Histochemistry of glandular trichomes and the structure of selected organs of Borago officinalis L.

Miroslawa CHWIL¹*, Andrzej BOROWY²

¹Department of Botany, University of Life Sciences in Lublin, Lublin, Poland
²Department of Horticultural Nursery and Seed Production, University of Life Sciences in Lublin, Lublin, Poland

Abstract: In Poland, Borago officinalis is a cultivated plant or a segetal weed. It is a medicinal, cosmetic, melliferous, ornamental, and edible species. Its leaves, herb, flowers, and seeds are a source of bioactive compounds. Given the high concentration of essential fatty acids, borage is used in many branches of industry. The aim of this study was to examine the structure of trichomes and to carry out histochemical labeling of selected groups of biologically active compounds contained in secretory cells. Anatomical traits of leaves, stems, and pedicels were investigated using bright-field light, fluorescence, and scanning electron microscopy. Characteristic features of the anatomical structure imply ecological adaptation of B. officinalis to xerothermic habitats. The trichomes in B. officinalis were divided into two groups. The first group included mechanical trichomes, whereas glandular trichomes formed the other group. Three hair types were distinguished in each group. Various groups of secondary metabolites were identified in exudates using histochemical assays. These investigations are a basis for identification of the location of selected groups of biologically active compounds such as essential oils, fatty compounds, neutral lipids, phenolic compounds, flavonoids, polysaccharides, and calcium in B. officinalis.

Key words: Borage, micromorphology, biologically active substances, stomatal index, cuticle, anatomy, leaves, stem, pedicel

1. Introduction

The family Boraginaceae comprises 156 genera and about 2500 species. The genus Borago is represented by 16 genera native to the Old World occurring in Europe, Africa, and Asia, and it is native to the Mediterranean region. In the natural environment, B. officinalis grows in the Atlantic European countries. It is cultivated in Europe, North Africa, and North America (Río-Celestino et al., 2008; Gupta and Singh, 2010). In Poland, it is a cultivated plant or a segetal weed (Warcholińska, 2008). The shoots of the species are 60 cm high. The lower leaves are large and oval-shaped while the upper leaves are smaller and clasp the stem. Flowers are arranged in a helicoid cyme inflorescence. The fruit is a schizocarp (Gupta and Singh, 2010). B. officinalis is a medicinal, cosmetic, melliferous, ornamental, and edible plant (Asadi-Samani et al., 2014). Bioactive compounds are derived from B. officinalis leaves, herb, flowers, and seeds (Río-Celestino et al., 2008). The borage herb mainly contains flavonoids, tannins, saponins, pyrrolizidine alkaloids, organic acids, essential oil, anthocyanin pigments, mucilage, vitamin C, calcium, potassium, iron, manganese, and cobalt (Gupta and Singh, 2010; Asadi-Samani et al., 2014).

In some countries, young aboveground parts of B. officinalis are used as a vegetable and seasoning for cheese, soups, salads, vegetable dishes, and cocktails. B. officinalis flowers are used to garnish snacks, confectionery, desserts, and beverages (Río-Celestino et al., 2008). Seeds are used to extract oil with a high concentration of essential unsaturated fatty acids, mainly γ-linolenic, linoleic, and oleic acid (Río-Celestino et al., 2008; Farhadi et al., 2012). The bioactive compounds contained in borage plants exhibit antibacterial, anti-inflammatory, healing and regenerating, diuretic, antioxidant, and anticancer activity (Basar et al., 2013; Asadi-Samani et al., 2014, Jaradat et al., 2016, Lozano-Baena et al., 2016). Various investigations demonstrated the possibility of using borage active compounds in the treatment of respiratory tract infections, arteriosclerosis, and cardiovascular diseases (Gupta and Singh, 2010; Farhadi et al., 2012). The aboveground parts of B. officinalis contain substantial amounts of essential omega-3 and omega-6 unsaturated fatty acids, which prevent infections, cancer, and cardiovascular diseases when used in the diet (Río-Celestino et al., 2008). Recently, there has been a growing interest in the phytotherapeutic activity of biologically active compounds used in the
prophylaxis and treatment of many diseases, as they are characterized by better bioavailability, greater efficacy, and higher safety than synthetic compounds (Jaradat et al., 2016).

Trichomes covering the stems and leaves of members of Boraginaceae have different densities, types, and natures, which are usually related to their taxonomy (Selvi and Bigazzi, 2001; Taia, 2006). The literature provides few reports on the glandular trichomes in B. officinalis (Selvi and Bigazzi, 2001). Currently, there is no such information referring to borage originating from the temperate parts of Europe. Weryszko-Chmielewska and Chwil (2008) found that glandular trichomes present in the epidermis of viper’s bugloss calyx (Echium vulgare L.) grown in Poland were composed of a single- or two-celled stalk with an average length of 18 µm and a unicellular head with a diameter of 25 µm. The head of the trichome was covered by a layer of cuticle, usually protruding at the site of accumulation of secretion. According to Gupta and Singh (2010), glandular trichomes in borage consist of a unicellular stalk and a unicellular, subepidermal head. Large trichomes have their base surrounded by several small cells and the walls are sometimes papillate. These types of trichomes are not as frequent as those with a bulbous base.

