

CHARACTERISTICS OF THE SECRETORY STRUCTURES IN THE FLOWERS OF *Rosa rugosa* Thunb.

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Abstract

Due to the presence of secondary metabolites exhibiting pharmacological activity, the flowers of *Rosa rugosa* Thunb. have found application in traditional and folk medicine. The essential oil obtained from them is also considered to be a phytoncide. The morphological and anatomical characters of glandular trichomes located on the sepals of *R. rugosa* were studied by light and scanning electron microscopy. Using histochemical tests, the type of secretion produced in the trichomes was determined and its contents were compared with the secretion produced by the papillae on the petals.

It was found that multicellular glandular trichomes, having the features of colleters, and non-glandular trichomes were located on the abaxial epidermis, while only non-glandular trichomes were situated on the adaxial epidermis. The stalk cells of the glandular trichomes are arranged in multiple rows, whereas the epidermal cells of the head are arranged radially. The capitate trichomes were classified into two types: short and long trichomes. The largest density of glandular trichomes was recorded in the basal abaxial epidermis and in the middle part of the sepals. During the initial stages of bud development, the glandular hairs were green colored, whereas in the next development stages they changed the color to red. The histochemical tests used allowed us to find that the trichomes on the sepals and the papille on the petals produced lipid substances, polyphenols, tannins, and flavonoids. Sesquiterpenes were found only in the secretion of the glandular hairs on the sepals.

Key words: *Rosa rugosa*, sepals, petals, colleters, glandular trichomes, histochemistry, secondary metabolites

INTRODUCTION

Petals of the *Rosa rugosa* Thunb. flowers are a valued raw material for the production of rose oil [1,2]. Not only the rugosa rose fruit (*Fructus Rosae*) is used in therapy, but also its flower (*Flos Rosae* syn. *Peta-*

lae Rosae) [3]. In China, where this species is found in natural stands, flower buds and petals, after being dried, are used to produce herbal tea utilized in traditional medicine [1,4,5].

Flavonoids, anthocyanins, tannins, and essential oil, whose main constituents are geraniol, citronellol and nerol, are found in rose flowers [1,6]. Flower decoction is used as an agent for activating blood circulation to relieve blood stasis as well as in indigestion and for wounds due to its anti-inflammatory and astringent effects [3,7]. The flowers of *Rosa rugosa* are also used as an agent counteracting toxins, among others as an antidote in antimony intoxication [7,8], and improving appetite and the functioning of the kidneys [8]. Research has also shown that rose oil can be included in active phytoncides affecting microorganisms resistant to antibiotics [1].

The intensely fragrant flowers of this species have a diameter of 6–10 cm. The color of the petals is most frequently purple, more rarely pink or white [9]. Because the flowers do not produce nectaries and nectar, they provide mainly pollen to pollinating insects [10,11]. In the flower, there are 5 sepals which reach a length of 2–3 cm and slightly expand at the tip. They belong to the persistent perianth segments, since they remain on the fruit. The oil produced by the petals and sepals of *R. rugosa* varies in its scent and chemical composition [12]. According to these authors, terpenoids and benzenoid alcohols predominate among odorous substances produced by the petals, whereas the sepals primarily contain sesquiterpenes. Many studies reveal that the flowers of *R. rugosa* contain polyphenols exhibiting antioxidant activity [7,13]. Dobson et al. [12] report that the abaxial surface of the *R. rugosa* sepals is covered by glandular hairs, but the chemical nature of their secretions has not been

investigated yet. Composition of secretions produced by the trichomes located on the sepals of moss roses *Rosa x damascena* and *Rosa x centifolia* was studied by Caissard et al. [14].

The aim of the present study was to determine the structure of glandular trichomes on the sepals of *R. rugosa* and to show the quality of the secretion produced in them using several histochemical tests as well as to compare its composition with the secretion produced by the petals.

MATERIALS AND METHODS

The study material consisted of *Rosa rugosa* Thunb. flowers collected in July over the period 2012–2014 from shrubs growing in the Botanical Garden of the Maria Curie-Skłodowska University in Lublin. The structure of glandular trichomes and the anatomy of sepals and petals were studied using light and scanning electron microscopy.

Light microscopy (LM). The length and width of sepals (at their widest place) were measured under a stereoscopic microscope (n=30). Hand-cut cross sections were prepared from fresh sepals and petals. The sepal thickness (n=30), the length of short glandular trichomes (type A) (n=30) and long glandular trichomes (type B) (n=30) as well as the diameter of their heads (n=30) were measured in glycerin-coated slides. The plant material was also subjected to histochemical tests (with neutral red) for the presence of secretory cells and some chemical compounds: lipids (Sudan III, neutral red), acid lipids (Nile blue), polyphenols (ferric trichloride), tannins (potassium dichromate), flavonoids (magnesium acetate), and sesquiterpenes (conc. H₂SO₄). Photographic documentation was made using a Nikon Coolpix 4500 camera.

Scanning electron microscopy (SEM). Petal and sepal sections were fixed in a 2% solution of glutaraldehyde with 2.5% paraformaldehyde in 0.75 M phosphate buffer (pH 6.8) at a temperature of 4°C for 12 h. Subsequently, the samples were dehydrated in an ethanol series and dried at the critical point in liquid CO₂. Using an EMITECH K 550x sputter coater, they were coated with gold. The slides were examined under a TESCAN/VEGA LMU scanning electron microscope at an accelerating voltage of 30 kV.

