci., 29(2), 493–499. https://doi.org/10.1007/s40199-021-00415-7

Amini, E., Nasrollahi, F., Sattarian, A., Haji Moradkhani, M., Habibi, M., Boozarpour, S. (2019). Morphological and anatomical study of the genus *Hedera* in Iran. Rostaniha, 20(2), 144–157. https://dx.doi.org/10.22092/botany.2019.127705.1171

Baharara, H., Moghadam, A. T., Sahebkar, A., Emami, S. A., Tayebi, T., Mohammadpour, A. H. (2021). The effects of Ivy (*Hedera helix*) on respiratory problems and cough in humans: A review. In: Natural products and human diseases. Advances in experimental medicine and biology, Sahebkar, A., Sathyapalan, T. (eds.), vol. 1328. Springer, Cham. https://doi.org/10.1007/978-3-030-73234-9_23, 361-376

Barnes, L.A., Leach, M., Anheyer, D., Brown, D., Carè, J., Lauche, R., Medina, D.N., Pinder, T.-A., Bugarcic, A., Steel, A. (2020). The effects of *Hedera helix* on viral respiratory infections in humans: A rapid review. Adv. Integr. Med., 7(4), 222–226. https://doi.org/10.1016/j.aimed.2020.07.012

Bartikova, H., Hanusova, V., Skalova, L., Ambroz, M., Bousova, I. (2014). Antioxidant, pro-oxidant and other biological activities of sesquiterpenes. Curr. Top. Med. Chem., 14(22), 2478–2494.

Berim, A., Gang, D.R. (2016). Methoxylated flavones: Occurrence, importance, biosynthesis. Phytochem. Rev., 15, 363–390. https://doi.org/10.1007/s11101-015-9426-0

Bezruk, I., Materiienko, A., Gubar, S., Proskurina, K., Budanova, L., Ivanauskas, L., Georgiyants, V. (2020a). Estimation of the influence of the environmental factors on the accumulation of phytochemicals and antioxidant capacity in the ivy leaves (*Hedera helix* L.). Nat. Prod. Res., 36(4), 1014–1019. https://doi.org/10.1080/14786419.2020.1843029

Bezruk, I., Georgiyants, V., Ivanauskas, L. (2020b). Comparison of components profile in herbal raw material, extract and pharmaceuticals of *Hedera helix*. Pharm. Sci., 4(26), 36–39. https://doi.org/10.15587/2519-4852.2020.210768

Chadwick, M., Trewin, H., Gawthrop F., Wagstaff, C. (2013). Sesquiterpenoids lactones: benefits to plants and people. Int. J. Mol. Sci., 14(6), 12780–12805. https://doi.org/10.3390/ijms140612780

Chandrika, M.P., Ram Rao, A.R., Narsaiah, B., Raju, M.B. (2008). Quinazoline derivatives with potent anti-infammatory and anti-allergic activities. Int. J. Chem. Sci., 6(3), 1119–1146.

Charrière-Ladreix, Y. (1976). Répartition intracellulaire du secrétat flavonique de *Populus nigra* L. [Intracellular localisation of secretory flavonoids from *Populus nigra* L.]. Planta, 129(2), 167–174 [in French]. https://doi.org/10.1007/BF00390024

ChemIDplus (2022). Emetine. United States National Library of Medicine. Available: https://chem.nlm.nih.gov/chemidplus/name/emetine [date of access: 22.03.2022].

Cheriet, T., Mancini, I., Seghiri, R., Benayache, F., Benayache, S. (2015). Chemical constituents and biological activities of the genus *Linaria* (Scrophulariaceae). Nat. Prod. Res., 29(17), 1589–1613. https://doi.org/10.1080/14786419.2014.999243

Chiej, R. (1984). Encyclopaedia of medicinal plants. MacDonald, Orbis.

Clark, G. (1981). Staining procedures, 4th ed. Williams and Wilkins, Baltimore.

Dumitriu, B., Olariu, L., Nita, R., Zglimbea, L., Rosoiu, N. (2013). Vascular anti-inflammatory effects of natural compounds from *Aesculus hippocastanum* and *Hedera helix*. Rom. Biotechnol. Lett., 18(1), 7963–7974.