The structure of glandular trichomes involved in exoscretion of secondary metabolites in Boraginaceae plants representing the genera Cordia, Echium, Anchusa, Pulmonaria, Symphytum, and Onosma is highly diverse (Akçın, 2007; Akçın and Baki, 2010; Ventrella and Marinho, 2008; Weryszko-Chmielewska and Chwil, 2008; Chwil and Weryszko-Chmielewska, 2009; Gostin, 2009). The secretion accumulated in the secretory cells of different types of trichomes differs in the composition of active compounds (Ventrella and Marinho, 2008). Some of these compounds, e.g., tocopherols, γ-linolenic acid, stearic acid, and flavonoids, can serve as chemotaxonomic indicators in Boraginaceae plants (Bagci et al., 2002).

So far, B. officinalis plants have been insufficiently explored in terms of epidermis micromorphology, in particular in terms of the structure of glandular trichomes involved in secretion of biologically active compounds, and histochemical identification of the major groups of these compounds. The available literature does not provide such information about borage. Therefore, an attempt was made to complete the knowledge.

The aim of this study was to determine the micromorphology of the epidermis and trichome structure and to carry out histochemical labeling of selected groups of bioactive compounds in secretory cells. Additionally, selected anatomical features of the leaves, stems, and pedicels of B. officinalis cultivated in the climatic conditions of Lublin, a city located in southeastern Poland, were characterized via comparative analysis.

2. Materials and methods

2.1. Study material

Borago officinalis L. plants were collected from an experimental plot of a collection of medicinal plants located on an experimental farm in Felin, University of Life Sciences in Lublin (215 m above sea level, 51°12’38.2″N, 22°38’18.2″E). The leaves, stems, and pedicels were sampled for microscopic analyses and determination of selected groups of bioactive substances in the initial phase of plant flowering. Fluorescence, bright field, and scanning electron microscopic techniques were used to compare the structure of the epidermis, trichomes, and cells of selected tissues and to localize active compounds in the trichomes.

2.2. Microscopy

2.2.1. Fluorescence microscope (FM)

Sections of hand-made cross-sections of leaves, stems, and pedicels were placed in a drop of 0.01% auramine O (Heslop-Harrison and Heslop-Harrison, 1981). The preparations were placed in a 50% glycerol solution and the structure of glandular trichomes was analyzed under a Nikon Eclipse 90i fluorescence microscope equipped with an FITC filter (EXP. 465–495; DM 505; BA 515–555). Autofluorescence of the trichomes and staining reactions of selected groups of secondary metabolites were observed upon application of magnesium acetate, aluminum chloride, and neutral red using a UV-2A filter (EXP. 378/11; DM 416; BA 416 LP).

2.2.2. Light microscope (LM)

Fragments of leaves, stems, and pedicels were sampled from fresh material. The sections were fixed in 4% glutaraldehyde for 6 h at room temperature and in 0.1 M phosphate buffer, pH 7.0, at 4 °C for 48 h.

To prepare semithin slides, fixed samples were rinsed in phosphate buffer and dehydrated in a series of ethanol solutions at concentrations of 15%, 30%, 50%, 70%, 90%, and 96% for 15 min, and twice in anhydrous ethanol. Dehydrated plant fragments were embedded in Spurr low-viscosity resin and polymerized at a temperature of 60 °C for 48 h. Cross-sections were made from the resin-embedded material. Sections of 1 µm in thickness were cut with a glass knife using a Reichert Ultracut S microtome and stained with 1% toluidine blue and 1% azure II (1:1) at a temperature of 60 °C for 5 min. Stained preparations were dried after rinsing with distilled water and 5% ethanol.

The anatomical structure of the epidermis, trichomes, and selected tissues of the analyzed organs were observed on the semithin slides. In turn, hand-made cross-sections of fresh material were analyzed with the use of various histochemical assays labeling selected biologically active compounds. Comparative analyses were carried out using a Nikon Eclipse 400 bright field microscope.
2.2.3. **Scanning electron microscope (SEM)**

After dehydration in an acetone series at concentrations of 15%, 30%, 50%, 70%, 90%, and 99.5% (anhydrous acetone was used twice), fixed plant samples were critical point-dried in liquid CO₂ using an Emitech K850 dryer. Next, the samples were coated with gold using an EMITECH K550X sputter coater (manufacturer: Quorum Technologies).

The surface of the stem, leaf, and pedicel epidermis was observed and photographic documentation was made using a SEM (TESCAN VEGA II LMU).

2.3. **Histochemical assays**

Selected bioactive compounds contained in the glandular trichomes of the stem, leaf, and pedicel epidermis of *B. officinalis* were determined using appropriate chemicals: lipids - Sudan Red 7B (Brundrett et al., 1991) or Sudan Black B (Lison, 1960) and neutral red (Kirk, 1970); neutral and acidic lipids - Nile Blue (Cain, 1947); terpenoids - Nadi reagent (David and Carde, 1964); phenolic compounds - ferric chloride (Johansen, 1940) and potassium dichromate (Gabe, 1968); flavonoids - aluminum trichloride, magnesium acetate, and neutral lead acetate (Charrière-Ladreix, 1976); and periodic acid-Schiff reagent (PAS) (Jensen, 1962), which was used for localization of polysaccharides (O’Brien and McCully, 1981). The presence of calcium was detected with alizarin (McGee-Russell, 1958).