RESULTS

Sepals. The sepals emit a delicate fruit scent. Capitulate glandular and non-glandular trichomes are present along their entire abaxial surface (Fig. 1 A, B, D–G; 2 A; 3 A, B), while the adaxial epidermis is covered only with non-glandular hairs (Fig. 2 A–C). The margins of the basal and middle part of the sepals are

particularly covered quite densely with non-glandular trichomes (Fig. 2 C), which can be seen with naked eye as pubescence on these parts. In the basal part of the sepals, there are long and short capitate trichomes (Fig. 1 C; 3 B), whereas short capitate hairs are predominant in their middle and apical part (Fig. 3 A). On the upper margins of the narrowing parts of the sepals, there are teeth equipped with round or conical glands with red contents (Fig. 1 A, F, H, I).

The thickness of the sepals in their widest place reaches 710 µm (Table 1). The stomata are located in small depressions and found only on the abaxial epidermis of the sepals (Fig. 2 F–H). The mesophyll of the sepals is not differentiated into palisade and spongy parenchyma. Chloroplasts occur only in the parenchyma cells from the side of the abaxial epidermis (Fig. 2 A, B). Small clusters of cells containing druses of calcium oxalate are present in the sepal mesophyll (Fig. 2 D, E).

The glandular trichomes (each type) greatly vary in terms of their size. This relates to the stalk length and diameter as well as to the head diameter (Fig. 3 A, B; 4 A–L; 5 A–H, K, L; Table 1). Irrespective of the size of the trichomes, their stalks and heads consist of many cells (Fig. 3 C, D; 4 A–L; 5 A–H, K, L). The length of type A trichomes (shorter ones) is in the range of 102 – 317 µm, for type B (longer ones) it ranges from 358 to 949 µm, whereas the head diameter is 71.5 – 153 µm and 112 – 175 µm, respectively (Table 1). The stalk cells are arranged in multiple rows, whereas the epidermal cells of the head are arranged radially (Fig. 4 A, D, E, G–J, L; 5 A, B, D–G, K, L). The secretion produced by the head cells accumulates in the subcuticular space (Fig. 4 E, F, H–J).

In living glandular trichomes obtained from fresh plant material, chloroplasts were observed in the cells located in the lower parts of the head and in the cells occurring in the stalk (Fig. 4 A–H, J, K; 5 A–C, G). During the initial stage of bud development, the heads and stalks of the glandular hairs were green colored (Fig. 1 A, C), while in the next development stages they gradually changed their color to red. In an opening bud, most hairs were dark red (Fig. 1 D–G). The red pigment was probably an anthocyanin. The substances producing the color of the head were located in the cells of the lower or upper region of the head (Fig. 4 G–K). The contents of the head cells containing pink- or red-stained vacuoles varied and were dependent on the stage of trichome development. The older trichomes had a darker color. The hairs remained on the sepals also during the fruiting stage. At this stage, we observed a dark red or brown secretion on the surface of their heads (Fig. 4 I, J).

The use of histochemical tests enabled the detection of lipid substances, polyphenols, tannins, flavonoids, and sesquiterpenes in the trichomes (Table 2). Depending on the degree of trichome development and

activity, stained lipids were observed in the apical part of the heads as different sized regions (Fig. 5 A–H). Polyphenolic compounds, stained black in the slides, occurred both in the stalk cells and in the cells forming the head (Fig. 5 L). The presence of tannins was revealed by the dark brown color observed in the stalk cells and in the trichome head (Fig. 5 J, K) as well as in the mesophyll of the sepals (Fig. 5 I). Having been treated with conc. H₂SO₄, the contents of the heads stained yellow, which indicates the presence of sesquiterpenes (not shown). On the other hand, the yellowish-green staining that was observed in the trichome head after the application of magnesium acetate allowed us to find the presence of flavonoids in its cells. Treatment of the sepal cross sections with neutral red revealed the presence of secretory cells not only in the glandular hairs but also in the epidermal and parenchyma cells (Fig. 2 B).

Petals. Papillae are found only on the adaxial epidermis of the petals (Fig. 6 A–H), while the abaxial epidermis cells do not have convex outer walls (Fig. 6 A, I, J). Large intercellular spaces are present in the petal mesophyll (Fig. 6 B). The external part of the cell walls of the adaxial and abaxial epidermis is covered

with a cuticle showing characteristic striation. In the adaxial epidermis, the striae converge at the apex of the papillae (Fig. 6 C–E), while in the abaxial epidermis they run longitudinally or irregularly, concealing the boundaries between the cells (Fig. 6 I).

Treatment of the petal cross sections with neutral red revealed higher secretory activity of the adaxial epidermis which stained more intensely; this is evidence of the higher content of lipids in the cells of this tissue (Fig. 6 A, F). Treatment of the sections with Nile blue (not shown) and Sudan III also indicates a high concentration of lipid compounds in the apical parts of the papillae (Fig. 6 G, H). After the application of the latter reagent, lipid compounds were found to be also concentrated in the vascular bundle regions (Fig. 6 K). Having performed histochemical tests for the presence of tannins and polyphenols, we found that both the papillae and parenchyma cells produced compounds of this type (Table 2.). Flavonoids were also present in the papillae, which is evidenced by the positive reaction with magnesium acetate. We observed negative histochemical tests only for the presence of sesquiterpenes (Table 2).