Dybka-Stępień, K., Otlewska, A., Góźdź, P., Piotrowska, M. (2021). The renaissance of plant mucilage in health pro-

motion and industrial applications: A review. Nutrients, 13(10), 3354. https://doi.org/10.3390/nu13103354

El'kina, O.V., Shramm, N.I., Molokhova, E.I., Petrichenko, V.M. (2014). Optimization of the extraction of biologically active substances from yellow toadflax (*Linaria vulgaris*) herb. Pharm. Chem. J., 48(4), 273–275. https://doi.org/10.1007/s11094-014-1092-9

EMA – European Medicines Agency Committee on Herbal Medicinal Products (2017). Assessment report on *Hedera helix* L., folium. EMA/HMPC/325715/2017. Available: https://www.ema.europa.eu/en/medicines/herbal/hederae-helicis-folium [date of access: 16.03.2022].

Fijałkowski D., Chojnacka–Fijałkowska, E. (2009). Rośliny lecznicze na Lubelszczyźnie [Medicinal plants in the Lublin region]. Redakcja Wydawnictw LTN, Lublin, 197.

Furr, Y., Mahlberg, P.G. (1981). Histochemical analysis of laticifers and glandular trichomes in *Cannabis sativa*. J. Nat. Prod. 44(2), 153–159.

Gabe, M. (1968). Techniques histologiques [Histological techniques]. Masson, Paris [in French].

Gahan, P.B. (1984). Plant histochemistry and cytochemistry. Academic Press, London.

Gaillard, Y., Blaise, P., Darré, A., Barbier, T., Pépin, G. (2003). An unusual case of death: suffocation caused by leaves of common ivy (*Hedera helix*). Detection of hederacosine c, α-hederin, and hederagenin by LC-EI/MS-MS. J. Anal. Toxicol., 27(4), 257–262. https://doi.org/10.1093/jat/27.4.257

Gardner, R.O. (1975). Vanillin-hydrochloric acid as a histochemical test for tannin. Stain Technol., 50(5), 315–317. https://doi.org/10.3109/10520297509117081

Geissmann, T.A., Griffn, T.S. (1971). Sesquiterpene lactones: acid-catalysed color reactions as an aid in structure determination. Phytochemistry, 10(10), 2475–2485. https://doi.org/10.1016/S0031-9422(00)89894-5

Góral, I., Wojciechowski, K. (2020). Surface activity and foaming properties of saponin-rich plants extracts. Adv. Colloid Interface Sci., 279, 102145. https://doi.org/10.1016/j.cis.2020.102145

Gruenwald, J., Brendler, T., Jaenicke, C. (2000). PDR for herbal medicines. Medical Economics Company, Montvale, NJ, 284–285.

Gutmann, M. (1995). Improved staining procedures for photographic documentation of phenolic deposits in semithin sections of plant tissue. J. Microsc., 179(3), 277–281.

Hanfer, M., Cheriet, T., Menad, A., Seghiri, R., Ameddah, S. (2019). HPLC profile and in vitro antioxidant properties of the n-butanol extract of *Linaria tingitana* Boiss. and Reut. J. Chem. Pharm. Res., 11(3), 50–58.

Haratym, W., Weryszko-Chmielewska, E., Konarska, A. (2020). Microstructural and histochemical analysis of aboveground organs of *Centaurea cyanus* used in herbal medicine. Protoplasma, 257(1), 285–298. https://doi.org/10.1007/s00709-019-01437-4

Hart, J.M., Henwood, M.J. (2006). A revision of Australian *Trachymene* (Apiaceae: Hydrocotyloideae). Austr. Syst. Bot., 19(1), 11–55. https://doi.org/10.1071/SB04051

Hexsel, D., Soirefmann, M. (2011). Cosmeceuticals for cellulite. Semin. Cutan. Med. Surg., 30(3), 167–170. https://doi.org/10.1016/j.sder.2011.06.005

Hua, H., Cheng, M., Li, X., Pei, Y. (2002). A new pyrroloquinazoline alkaloid from *Linaria vulgaris*. Chem. Pharm. Bull., 50(10), 1393–1394. https://doi.org/10.1248/cpb.50.1393

Hua, H., Hou, B., Li, W., Li, X., Zhang, Y. (2000). Triterpenes from butter-and-eggs (*Linaria vulgaris*). Chin. Tradit. Herb. Drug., 31(6), 409–412.