2.4. **Morphometric analyses**

Length and width of stomata, diameter of stomatal complex, and number of epidermis cells and stomata per 1 mm² were determined in the adaxial and abaxial epidermis of *B. officinalis* leaves. Stomatal index was calculated according to the method proposed by Meidner and Mansfield (1968). The length of nonglandular trichomes as well as the height and width in the basal and apical part of the pedestal were measured. The size of glandular trichomes was specified as well by comparison of the height, width, and surface of the head and stalk. In the leaf cross-section, the size of stomata, as well as other epidermal and palisade parenchyma cells, and the thickness of the palisade and spongy layers and lamina were determined. In the anatomical structure of the stem and pedicel, we measured the height and width of epidermis and hypodermis cells, the cell diameter and thickness of the collenchyma layer, and the width of vascular bundles.

Morphometric measurements of epidermis micromorphology and tissue structure were carried out using computer software for microscope image analysis Nikon NIS-Elements, version 3.0, Advance Research. Morphometric measurements were performed in 16 replicates for each trait of epidermis micromorphology and structure of the selected tissues. Mean results of the measurements and standard deviation were calculated in Microsoft Excel 2013.

3. **Results**

Stomata and mechanical and glandular trichomes were present at a varied density in the epidermis of the three analyzed organs of *B. officinalis*. Results of these studies and the histochemical analyses are presented below in Figures 1A–1D, 2A–2D, 3A–3D, 4A–4H, 5A–5H, 6A–6N, 7A–7E, 8A–8F, and 9A–9D.

3.1. **Micromorphology of the epidermis**

3.1.1. **Leaves**

Stomata were located on both surfaces of the *B. officinalis* leaf epidermis (Figures 1A–1D, 2C, 2D). The cuticle on the stomatal cell surface formed semicircular striae on the midrib, parallel to the long axis of the cells. Striae on the other epidermis cells were gently undulating, or the cuticle was smooth (Figures 1B, 1D, 2C, 2D). Stomata represented the anomocytic type and were located at the level of the other epidermis cells. They were surrounded by 3 or 4 guard cells (Figures 1D, 2C, 2D). The adaxial epidermis exhibited substantially larger but fewer stomata than the abaxial epidermis (Table 1). The diameter of the stomatal complex on both epidermis surfaces was in the range of 61–73 µm. The number of stomata per 1 mm² in the adaxial epidermis accounted for 71% of the number of stomata in the abaxial epidermis. The stomatal index was 12.3 and 13.9, respectively (Table 1).

3.1.2. **Stem and pedicel**

On the surface of stems and pedicels, the epidermis grew dense trichomes (Figures 3A, 4A, 4B). The cuticle on the *B. officinalis* stem and pedicel epidermis had striated ornamentation. The striae were semicircular on the guard cells and were arranged along the longer axis in other epidermis cells (Figures 3B, 3C). Stomata had thick cuticular ledges. They exhibited different developmental stages and were open, semiclosed, and closed (Figure 3C).

3.1.3. **Trichomes**

The epidermis of both surfaces of the borage leaves, stem, and pedicel exhibited glandular and mechanical trichomes (Figures 1A–1C, 2A–2C, 3A, 3B, 3D, 4A–4C).

3.1.4. **Mechanical trichomes**

Unicellular, bristly, sharply pointed, mechanical trichomes were present in the epidermis of the analyzed organs (Figures 1A–1C, 2A–2C, 3A, 4A–4C). Three types were distinguished according to the number of basal cells and trichome length:

I. Short (326–451 µm), with 2–4 typically outlined basal cells (Figures 1A, 1B, 2A, 3A, 4B);

II. Medium-length (650–865 µm), with 5–8 large, linearly arranged basal cells (Figures 1A, 2A, 3A, 4B);

III. Long (1043–21964 µm) cells (Figures 1A, 2A, 3A, 4B) located on a massive, multicellular, multiple-row pedestal with a height of 137–167 µm and width of 185–212 µm (Figures 3A, 4A, 4B, 4D, 4E, 8A, 8B, Table 2).
The trichome wall had distinct, short striae arranged along the longer axis (Figures 3D, 4C, 4F, 4G), or strongly shortened striae forming micropapillae located mainly near the pedestal (Figures 4D, 4H). The trichomes had a thick wall (Figures 7D, 9D).

3.1.5. Glandular trichomes

The glandular trichomes in the adaxial leaf epidermis were denser on the surface of veins than on the intercostal fields (Figures 2A, 2B). Capitate glandular trichomes were present in the epidermis of the analyzed organs (Figures 1A, 1B, 2A, 2B).
The trichomes had a single-celled secretory head and a 1-, 2-, or 3-celled stalk. The secretory head was spherical or elliptical (Figures 2B, 3B, 3D). Its height and width (group A) were 36–39 µm and 27–33 µm, respectively. The surface of the head accounted for 47%–67% of the total area of glandular trichomes (1362–1823 µm²) (Table 3). The cuticle on the surface of the glandular trichomes was smooth. At the sites of accumulation of essential oil, the cuticle formed protuberances over the subcuticular space (Figures 5D–5F).