Table 1
Some features of sepals and glandular trichomes

Studied feature	Min - Max	Mean
Sepal length (mm)	21.0 – 33.0	25.0
Sepal width (mm)	5.5 – 10.0	7.7
Sepal thickness (µm)	530.8 – 1000.4	710.2
Length of type A trichomes* (µm)	102.1 – 316.5	215.9
Diameter of type A glandular head (µm)	71.5 – 153.3	118.2
Length of type B trichomes* (µm)	357.3 – 949.4	559.6
Diameter of type B glandular head (µm)	112.3 – 175.0	136.2

*type A – short trichome, type B – long trichome

Table 2
Compounds identified by histochemical tests
in the glandular trichomes of *R. rugosa* sepals and in the papillae on the petals

Test	Compounds	Color observed	Capitate trichomes on sepals	Papillae on petals
Sudan III	lipids	orange	+	+
Nile blue	acid lipids	blue	+	+
	neutral lipids	pink	-	-
Neutral red	lipids	red	+	+
Potassium dichromate	tannins	brown	+	+
Ferric trichloride	polyphenols	black	+	+
Magnesium acetate	flavonoids	yellow-greenish	+	+
Conc. H ₂ SO ₄	sesquiterpenes	yellow	+	-

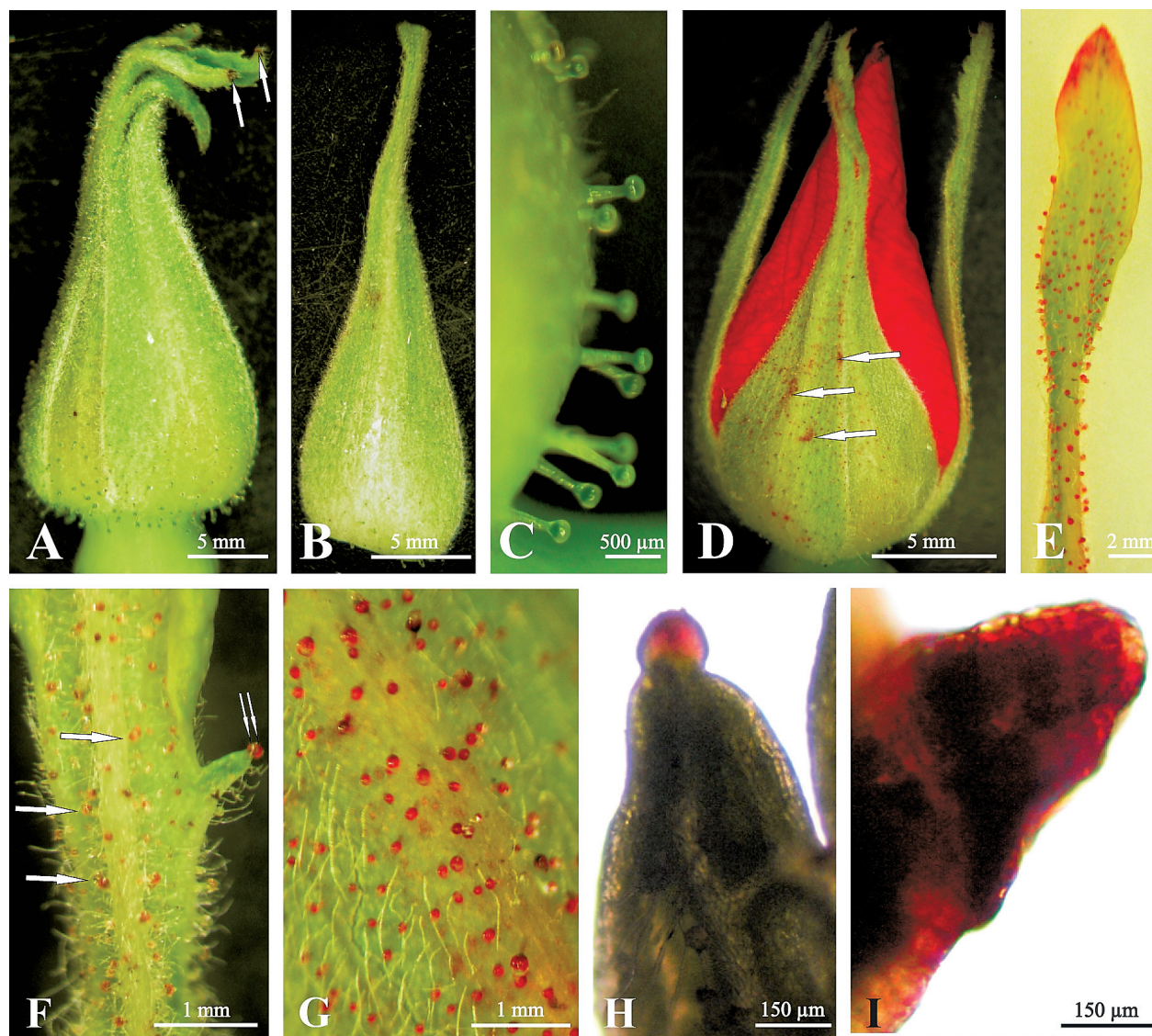


Fig. 1. *R. rugosa* calyx and its details.

A – Closed bud covered by sepals. Teeth with glands (arrows) can be seen at the apical part.

B – Sepal of a closed bud.

C – Section of a sepal with green glandular trichomes.

D – Open bud. Dark red glandular trichomes (arrows) can be seen on the sepals.

E – Apical part of a sepal with numerous glandular trichomes on its surface.

F – Section of the apical part of a sepal with visible non-glandular trichomes on its entire surface, glandular trichomes (arrows), and a gland on the tooth (double arrow).

G – Section of the middle part of a sepal with numerous glandular and non-glandular trichomes.