Huang, Y., Wang, Y., Tan, L., Sun, L., Petrosino, J., Cui, M. Z., Hao, F., Zhang, M. (2016). Nanospherical arabinogalactan proteins are a key component of the high-strength adhesive secreted by English ivy. Proc. Natl Acad. Sci., 113(23), 3193–3202. https://doi.org/10.1073/pnas.1600406113

Ibezim, E.C. (2012). Natural bioactive principles in the treatment of skin diseases. In: Bioactive natural products: opportunities and challenges in medicinal chemistry, Brahmachari, G. (ed.). 1st ed. World Scientific Publishing Company, Singapore, 499–518. https://doi.org/10.1142/9789814335386_0011

INCI Beauty, 2022. *Hedera helix* leaf/stem extract (Ivy extract). Ingredient INCI Beauty. Available: https://incibeauty.com/en/ingredients/18882-hedera-helix-leaf-stem-extract [date of access: 4.03.2022].

Jabłońska, M. (2019). Eko Certyfikowany Kosmetyk Naturalny, jako nowa alternatywa dla istniejących znaków ekologicznych. [ECO Certified Natural Cosmetic as an alternative to the existing ecological signs]. Chemia Biznes, 1, 50-53.

Jachuła, J., Konarska, A., Denisow, B. (2018). Micromorphological and histochemical attributes of flowers and floral reward in *Linaria vulgaris* (Plantaginaceae). Protoplasma, 255(6), 1763–1776. https://doi.org/10.1007/s00709-018-1269-2

Jensen, W.A. (1962). Botanical histochemistry principles and practice, 1st ed., W.H. Freeman and Company, San Francisco.

Johansen, D.A. (1940). Plant microtechnique, 1st ed., London McGraw Hill, London.

Kamińska, M., Sulborska, A., Weryszko-Chmielewska, E. (2018). Skin lesions caused by ivy (*Hedera helix* L.) leaves. Alergoprofil, 14(3), 63–68. https://doi.org/10.24292/01.AP.110718

Kelemen, L., Scedo, C. (2003). Data about the polyphenol content of *Linaria vulgaris* Mill species. Note I. Farmacia (Bucharest, Romania), 51, 86–89.

Kirk, P.W. Jr. (1970). Neutral red as a lipid fuorochrome. Stain Technol., 45(1), 1–4.

Konarska, A., Domaciuk, M. (2018). Differences in the fruit structure and the location and content of bioactive substances in *Viburnum opulus* and *Viburnum lantana* fruits. Protoplasma, 255(1), 25–41. https://doi.org/10.1007/s00709-017-1130-z

Konarska, A., Łotocka, B. (2020). Glandular trichomes of *Robinia viscosa* Vent. var. *hartwigii* (Koehne) Ashe (Faboideae, Fabaceae) – morphology, histochemistry and ultrastructure. Planta, 252, 102. https://doi.org/10.1007/s00425-020-03513-z

Kuang, W., Zhang, X., Lan, Z. (2018). Flavonoids extracted from *Linaria vulgaris* protect against hyperlipidemia and hepatic steatosis induced by western-type diet in mice. Arch. Pharm. Res., 41(12), 1190–1198. https://doi.org/10.1007/s12272-017-0941-y

Lestari, S.M., Elya, B. (2019). Macroscopic and microscopic studies of *Polyscias guilfoylei* LH Bailey leaves (Araliaceae). Pharmacogn. J., 11(4). http://dx.doi.org/10.5530/pj.2019.11.132

Lewkowicz Mosiej, T. (2012). Rośliny lecznicze świata [Medicinal plants of the Word]. Świat Książki, Warszawa, 173.

Lognay, G., Marlier, M., Séverin, M., Wathelet, J.P. (1987). Study of the lipids from ivy (*Hedera-helix* l) – identification of isomeric monounsaturated fatty-acids. Rev. Fr. Corps Gras, 34(9), 407–411.

Lutsenko, Y., Bylka, W., Matlawska, I., Darmohray, R. (2010). *Hedera helix* as a medicinal plant. Herba Pol., 56(1), 83–96.