The trichomes were covered by a wax layer (Figures 5G–5H). Three different types of glandular trichomes were distinguished according to the number of stalk cells:
A - 1-celled head and 1-celled stalk (Figure 5A);
B - 1-celled head and 2-celled stalk (Figure 5C);
C - 1-celled head and 3-celled stalk (Figure 5E).
Figure 3. Fragments of epidermis in the *Borago officinalis* stem. A- Glandular trichomes (arrowhead); short (arrow), medium-length (double-headed arrow), and long mechanical trichomes on a pedestal (two arrows); B- glandular trichomes (arrowhead) with a unicellular stalk and a unicellular head; C- stomata with a thick cuticular ledge; cuticular striation of the surface of stomata and other epidermis cells; D- cuticular striae (st) on the surface of the wall of a mechanical trichome, capitate trichome with accumulated secretion at the head apex (asterisk). A–D- SEM.
Figure 4. Fragments of epidermis in the *Borago officinalis* pedicel. A- Glandular (arrowhead) and mechanical trichomes with a different length and base; B- short mechanical trichome - type I (arrow), medium-length - type II (double-headed arrow), and long trichome with a multicellular pedestal (mp) - type III (two arrows); C- glandular (arrowhead) and mechanical trichomes with longitudinal striae on the wall; D, E- multicellular pedestal (mp) of the long trichomes - type III; F, G- cuticular striae (asterisk) on the wall of mechanical trichomes; H- micropapillae (m) on the surface of the mechanical trichome wall near the base. A–H- SEM.
Figure 5. Glandular trichomes in *Borago officinalis*. A, B- Glandular trichomes - type A with a unicellular stalk and a unicellular head; visible vesicular cuticle protuberance (asterisk) formed by accumulated essential oil (B); C- glandular trichome - type B - formed of a two-celled stalk and a unicellular head; D–F- lateral (D, E) and apical (F) cuticular protuberances on the head, glandular trichome - type C - formed by a free-celled stalk (arrowhead) and a unicellular head (E); G, H- smooth cuticle on the surface of a glandular trichome with a fine wax coating (arrow). A- LM, B–H- SEM.
Figure 6. Histochemical assays of glandular (A–L) and mechanical (M, N) trichomes of *Borago officinalis*. A- Epifluorescence of the cell wall of the secretory head; visible fluorescence of secondary metabolites in the protoplast - auramine O fluorochrome; B- red-stained lipids in the cytoplasm and subcuticular space - Sudan Red B; C- dark blue-stained lipid compounds - Sudan Black; D- blue fluorescence of fatty compounds accumulated in the subcuticular space - neutral red; E- blue-stained acidic lipids - Nile Blue; F- slight purple staining of terpene compounds - Nadi reagent; G–I- yellow-green fluorescence of flavonoids - aluminum chloride (G), lead acetate (H), magnesium acetate (I); J- brown-stained phenolic compounds - potassium dichromate; K- blue autofluorescence of secondary metabolites accumulated in the subcuticular space; L, M- red-stained calcium-mineralized cell wall (arrow) of a glandular (at the stalk and head junction) (L) and mechanical trichome near the base and apex (double-headed arrow) (M) - alizarin; N- pink-stained polysaccharides in the cell wall (two arrows) - periodic-Schiff reagent. A, D, G–I, K - MF; B, C, E, F, J, L–N - LM.
The first type (A) of trichomes was observed frequently in the epidermis of the examined organs, whereas the presence of the second type (B) was rare and that of the third type (C) was sporadic. The height of the glandular trichomes was in the range of 62–77 µm (Table 3).

3.2. Histochemical assays
Groups of bioactive substances were identified in the exudates from the glandular trichomes based on staining reaction to chemical compounds. In the presence of auramine O, the secretory head emitted green fluorescence.
Figure 8. Fragments of cross-sections of the B. officinalis pedicel (A) and stems (B–F). A, B - Pedicel (A) and stem (B); visible glandular (arrowhead) and mechanical trichomes located on a multicellular pedestal (asterisk), large cortical cells (co), vascular bundles (vb) (A); C - epidermis cells (e) with visible autofluorescence of the cuticle (double-headed arrow) and a glandular trichome (arrowhead), hypodermis (h), collenchyma cells (c), cortical cells (co), and vascular bundles (vb); D - thick outer cell wall (cw) of epidermis (e), hypodermis cells (h), and angular or lacunar collenchyma cells (c); E - cortical parenchyma cells (co), starch grains (arrow) in endodermis cells (en), vascular elements (ve); F - starch grains (arrow) in the endodermis (en), cortical parenchyma cells (co), vascular bundles (vb) connected with a sclerenchyma band (sc). A, B - SEM, C - FM, D–F - LM.
Figure 9. Fragments of cross-sections of the *B. officinalis* stem (A) and pedicel (B–D). A- Cells of the epidermis (e), hypodermis (h), collenchyma (c); large parenchyma cortex cells (co), vascular bundles (vb); B- multicellular pedestal (asterisk), epidermis cells (e), collenchyma (c), elongated hypodermis cells (h), parenchyma cortex cells (co) with invaginations of the cell wall, vascular bundles (vb); C, D- thick outer cell wall (cw) of epidermis (e) with a distinct cuticle band (double-headed arrow), loosely arranged hypodermis cells (h), bilayered lacunar collenchymas (c), stomata (s), mechanical trichome with a thick wall (two arrows) (D). A–D- LM
and the fatty compounds contained in the head protoplast emitted stronger fluorescence than the epifluorescence of the cell wall (Figure 6A). After application of Sudan Red B and Sudan Black B, lipids were stained red (Figure 6B) and dark blue (Figure 6C), respectively. In the presence of neutral red, fats accumulated in the subcuticular space emitted blue fluorescence and those contained in the protoplast had yellow fluorescence (Figure 6D). Acidic lipids were stained blue when treated with Nile Blue (Figure 6E). Essential oil and accumulated terpene compounds were purple after treatment with the Nadi reagent (Figure 6F). Flavonoids treated with aluminum chloride, magnesium acetate, or neutral lead acetate exhibited yellow fluorescence (Figures 6G–6I). Phenolic compounds were stained brown after the application of potassium dichromate (Figure 6J). Secondary metabolites accumulated in the subcuticular space exhibited blue autofluorescence (Figure 6K). After treatment with alizarin, calcium present in the trichome wall at the stalk and head junction (Figure 6L) and in the basal and apical part of the glandular trichomes was stained red (Figure 6M). After application of PAS, polysaccharides contained in the cell wall of the trichomes (Figure 6N) and other tissues, e.g., the epidermis and collenchyma, were stained pink.