H – Apical part of a sepal tooth with a round gland visible at the apex.

I – Conical gland from the apical part of a sepal tooth.

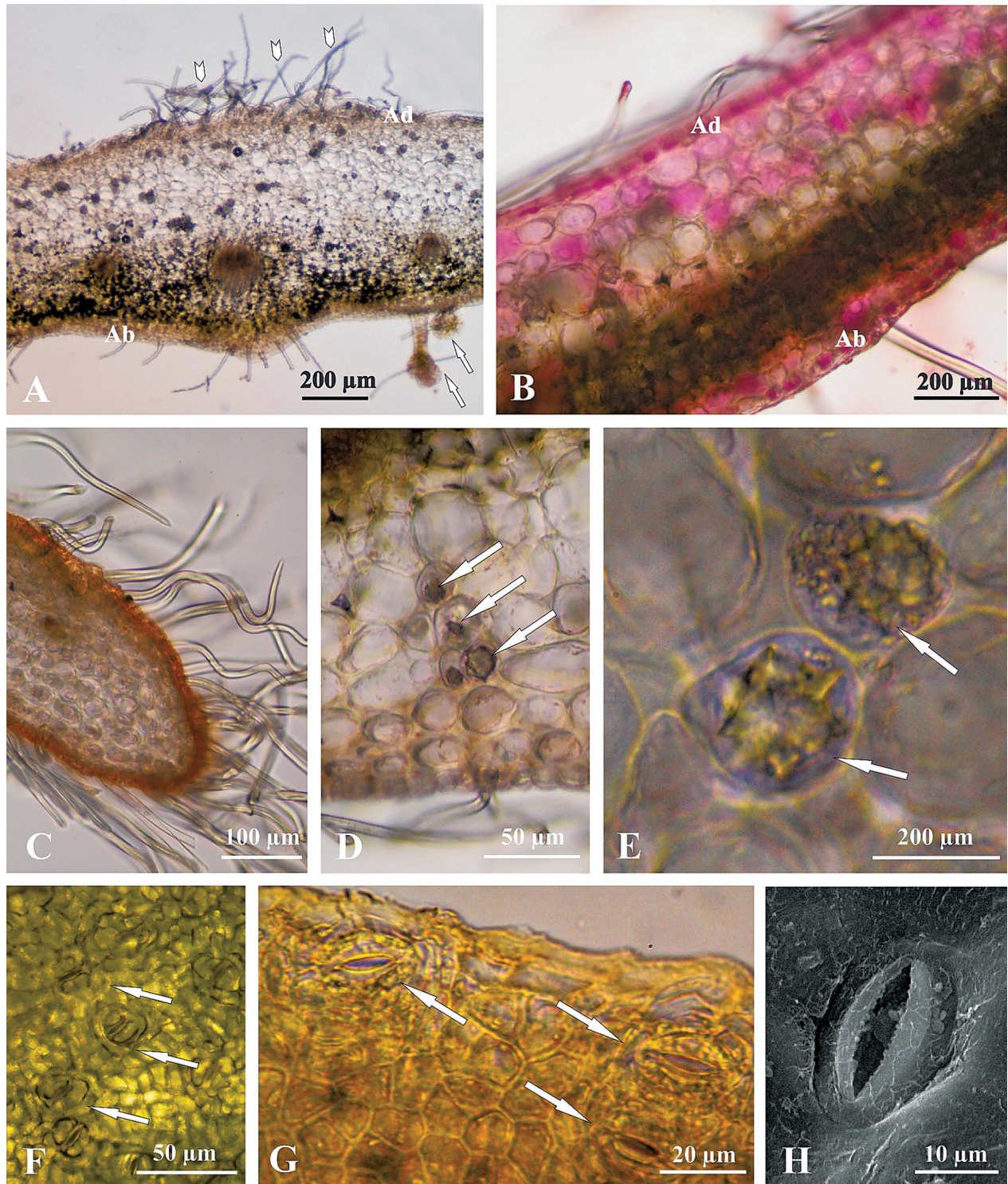


Fig. 2. Anatomy of the sepal.

- A – Cross section of a sepal. The arrows indicate glandular trichomes, the arrowheads – non-glandular trichomes.
- B – Cross section of a sepal. The pink staining of the cells (with neutral red) is evidence of their secretory activity.
- C – Section of a sepal with visible non-glandular trichomes (stained with Sudan III).
- D, E – Cross sections of a sepal. The arrows indicate calcium oxalate crystals in the parenchyma cells.
- F, G – Sections of abaxial epidermis. The arrows indicate stomata.
- H – Stoma from the outer surface of a sepal.