Ma, C., Higashi, N., Ishiguro, K., Zhao, Y., Zhang, L., Zhao, C., Cheng, M., Oku, H. (2018). Allergy-preventive effects of linarinic acid and its tetrahydropyrrolo [2, 1-b] quinazoline derivatives isolated from *Linaria vulgaris*. J. Nat. Med., 72(2), 582–587. https://doi.org/10.1007/s11418-018-1187-9

Mahran, G.H., Hilal, S.H., El–Alfy, T.S. (1975). The isolation and characterisation of emetine alkaloid from *Hedera helix*. Planta Med., 27(2), 127–132

Metcalfe, D.J. (2005). *Hedera helix* L. J. Ecol., 93(3), 632–648.

Minarchenko, V., Futorna, O., Pidchenko, V., Tymchenko, I., Dvirna, T., Makhynia, L. (2021). Morphological investigations on the diagnostic features of six *Hypericum* species of the Ukrainian flora. FABAD J. Pharm. Sci., 46(1), 31–42.

Nafis, A., Saad, F.E., Khalloufi, F.E., Kasrati, A., Abbad, A., Mezrioui, N., Oudra, B., Vasconcelos, V., Hassani, L. (2021). New insight into antimicrobial activities of *Linaria ventricosa* essential oil and its synergetic effect with conventional antibiotics. Arch. Microbiol., 203(7), 4361–4366. https://doi.org/10.1007/s00203-021-02391-7

Pearse, A.G.E. (1985). Histochemistry: theorical and applied. Vol II, Churchill Livingstone, New York, pp. 1055.

Pizzi, A. (2019). Tannins: prospectives and actual industrial applications. Biomolecules, 9(8), 344. https://doi.org/10.3390/biom9080344

Plunkett, G.M., Wen, J., Lowry, P.P., Mitchell, A.D., Henwood, M.J., Fiaschi, P. (2018). Araliaceae. In: Flowering plants. Eudicots, Kadereit, J., Bittrich, V. (eds.). The families and genera of vascular plants, vol. 15. Springer, Cham., 413–446. https://doi.org/10.1007/978-3-319-93605-5_4

Pop, C.E., Parvu, M., Arsene, A.L., Pârvu, A.E., Vodnar, D.C., Tarcea, M., Toiu, A.M., Vlase, L. (2017). Investigation of antioxidant and antimicrobial potential of some extracts from *Hedera helix* L. Farmacia, 65(4), 624–629.

Puvača, N. (2018). Bioactive compounds in selected hot spices and medicinal plants. J. Agron. Technol. Eng. Manag., 1(1), 8–17. https://orcid.org/0000-0002-5500-7010

Rehman, K.U., Hamayun, M., Yaseen, T., Butt, Z.A., Ahmad, G., Wali, S. (2022). The usefulness of common ivy (*Hedera helix* L.) extracts against bacterial and fungal pathogens found in local hospitals. Pak. J. Sci. Ind. Res. B: Biol. Sci., 65(1), 45–49. https://doi.org/10.52763/PJSIR.BIOL.SCI.65.1.2022.45.49

Saner, M.A., Clements D.R., Hall, M.R. Doohan D.J., Crompton C.W. (1995). The biology of Canadian weeds. 105. *Linaria vulgaris* Mill. Can. J. Plant Sci., 75(2), 525–537. https://doi.org/10.4141/cjps95-092

Săvulescu, E., Luchian, V. (2009). Comparative anatomy of the vegetative organs of the *Hedera helix* L. (Araliaceae). Sci. Papers Ser. A, Agron. (Univ. Agron. Sci. Vet. Med. Buchar.), 52, 487–490.

Schulte-Michels, J., Keksel, C., Häberlein, H., Franken, S. (2019). Anti-inflammatory effects of ivy leaves dry extract: influence on transcriptional activity of NFκB. Inflammopharmacology, 27(2), 339–347. https://doi.org/10.1007/s10787-018-0494-9

Segarra, J.G., Mateu, I. (2001). Taxonomic study of *Linaria depauperata* and *L. supina* complexes in Eastern Spain. Ann. Bot., 87(2), 157–177. https://doi.org/10.1006/anbo.2000.1311

Senderski, M.E. (2016). Prawie wszystko o ziołach i ziołolecznictwie [Almost everything about herbs and herbal medicine]. Wyd. M.E. Senderski, Podkowa Leśna, 386–387.