### 3.3. Anatomical structure

#### 3.3.1. Leaf

The cells of both epidermis surfaces were well developed (Figures 7A–7C). The adaxial epidermis had a thick outer cell wall (Figures 7C, 7D) and a distinct cuticle layer (Figure 7E). The adaxial epidermis had larger cells than those building up the abaxial surface. The stomata were approximately 3- or 4-fold smaller than those located in other epidermis cells. The cells of the palisade mesophyll (65 µm height/39 µm width) formed one or two layers, whereas the spongy mesophyll was composed of four, five, or six layers (Figures 7A–7C). The ratio of the palisade to spongy mesophyll was 1:2.

### Table 1. Micromorphology of the surface of epidermis cells in *Borago officinalis* leaves.

<table>
<thead>
<tr>
<th>Feature examined</th>
<th>Adaxial epidermis</th>
<th>Abaxial epidermis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of stomata</td>
<td>Min–max</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Width of stomata</td>
<td>µm</td>
<td>34.51–22.73</td>
</tr>
<tr>
<td>Diameter of stomatal complex</td>
<td>Min.</td>
<td>45.46–80.95</td>
</tr>
<tr>
<td></td>
<td>Max.</td>
<td>55.46–100</td>
</tr>
<tr>
<td>Number of epidermis cells/1 mm²</td>
<td>1294.17–1798.68</td>
<td>1523.12 ± 171.16</td>
</tr>
<tr>
<td>Number of stomata/1 mm²</td>
<td>197.42–230.32</td>
<td>212.50 ± 13.03</td>
</tr>
<tr>
<td>Stomatal index (%)</td>
<td>10.38–14.00</td>
<td>12.34 ± 1.30</td>
</tr>
</tbody>
</table>

### Table 2. Length of mechanical trichomes and the size of the pedestal in the epidermis of the analyzed organs in *Borago officinalis*.

<table>
<thead>
<tr>
<th>Feature examined</th>
<th>Stem</th>
<th>Pedicels</th>
<th>Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of nonglandular trichomes</td>
<td>µm</td>
<td>282.80–616.10</td>
<td>451.34 ± 107.95</td>
</tr>
<tr>
<td>Height of pedestal</td>
<td>Type III</td>
<td>97.28–179.20</td>
<td>134.40 ± 20.61</td>
</tr>
<tr>
<td>Width of pedestal in the apical</td>
<td>Type III</td>
<td>128.00–284.16</td>
<td>203.04 ± 49.79</td>
</tr>
<tr>
<td>Width of pedestal in the basal part</td>
<td>Type III</td>
<td>89.60–202.24</td>
<td>136.48 ± 37.48</td>
</tr>
</tbody>
</table>
lamina thickness, respectively (Table 4). The anatomical structure of *B. officinalis* leaf cells indicates that the leaves represent a bifacial type (Figures 7A–7C).

### 3.3.2. Stem

Stem epidermis cells had a thick outer wall with a distinct cuticle band (Figures 8C, 8D). The hypodermis was composed of two layers of cells. Larger, cylindrical cells were present in the layer directly under the epidermis (Figures 8C, 8D). Angular or lacunar collenchyma formed a 102-µm thick four- or five-layered stratum (Figures 8D, 9A). Cortical parenchyma cells formed from 3 to 6 rows. The starch grains present in the endodermis cells of the stem (Figure 8E) and pedicel exhibited a positive PAS reaction (Figure 8F). The vascular bundles arranged in a ring were connected with a sclerenchyma band (Figure 8F; Table 5). An air canal was present in the central part.

### 3.3.3. Pedicel

The cells of the pedicel epidermis (Figures 8A, 9B–9D) had a similar structure as that in the stem (Figures 8B, 8D, 9A). The hypodermis was formed by two layers of cells: one contained larger and the other smaller cells (Figure 9C; Table 5). Collenchyma cells formed two layers (Figures 9B, 9C). The cells of the cortical parenchyma formed 4 or 5 rows (Figure 5B). The cross-section exhibited 10 vascular bundles.