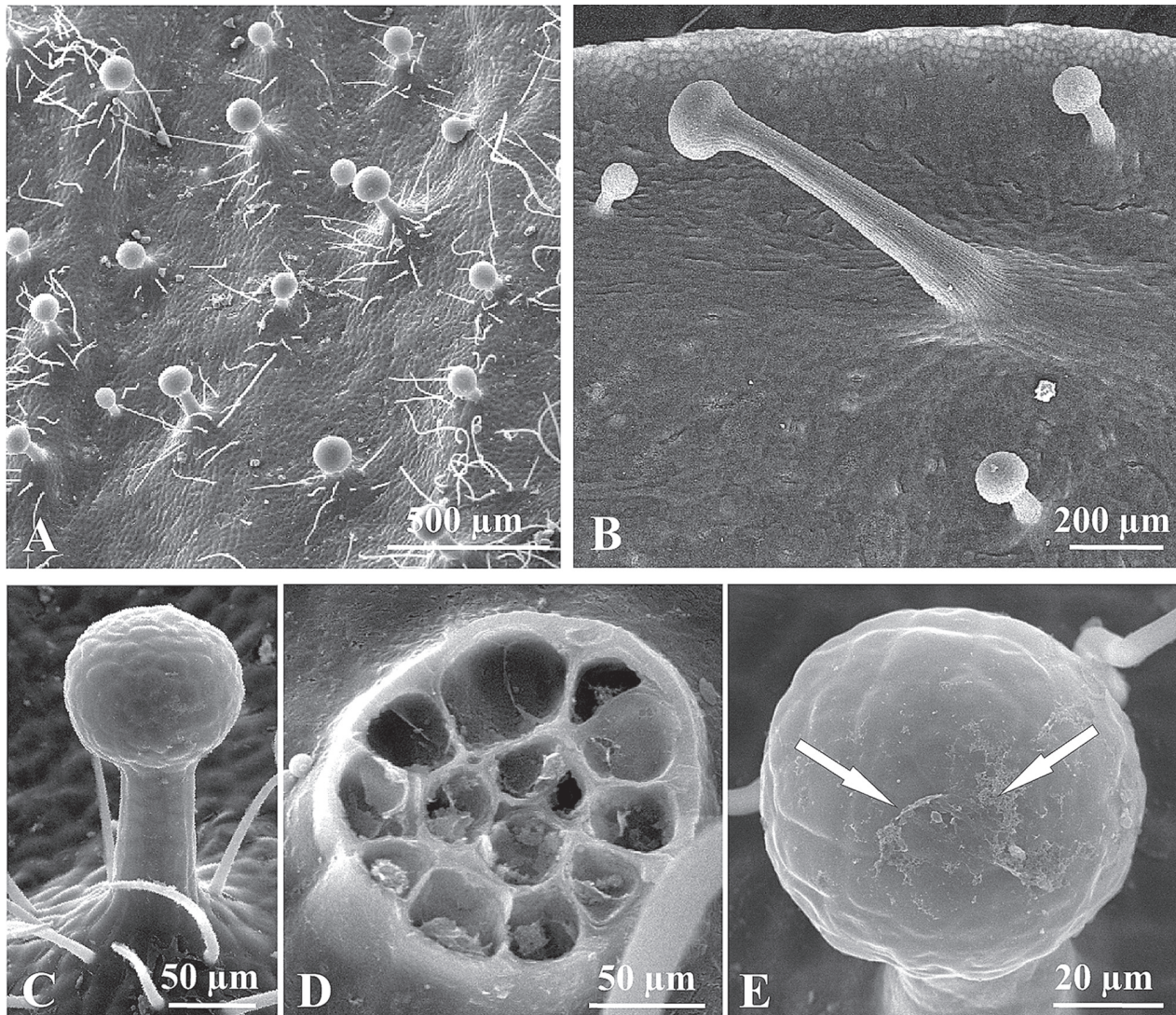


Fig. 3. Surface of the abaxial epidermis of the sepals viewed by SEM.

- A – Section of the middle surface of a sepal covered with glandular and non-glandular trichomes.
- B – Section of a sepal (basal part) with visible type A and B glandular trichomes.
- C – Short glandular trichome and non-glandular trichomes.
- D – Cross section of the stalk of a short glandular trichome.
- E – Head of a short trichome with visible secretion (arrows).

