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Original Scientific Paper

The anatomy and essential oil composition of the different organs of *Pimpinella tragium* and *P. saxifraga* (Apiaceae)

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ABSTRACT:
This work was aimed at investigating the anatomy and composition of the essential oils from different organs of two *Pimpinella* species, *P. tragium* and *P. saxifraga*, originating from the central part of the Balkan Peninsula. The results of the analysis of the anatomy of the different organs of *P. tragium* and *P. saxifraga* showed that they were very similar in terms of the structure of the roots, stems and petioles. The roots showed a secondary structure and the stems had a primary structure with closed collateral vascular bundles. The petioles were cordatum, ribbed with an adaxial groove, and sparsely covered with short uni- or bicellular non-glandular trichomes with a pointed top (both species) or with long uniseriate, multicellular, non-glandular trichomes (*P. saxifraga*). Differences were observed in the anatomy of the leaves and in the indumentum of the fruits. The leaves of *P. tragium* were isobilateral, while those of *P. saxifraga* were dorsiventral. The *P. tragium* fruits were pubescent, covered with numerous, multicellular, uniseriate, straight or curved, gradually acuminate non-glandular trichomes, whereas the fruits of *P. saxifraga* were glabrous. These differences are of ecological and taxonomic significance. Secretory canals were observed in all the investigated organs of both species. The essential oils, isolated by hydrodistillation from the aerial parts with inflorescences, the roots from the flowering and fruiting period, and the fruits of both species, were analysed by GC-FID/MS. Although *P. tragium* and *P. saxifraga* essential oils showed certain differences in qualitative and quantitative patterns, all the oils were characterised by the presence of pseudoisoeugenol type phenylpropanoid epoxy-pseudoisoeugenyl-2-methylbutyrate, as well as by that of trinorsesquiterpenes pregeijerene, geijerene and trinoranastreptene. In addition, azulenes were present in all the root essential oils, whereas in the majority of the oils from the fruits and flowering aerial parts the common compound was the sesquiterpene β-bisabolene. The occurrence of phenylpropanoids of pseudoisoeugenol type and geijerenes is important from the chemotaxonomic point of view and confirms them as chemical markers of the *Pimpinella* species analysed so far.

Keywords:
vegetative organs and fruit anatomy, secretory canals, trichomes, GC-FID/MS analysis

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INTRODUCTION

The genus *Pimpinella* L. belongs to the tribe Pimpinelleae Spreng. (Fernández Prieto *et al.* 2018), subfamily Apioideae Drude and family Apiaceae Lindl. It is divided into three sections: *Tragoselinum* (Mill.) DC., *Tragium* (Spreng.) DC. and *Reutera* (Boiss.) Benth. based on the colour of the petals and the presence or absence of trichomes on the fruits (Abebe 1992; Fernández Prieto *et al.* 2018).

This genus is represented by approximately 150 species distributed in Europe, Asia and Africa. These are annuals, biennials and perennials which usually grow in dry rocky places, rock crevices, meadows, mountain pastures and grasslands. There are 16 species of this genus in the flora of Europe, and nine in the flora of the Balkan Peninsula (Tutin 1968), of which six species are represented in the flora of the central part of the Balkans: *P. major* (L.) Hudson, *P. alpina* Host, *P. peregrina* L., *P. serbica* (Vis.) Bentham, *P. tragium* Vill. and *P. saxifraga* L. (Rohlena 1942; Nikolić 1973; Diklić & Nikolić 1986; Matevski 2005).

A number of plants of the *Pimpinella* genus are used for their medicinal properties, which are, in part, based on the presence of the essential oils which exhibit various pharmacological/biological effects (Sihoglu Tepe & Tepe 2015; Nasir & Yabalak 2021). The essential oils are localised in the secretory canals of plants of the genus *Pimpinella*, as in other Apiaceae species (Jančić & Lakušić 2017). The most well known species of this genus is *P. anisum* L. The fruit and fruit essential oil of *P. anisum* are used in phytotherapy as expectorants and for the treatment of certain gastrointestinal complaints (EMA/HMPC/321184/2012; EMA/HMPC/321185/2012). The roots of *P. saxifraga* and *P. major* were an official herbal drug ("*Radix Pimpinellae*") in earlier editions of German and Swiss pharmacopoieas, with expectorant, bronchosecretolytic and antiphlogistic effects (Bohn *et al.* 1989). Other *Pimpinella* species are used in traditional medicines in different parts of the world for the treatment of various diseases, such as asthma, bronchitis, menstrual and gastrointestinal disorders, persistent cough, colds and constipation (Sihoglu Tepe & Tepe 2015).

The data published so far on the anatomy of the plants of the genus *Pimpinella* are mainly related to the anatomic structure of the fruits (Liu *et al.* 2006; Khajepiri *et al.* 2010; Akalin *et al.* 2016; Kljuykov *et al.* 2021) which represent a significant taxonomic character, not only for the species of this genus, but also for the entire Apiaceae family. On the other hand, the anatomical properties of the vegetative organs of *Pimpinella* species has rarely been investigated so far (Abebe 1992; Yurtseva & Tikhomirov 1998; Marchyshyn *et al.* 2015). Thus one of the goals of this work was the anatomical analysis of the roots, stems, leaves and petioles of *P. tragium* and *P. saxifraga*. The fruits of these two species were also included in the anatomical analysis.