**Table 3. Size of glandular trichomes (type A) in the epidermis of the analyzed organs in *Borago officinalis*.**

<table>
<thead>
<tr>
<th>Feature examined</th>
<th>Stem</th>
<th>Pedicels</th>
<th>Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min–max</td>
<td>Mean ± SD</td>
<td>Min–max</td>
</tr>
<tr>
<td>µm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height of secretory head</td>
<td>24.49–46.12</td>
<td>38.88 ± 5.710</td>
<td>25.95–45.52</td>
</tr>
<tr>
<td>Width of secretory head</td>
<td>24.69–43.38</td>
<td>29.35 ± 6.65</td>
<td>17.90–37.27</td>
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<tr>
<td>Surface of secretory head</td>
<td>812.73–1082.81</td>
<td>905.65 ± 96.69</td>
<td>1149.73–592.32</td>
</tr>
<tr>
<td>Height of stalk</td>
<td>24.93–46.51</td>
<td>32.70 ± 5.92</td>
<td>29.41–42.09</td>
</tr>
<tr>
<td>Width of stalk</td>
<td>14.08–20.89</td>
<td>16.06 ± 2.73</td>
<td>15.03–18.37</td>
</tr>
<tr>
<td>Surface of stalk</td>
<td>320.83–828.92</td>
<td>456.35 ± 121.51</td>
<td>809.78–1149.73</td>
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<tr>
<td>Height of glandular trichomes</td>
<td>50.70–81.31</td>
<td>62.05 ± 9.00</td>
<td>66.75–86.32</td>
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<tr>
<td>Surface of glandular trichomes</td>
<td>1139.94–1798.47</td>
<td>1362.00 ± 164.79</td>
<td>1469.2–2140.27</td>
</tr>
</tbody>
</table>

**Table 4. Size of epidermis and mesophyll cells and thickness of *Borago officinalis* leaves.**

<table>
<thead>
<tr>
<th>Feature examined</th>
<th>Min–max</th>
<th>Mean ± SD</th>
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</thead>
<tbody>
<tr>
<td>µm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height of cells on epidermis surface</td>
<td>Adaxial</td>
<td>36.74–58.45</td>
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<tr>
<td></td>
<td>Abaxial</td>
<td>28.39–55.11</td>
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<tr>
<td>Width of cells on epidermis surface</td>
<td>Adaxial</td>
<td>41.75–51.17</td>
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<td></td>
<td>Abaxial</td>
<td>35.07–86.84</td>
</tr>
<tr>
<td>Height of stomatal cells on epidermis surface</td>
<td>Adaxial</td>
<td>8.63–15.03</td>
</tr>
<tr>
<td></td>
<td>Abaxial</td>
<td>8.61–11.69</td>
</tr>
<tr>
<td>Width of stomatal cells on epidermis surface</td>
<td>Adaxial</td>
<td>8.95–15.03</td>
</tr>
<tr>
<td></td>
<td>Abaxial</td>
<td>8.61–10.02</td>
</tr>
<tr>
<td>Height of palisade cells</td>
<td>53.44–90.18</td>
<td>64.50 ± 8.06</td>
</tr>
<tr>
<td>Width of palisade cells</td>
<td>30.06–50.10</td>
<td>39.04 ± 6.81</td>
</tr>
<tr>
<td>Thickness of palisade layers</td>
<td>76.80–117.76</td>
<td>95.36 ± 12.87</td>
</tr>
<tr>
<td>Thickness of spongy layers</td>
<td>89.60–133.12</td>
<td>113.92 ± 19.50</td>
</tr>
<tr>
<td>Thickness of lamina</td>
<td>250.79–333.49</td>
<td>293.09 ± 29.75</td>
</tr>
</tbody>
</table>
4. Discussion

4.1. Micromorphology of the epidermis

The leaves of the B. officinalis species analyzed and described in the literature represented the amphistomatic type (Selvi and Bigazzi, 2001). Similarly, stomata were present in both epidermis surfaces in Anchusa (Akçin et al., 2010), Euploca (Tölke, 2015), Heliocarya (Yousefi, 2010), Heliotropium (Tölke, 2015), Moltkia (Doğu et al., 2012), Onosma (Akçin and Engin, 2005), Pulmonaria (Gostin, 2009), and in other Boraginaceae taxa (Selvi and Bigazzi, 2001).

Anomocytic stomata were present in the epidermis of the analyzed B. officinalis leaves, as well as plants described by other authors and representing the genera Anchusa (Akçin et al., 2010), Asperugo, Heliotropium, Lappula (Zarinkamar, 2007), Onosma (Azizian et al., 2000), Pulmonaria (Gostin, 2009), and Trichodesma (Kumar and Kumar, 2016). In Borago morissiana, anisocytic stomata were identified (Bigazzi and Ricceri, 1992). In turn, other Boraginaceae taxa exhibited both anomocytic and anisocytic stomata (Akçin, 2007; Zarinkamar, 2007; Akçin and Binzet, 2010), e.g., representatives of the genera Cynoglossum (Akçin, 2012), Moltkia (Doğu et al., 2012), and Symphytum (Akçin and Baki, 2007). Symphytum asperum had anomo-, aniso-, and diacytic stomata (Zarinkamar, 2007). This diversity of stomata is one of the criteria for determination of the taxonomic hierarchy of plants at the family level within the genus or species.

The stomata in the adaxial epidermis of the analyzed leaves were larger and less dense than in the abaxial epidermis. A similar pattern was observed in the leaves of three Anchusa species (Akçin et al., 2010). The number of stomata in the adaxial and abaxial epidermis was higher than that in the epidermis of two species representing the genus Borago (Selvi and Bigazzi, 2001).

The values of the stomatal index in the adaxial and abaxial epidermis of the analyzed leaves were 12.34 and 13.94, respectively. As shown by literature data, the value of the stomatal index and the size of stomata are positively correlated in related taxa. The values of these parameters are similar in species from the same family (Akçin et al., 2010; Kumar and Kumar, 2016). The stomatal index was in the range of 28.6–44.9 in Heliotropium (Kumar and Kumar, 2016), 12–28 in Moltkia (Doğu et al., 2012), and 12.7–17.33 in Onosma (Akçin and Binzet, 2010).