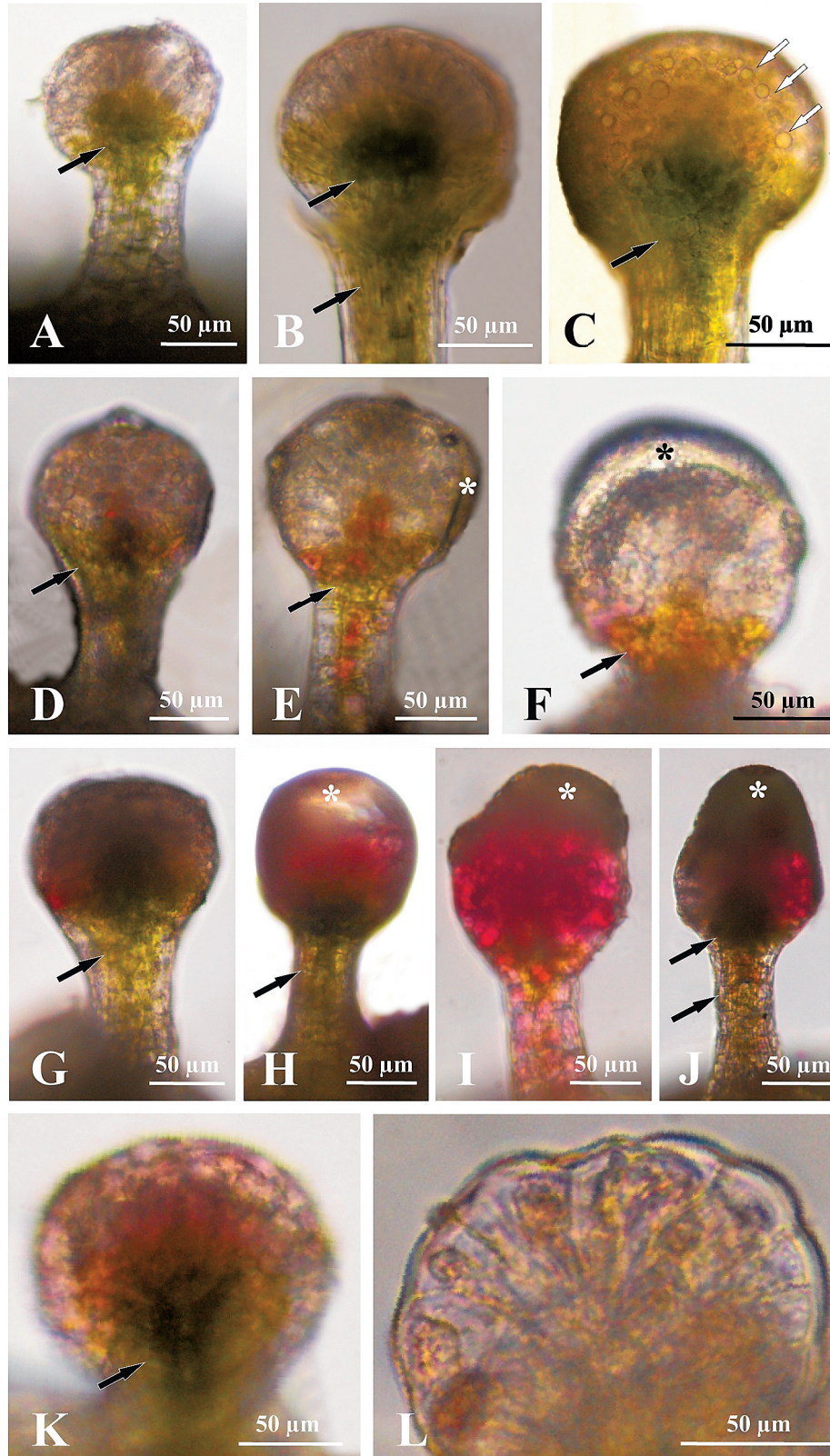


Fig. 4. Short glandular trichomes from the abaxial surface of a sepal (fresh unstained material).

A-F – Younger trichomes with visible chloroplasts (black arrows). The white arrows in Fig. C indicate lipid drops in the cells of the trichome head, while the asterisks in Fig. E, F indicate the secretion in the subcuticular space.

G-K – Older trichomes with visible secretion (asterisks).

L – Longitudinal section of a trichome head. The radially arranged cells are seen.

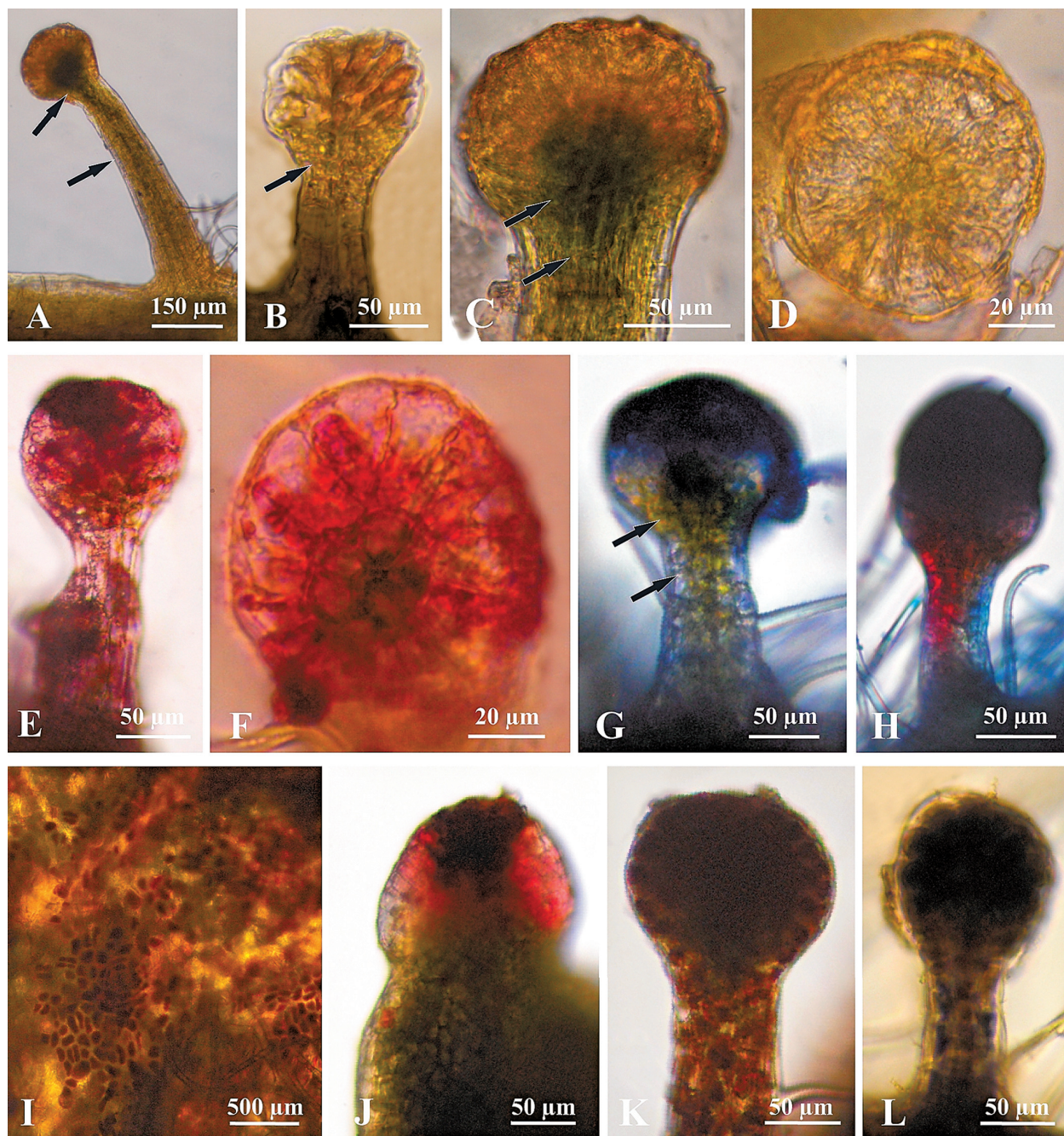


Fig. 5. Glandular trichomes after histochemical tests.