Shokry, A.A., El-Shiekh, R.A., Kamel, G., Bakr, A.F., Sabry, D., Ramadan, A. (2022). Anti-arthritic activity of the flavonoids fraction of ivy leaves (*Hedera helix* L.)

standardized extract in adjuvant induced arthritis model in rats in relation to its metabolite profile using LC/MS. Biomed. Pharmacother., 145, 112456. https://doi.org/10.1016/j.biopha.2021.112456

Sing, S.E., Peterson, R.K. (2011). Assessing environmental risks for established invasive weeds: Dalmatian (*Linaria dalmatica*) and yellow (*L. vulgaris*) toadflax in North America. Int. J. Environ. Res. Public Health, 8(7), 2828–2853. https://doi.org/10.3390/ijerph8072828

Sokolowska-Wozniak, A., Szewczyk, K., Nowak, R. (2003). Phenolic acids from the herb of *Linaria vulgaris* [L.] Mill. Herba Pol., 49(3–4), 161–165.

Sokornova, S.V., Matveeva, T.V. (2021). Iridoid glycosides of the tribe Antirrhineae. Phytochem. Rev., 1–23. https://doi.org/10.1007/s11101-021-09774-0

Suica-Bunghez, I.R., Sorescu, A.A., Doncea, S.M., Constantin, M., Raut, I., Rodica Mariana, I.O.N. (2020). Phytochemical, antioxidant and antimicrobial characterization of *Hedera helix* L. extract. J. Plant Dev., 27, 47–53. https://doi.org/10.33628/jpd.2020.27.1.47

Sulborska, A., Konarska, A., Matysik-Woźniak, A., Dmitruk, M., Weryszko-Chmielewska, E., Skalska-Kamińska, A., Rejdak, R. (2020). Phenolic constituents of *Lamium album* L. subsp. *album* flowers: Anatomical, histochemical, and phytochemical study. Molecules, 25(24), 6025. https://doi.org/10.3390/molecules25246025

Sultana, S., Khan, M.A., Ahmad, M., Bano, A., Zafar, M., Shinwari, Z.K. (2011). Authentication of herbal medicine neem (*Azadirachta indica* A. Juss.) by using taxonomic and pharmacognostic techniques. Pak. J. Bot., 43, 141–150.

Tajik, S., Zarinkamar, F., Soltani, B.M., Nazari, M. (2019). Induction of phenolic and flavonoid compounds in leaves of saffron (*Crocus sativus* L.) by salicylic acid. Sci. Hortic., 257, 108751. https://doi.org/10.1016/j.scienta.2019.108751

Talamond, P., Verdeil, J.L., Conéjéro, G. (2015). Secondary metabolite localization by autofluorescence in living plant cells. Molecules, 20, 5024–5037. https://doi.org/10.3390/molecules20035024

Terlecka, P., Terlecki, K., Makuch, M. (2020). Spasmolytic, expectorant, and anti-inflammatory activity of *Hedera helix* L. saponins. In: Biologically active compounds of plant origin in medicine, Chwil, M., Skoczylas, M.M. (eds). Wydawnictwo Uniwersytetu Przyrodniczego w Lublinie, Lublin, 91–99. https://doi.org/10.24326/mon.2020.10

Ucciani, E., Mallet, G., Gamisans, J., Gruber, M. (1994). Fatty acid composition of seed oils of Mediterranean herbaceous species. Grasas Aceites, 45(3), 107–112.

Vrchovská, V., Spilková, J., Valentão, P., Sousa, C., Andrade, P.B., Seabra, R.M. (2008). Assessing the antioxidative properties and chemical composition of *Linaria vulgaris* infusion. Nat. Prod. Res., 22(9), 735–746. https://doi.org/10.1080/14786410601132360

Wang, Y., Xie, M., Ma, G., Fang, Y., Yang, W., Ma, N., Fang, D., Hu, Q, Pei, F. (2019). The antioxidant and antimicrobial activities of different phenolic acids grafted onto chitosan. Carbohydr. Polym., 225, 115238. https://doi.org/10.1016/j.carbpol.2019.115238

Wen, J. (2011). Systematics and biogeography of *Aralia* L. (Araliaceae): Revision of *Aralia* sects. *Aralia*, *Humiles*, *Nanae*, and *Sciadodendron*. Contr. United States Nat. Herb., 57, 1–172.

Wichtl, M. (2004). *Hedera* folium. Herbal Drug and Phytopharmaceuticals, a handbook for practice on a scientific basis. 3rd ed. MedPharm GmbH Scientific Publishers, Marburg, 274–277.