The cuticle on the epidermis of the analyzed B. officinalis organs was thick and formed parallel, slightly undulating, or semicircular striae, or was smooth elsewhere. A thick cuticle band was found on the leaf surface in Moltkia (Doğu et al., 2012). The cuticle in Boraginaceae plants exhibits a complex and specific ornamentation type that can serve as a diagnostic trait. This hydrophobic and nonuniform layer composed mainly of cutin, waxes, alkanes, primary alcohols, and hydroxy-fatty acid is an extracellular barrier between the plant and the environment. It is involved in gas and nutrient exchange. The cuticle layer and secreted essential oil serve adaptive functions in xerothermic habitats and protect plants against excessive transpiration and light diffraction (Kunst and Samuels, 2003).

4.2. Trichomes

4.2.1. Mechanical trichomes

The mechanical trichomes in B. officinalis represented three groups: short with typical basal cells, medium-length with a
single-layered base, and long with a multilayered pedestal. There is a wide range of classifications of mechanical trichomes in Boraginaceae. Considering the number of basal cells as well as the wall surface and density of trichomes in the epidermis, Taia (2006) distinguished three main groups and five subgroups in several Boraginaceae species. The first one comprised trichomes with no distinct basal cells, the second group included trichomes with a single-layered multicellular base, and the trichomes from the third group exhibited a two-layered multicellular base. In their analysis of fourteen Boraginaceae species, Selvi and Bigazzi (2001) distinguished seven trichome groups, including five groups of mechanical trichomes and two groups of glandular trichomes. The authors classified *Borago officinalis* trichomes into a third group. They found that *Borago officinalis* trichomes were similar to those developed by *Nonea pulla* and *Pulmonaria picta* plants. They were characterized by an inconspicuous basal tubercle of only 6–8 cells arranged in a single ring around a slender head cell. In this study, the cross-section of analyzed organs demonstrated 2–4 (type I) or 6–8 (type II) basal cells of the mechanical trichomes and a multicellular multilayered pedestal (type III). In turn, in plants from the genus *Cordia*, one group comprised trichomes without distinct basal cells and the second group included trichomes with a ring-shaped base (Amer et al., 2016).

The walls of the analyzed *B. officinalis* trichomes exhibited papillae, striae, or a smooth cuticle. Similar micropapillae were observed in *Borago morrisiana* (Bigazzi and Ricceri, 1992). The trichome cell wall in other species from the family Boraginaceae was granular, scaly, papillate, micropapillate, or smooth (Selvi and Bigazzi, 2001; Taia, 2006; Gostin, 2009; Amer et al., 2016). These characteristic microstructural patterns of ornamentation of the trichome cell wall can have taxonomic value (Selvi and Bigazzi, 2001; Taia, 2006; Amer et al., 2016).

### 4.2.2. Glandular trichomes

The analyzed *B. officinalis* glandular trichomes had a 1-, 2-, or 3-celled stalk and a spherical or elliptical unicellular head. They represented three groups. A similar structure of glandular trichomes was described in other species of Boraginaceae as well (Gostin, 2009; Akçin and Binzet, 2010; Basar et al., 2013). In turn, Selvi and Bigazzi (2001) distinguished small glandular trichomes with a spherical cell and a stalk formed by three or more cells, as well as long trichomes with elongated secretory cells and a stalk composed of three or more cells. The spherical shape of the secretory head noted in this study is consistent with the description of the first group of glandular trichomes reported by Selvi and Bigazzi (2001) and by other authors (Ventrella and Marinho, 2008; Amer et al., 2016). The cuticle on the surface of the examined trichomes was smooth and exhibited a slight protuberance over the subcuticular space, where essential oil was accumulated. The exudate can be released by cuticle rupture. The secretion accumulated in different types of trichomes differs in the composition of secondary metabolites (Ventrella and Marinho, 2008).

### 4.3. Histochemical assays

The present study provides, for the first time, the results of histochemical assays of selected groups of biologically active compounds in *B. officinalis* glandular trichomes. Literature data show the presence of fatty compounds in the head protoplast of *Cordia verbenacea* trichomes (Ventrella and Marinho, 2008). The fatty acids in the *B. officinalis* leaves were dominated by palmitic, α-linolenic, and stearic acids. Unsaturated fatty acids prevent the development of infectious and cardiovascular diseases and have an anticancer effect (Río-Celestino et al., 2008). Borage is a source of γ-linolenic acid, i.e. a compound applied mainly in the pharmaceutical industry and in production of healthy foods and dietary supplements (Basar et al., 2013).

The stain reaction confirmed the presence of phenolic compounds in the head of the analyzed glandular trichomes. A similar effect was observed in *Cordia verbenacea* trichomes and in powdered *Sericostoma pauciflorum* Stocks raw material (Ventrella and Marinho, 2008; Kevalia et al., 2015). The content of phenolic compounds is associated with antioxidant, antimicrobial, anticancer, and cytotoxic properties of borage (Zemmouri, 2014; Lozano-Baena et al., 2016). The compounds also have economic importance, as they determine the quality of food: color, odor, and flavor. Phenolic compounds and flavonoids accumulated in secretory structures provide protection against UV-B radiation during exposure to sunlight (Liakoura et al., 1997).

The investigations of borage presented in this paper and literature reports of *Cordia verbenacea* showed the presence of yellow-stained flavonoids in the head protoplasts (Ventrella and Marinho, 2008). Ten different flavonoids with antioxidant activity have been identified in *B. officinalis* leaves (Zemmouri et al., 2014). These compounds have a wide spectrum of biological functions. They play an important role in the interactions between the plant and the environment with different effects on insects, particularly in the reproduction process. Additionally, the flavonoids, anthocyanins, and flavonols present in the exudates of glandular trichomes are involved in pollination biochemistry (Simmonds, 2003).