A–D – Sudan III. A – Long trichome (type B). B – Short trichome (type A). C – Head of a long trichome and a section of the stalk. D – Head of a short trichome in dorsal view.

E–F – Neutral red. E – Short trichome. F – Head of a short trichome in dorsal view.

G–H – Nile blue. G – Younger trichome. H – Older trichome.

I–K – Potassium dichromate. I – Section of abaxial epidermis. J – Gland of a sepal tooth. K – Short trichome.

L – Ferric trichloride. Short trichome.

The arrows in Fig. A–C and G indicate chloroplasts.

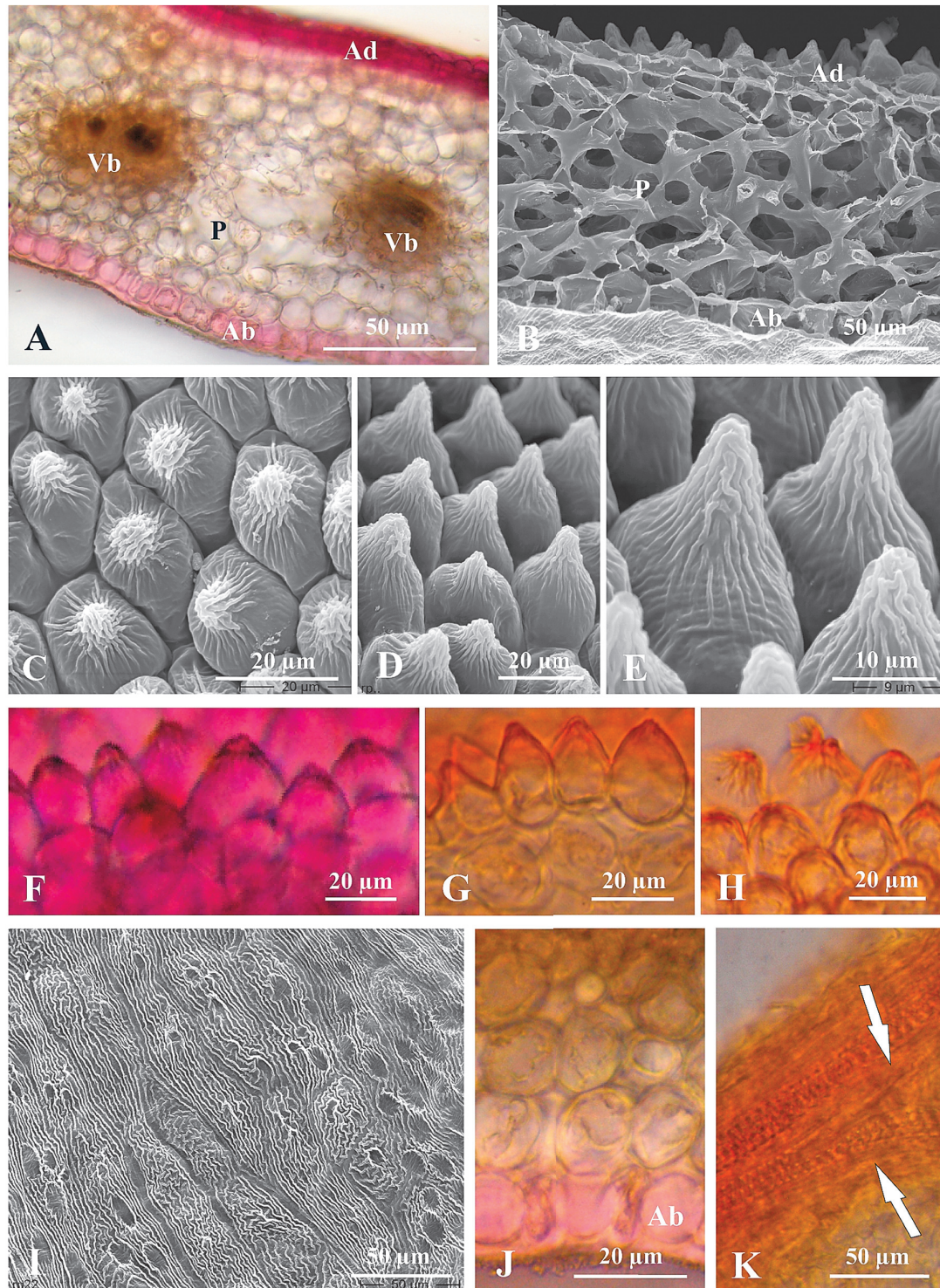


Fig. 6. Morphology and anatomy of *R. rugosa* petals.

A, B – Cross sections of petals. Ad – adaxial surface,
Ab – abaxial surface, P – parenchyma
Vb – vascular bundle.

C–E – Sections of the adaxial surface of a petal with visible papillae.

F–H – Sections of the adaxial surface of a petal after histochemical tests;

F – Neutral red, G, H –Sudan III.