The alizarin-based method confirmed the presence of calcium compounds in the trichome cell walls. There are numerous literature reports indicating calcium and silicon mineralization of trichome cell walls in the genera *Anchusa*, *Anchusella*, *Brunnera*, *Cordia*, *Cynoglossitis*, *Hormuzakia*, *Nonea*, *Pentaglottis*, *Pulmonaria*, and
Cutler, 1992). Similarly, investigations conducted by Selvi and Bigazzi (2001) indicated that calcium was the main structural element of the head cells (68.3 wt.%). The content of silica in basal and head cells of borage trichomes was slightly above 8 wt.% The studies cited above were carried out in the Mediterranean region. Calcium carbonate crystals observed in several Onosma species were detected in trichome walls and basal cells of large filaments (Azizian et al., 2000). Deposition of these elements in trichomes not only modifies their strength but also represents environmental adaptations.

Secretions accumulated in trichomes have economic importance. Currently, there is a growing interest in the health-promoting activity of plant-derived compounds and their role in dietary therapy. Given its ability to inhibit bacterial growth, an extract from B. officinalis leaves can be used as a natural preservative and antioxidant in food products. However, the presence of pyrrolizidine alkaloids, e.g., lycopsamine and thesine, which exert carcinogenic and hepatotoxic effects, should also be underlined. The high antioxidant activity of the borage herb indicates its potential to be used in the prophylaxis of many diseases (Jaradat et al., 2016). The antimicrobial activity of borage can be used in the treatment of infectious diseases, which are currently a huge problem in many countries due to bacterial resistance to antibiotics.

4.4. Anatomical structure

The well-developed cells of the stem, pedicel, and leaf epidermis, in particular on the adaxial surface, with a thick, cutinized outer wall, were found to be elements of an ecological strategy for adaptation to environmental conditions, strong insolation, and high temperature. The analyzed plants developed bifacial leaves. This type of leaf blade was found in Boraginaceae species from the genera Heliotropium (Alwahibi and Bukhary, 2013), Pulmonaria (Gostin, 2009), Symphytum (Akcın and Baki, 2007), and Trachystemon (Akcın et al., 2004). Present investigations and literature data demonstrated that the palisade cells in B. officinalis, B. pygmaea, and several other Boraginaceae species formed one or two layers (Selvi and Bigazzi, 2001; Gostin, 2009). The thickness of the palisade mesophyll in the analyzed leaves amounted to 83% of the thickness of the spongy mesophyll (114 µm). Leaves exposed to strong sunlight are characterized by a well-developed palisade mesophyll, which is confirmed by the ecological form of borage, which is a plant growing in xerothermic habitats. The thick palisade layer is also associated with a higher proportion of mesophyll tissues, which is regarded in the literature as a structural mechanism to increase photosynthetic activity and an anatomical indicator of xeromorphic nature (Fahn and Cutler, 1992).

Hypodermis cells in the examined stems and pedicels were arranged in one, two, or three layers. Two or three layers of chlorenchyma cells were also observed in plants from the genera Heliotropium and Trichodesma (Kumar and Kumar, 2016). The several-layered chlorenchyma accumulates water and is thus an important structure serving a xeromorphic function in the ecology of dry habitat plants (Lyshede, 1979).

In the present study, the collenchyma was formed by one or two layers in the pedicel and four or five layers in the stem. Literature data showed that there were 2–6 layers of this tissue cells in the stems of Heliocarya, Onosma (Akcın and Binzet, 2010; Yousefi, 2010), and Moltkia (Doğu et al., 2012). A positive correlation was noted between the development of the supporting tissue and a xerothermic habitat (Torres-Boeger et al., 2010).

Cortical cells characterized by a large diameter formed 3–6 layers in the stem and 4–5 layers in the pedicel. Cortical cells have been found to form 3–5 layers in Heliocarya (Yousefi, 2010) and 1–3 layers in Moltkia (Doğu et al., 2012). Both the present results and the literature data confirm the content of starch grains in the single-layered B. officinalis endodermis (Gupta and Singh, 2010). Similar observations were reported in species from the genera Heliocarya (Yousefi, 2010), Onosma (Akcın and Binzet, 2010), and Moltkia (Doğu et al., 2012).

In summary, micromorphological features of the epidermis, i.e., cuticular ornamentation, wall microstructure and trichome type, type of stomata, and stomatal index, can be useful for identification of closely related species. Characteristic traits of the anatomical structure of the epidermis, palisade parenchyma, and hypodermis indicate ecological adaptation of B. officinalis to xerothermic habitats. The B. officinalis glandular trichomes involved in exosecretion of secondary metabolites represented three types. Histochemical assays revealed the presence of essential oils, fatty compounds, neutral lipids, phenolic compounds, flavonoids, polysaccharides, and calcium compounds in the exudates. Determination of the relationship between the chemical composition and anatomy of trichomes and the analyzed organs provides knowledge of the location of secondary metabolites in B. officinalis. The knowledge of the phytotherapeutic significance of biologically active compounds prompts the development of various methods for modification of secretion composition and biosynthesis pathways, cloning appropriate genes, and application of genetic transformation techniques in industrially important essential oil-bearing plants.

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References