I – Section of the abaxial surface of a petal viewed by SEM.

J – Cross section of a petal with the visible lower epidermis (Ab) (without staining).

K – Cross section of a petal treated with Sudan III with visible xylem elements (arrows).

DISCUSSION

On the sepals of *R. rugosa*, we found different sized glandular trichomes with a structure typical of colleters, which are characterized by a multi-row stalk and a multicellular head. Colleters have been described in various plant species. They are produced on bud scales in many woody plants, e.g. *Aesculus* [15,16]. In *Aesculus hippocastanum*, Chwil et al. [17] found colleters to occur also on the ovary of the pistil. Colleters are also present on bud scales and young leaves in *Alnus* [18]. These trichomes give off a sticky substance containing terpenes and mucilages that provide protection for the buds. Colleters were also found on the stipule in *Pentas* (Rubiaceae) and 72 ingredients, among others tannins and phenolic acids, were revealed in their extract [19].

Dobson et al. [12] found that the scent of the *R. rugosa* sepals was determined by the presence of glandular trichomes and that it may have a defensive role, in particular at the bud stage when the sepals perform a protective role for the other parts of the flower.

Our study demonstrates that the glandular trichomes of *R. rugosa*, which are found on the sepals and on the epidermis of the petals, and the parenchyma contain polyphenols. The content of these substances at different flower development stages of *R. rugosa* was studied by Youwei and Yonghong [7]. These authors found the highest polyphenol content and antioxidative activity at the bud stage when the petals are still colorless or are beginning to take on the pink color. Karabourniotis and Easseas [20] report that at the initial leaf development stages the polyphenol-containing trichomes may perform a protective role against UV-B radiation damage. Other authors claim that the secretory trichomes may be a defense against insects. Some substances secreted by trichomes are poisonous for this group of animals, while some other ones can inhibit their growth [21–23].

The histochemical tests used in our study also enabled the detection of tannins in the sepals (trichomes, parenchyma) and in the petals of *R. rugosa*. Kamijo et al. [24] showed tannins in *R. rugosa* to have antibacterial activity against 10 pathogenic bacteria. Dobson et al. [12] found sesquiterpenes to be dominant in the essential oils secreted by the sepals of *R. rugosa*. As reported by Harborne [25] and Kohlmeüner [26], these compounds can play an important role in the protection of plants against pests, inhibiting the growth and development of their larvae. In our research, we also found the presence of flavonoids in the glandular trichomes and papillae of *R. rugosa*. Flavonoids are the most important plant pigments for flower coloration and they act as attractants to pollinators and symbionts. Moreover, flavonoids

perform the role of sunscreens to protect against UV irradiation, as allelochemicals, and as antimicrobial and antiherbivory factors [27].

The trichomes observed on the sepals of *R. rugosa* had different colors in the successive development stages, from green to intensely red. Secretion was seen at their apex. According to Esau [15], the secretion is released onto the surface of the colleters by rupturing the unstretched cuticle. In *R. rugosa*, the glandular trichomes remained on the sepals also during fruit ripening. However, Esau [15] reports that the colleters occurring on young leaves dry out after the leaf blades open up.

Regardless of the size of the trichomes, we observed their different colors which came from, among others, the secretion contained in them. Green trichomes were found alongside hairs with an intensely red head even at the colored bud stage. This may be evidence of asynchronous maturation of the trichomes and their varying secretory activity. In our previous study, we found the papillae on the petals of *R. rugosa* to function asynchronously [28].

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Authors' contributions:

Concept of the study: EW-C; microscopical analysis: AS, EW-C; photographs: AS; writing of the manuscript: EW-C, AS.

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Charakterystyka struktur wydzielniczych w kwiatach *Rosa rugosa* Thunb.

Streszczenie

Kwiaty *Rosa rugosa* Thunb. z uwagi na obecność metabolitów wtórnych wykazujących działanie farmakologiczne znalazły zastosowanie w tradycyjnej i ludowej medycynie. Pozyskiwany z nich olejek eteryczny zaliczany jest także do fitoncydów. Wykorzystując mikroskopię świetlną i skaningową elektronową badano cechy morfologiczno-anatomiczne włosków gruczołowych zlokalizowanych na działkach kielicha *R. rugosa*. Przy użyciu testów histochemicznych określono typ wytwarzanej we włoskach wydzieliny, a także porównano jej zawartość z wydzieliną produkowaną przez papille płatków korony.

Stwierdzono, że w epidermie odosiowej działek kielicha zlokalizowane są wielokomórkowe włoski gruczołowe posiadające cechy koleterów oraz włoski mechaniczne, natomiast w epidermie doosiowej usytuowane są tylko włoski mechaniczne. Komórki trzonka włosków gruczołowych ułożone są wielorzędowo, zaś komórki epidermy główki mają układ promienisty. Główkowate trichomy zaklasyfikowano do dwóch typów: włosków krótkich i długich. Największe zagęszczenie włosków gruczołowych zarejestrowano w epidermie odosiowej bazalnej i środkowej części działek kielicha. We wczesnych fazach rozwoju pąków włoski wydzielnicze miały zielone zabarwienie, zaś w kolejnych stadiach rozwojowych stopniowo zmieniały barwę na czerwoną. Zastosowane testy histochemiczne pozwoliły stwierdzić, że trichomy na działkach kielicha i papille płatków korony produkują substancje lipidowe, polifenole, taniny oraz flawonoidy. Natomiast jedynie seskwiterpeny zarejestrowano tylko w wydzielinie włosków gruczołowych działek kielicha.

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