While the chemical composition of the essential oils of *P. tragium* originating from Turkey (Tabanca *et al.* 2006), Sicily (Maggio *et al.* 2013) and Iran (Askari & Sefidkon 2005; Hosseini *et al.* 2014) and *P. saxifraga* from Germany and Italy (Kubezcka *et al.* 1989), Iran (Massoudi *et al.* 2009), Turkey (Tabanca *et al.* 2006) and Tunisia (Ksouda *et al.* 2019) have been analysed, the essential oils of these plants originating from the central part of the Balkan Peninsula have not yet been investigated. In that context, the present study aimed to investigate the content and composition of the essential oils of the flowering aerial parts, the roots collected during the flowering and fruiting stage, and the fruits of *P. tragium* and *P. saxifraga* collected from different localities in Serbia and North Macedonia. Furthermore, the results of the chemical analysis will provide insight into the potential for further studies, especially with regard to the bioactivity of essential oils. The obtained results on anatomy and essential oil composition will be discussed in relation to their biological significance.

MATERIALS AND METHODS

Plant material. The plant material of *P. tragium* was collected in the village of Izvor (near Bosilegrad, Southeast Serbia) and on Mt. Galičica (Southwest North Macedonia), whereas *P. saxifraga* was collected in southeast Serbia at the localities of Bojanine vode (Mt. Suva Planina) and Ostrovica (Sićevo Gorge). The plants were gathered in two phenological stages, in the flowering and fruiting stage. The voucher specimens are deposited in the Herbarium of the Department of Botany, University of Belgrade - Faculty of Pharmacy (HFF) (Table 1).

Anatomical analysis. The collected plant material was fixed in 50% ethanol. Anatomical sections of the roots, stems, leaves, petioles and fruits were carried out on permanent and temporary slides. Permanent slides were prepared by the standard method for light microscopy observation. Cross-sections of the vegetative organs and fruits were cut on a Reichert sliding microtome (10–15 μm thick). The sections were cleared in Parazone (NaClO) and thoroughly washed before staining in safranin solution (1% w/v in 50% ethanol) and alcian blue solution (1% w/v in water). After that, the cross-sections were passed through a series of ethanol solutions of increasing concentration (50%, 70%, 96% and absolute ethanol). All of the slides were mounted in Canada balsam after dehydration. The temporary slides were stained with a general reagent according to Tucakov (Kundaković *et al.* 2017). The anatomical sections were analysed on a Light Microscopy system (LM) with triocular tubus, BX41, with an Olympus colour camera for Light Microscopy SC30.

Table 1. Collection data, essential oils (EO) yield and colour.

Species	Collection site	Voucher number	Plant part	EO yield, %, (w/w)	EO Colour
P. tragium	Village Izvor (near to Bosilegrad, Southeast Serbia)	3783 HFF	Root (flowering)[1]	0.3	Dark blue
			Root (fruiting)[2]	0.8	Dark blue
			Flowering aerial part	0.2	Dark green
			Fruit	0.8	Light yellow
	Mt. Galičica (Southwest of North Macedonia)	3885 HFF	Root (flowering)	1.1	Dark blue
			Root (fruiting)	1.0	Dark blue
			Flowering aerial part	0.6	Dark bluegreen
			Fruit	0.9	Bluegreen
P. saxifraga	Bojanine vode - Mt. Suva Planina (Southeast Serbia)	3733 HFF	Root (flowering)	0.4	Dark blue
			Root (fruiting)	0.5	Dark blue
			Flowering aerial part	0.1	Dark green
			Fruit	1.5	Olive green
	Ostrovica - Sićevo Gorge (Southeast Serbia)	3734 HFF	Root (flowering)	0.2	Dark blue
			Root (fruiting)	0.7	Dark blue
			Flowering aerial part	0.2	Olive green
			Fruit	1.5	Yellow

[1] Collected during the flowering period
[2] Collected during the fruiting period

Isolation of the essential oils. The essential oils were isolated from air-dried plant material: the aerial parts with inflorescences, the roots from the flowering and fruiting period, and the fruits of both species. The flowering aerial parts were cut, whereas the fruits and roots were ground in an electric grinder and subjected to hydrodistillation for 2.5 h, using a Clevenger-type apparatus, with *n*-hexane as a collecting solvent. The essential oil yields and colours are given in Table 1.

GC-FID/MS analysis. Gas chromatographic analysis (GC-FID/MS) was performed on an Agilent 6890N gas chromatograph equipped with a flame-ionization detector (FID) and Agilent 5975C MS detector, using a capillary column HP-5MS (30 m × 0.25 mm i.d., film thickness 0.25 μm). The essential oil samples were dissolved in hexane (1%) and 1 μl was injected in split mode (10:1). The carrier gas used was He with a constant flow rate of 1.0 mL/min, the injector temperature was 200°C and the oven was linearly heated from 60°C to 280°C at a rate of 3°C/min. The transfer line temperatures were set at 250°C for MS and 300°C for FID. EI mass spectra (70 eV) were acquired over the *m/z* range of 35-550.

The constituents of the essential oils were identified by comparing their retention indices (RI) and mass spectra with those from NIST/NBS, Wiley libraries and the literature (Jordan *et al.* 1986; Kubezcka *et al.* 1989; Tabanca *et al.* 2003; Adams 2007). The linear RIs were determined in relation to a homologous series of *n*-alkanes (C_8-C_{40}) run under the same operating conditions (Adams 2007). The quantitative analysis was based on the calculation of the peak areas obtained from the FID data.

RESULTS AND DISCUSSION

The root anatomy of *P. tragium*. The root showed a secondary structure, with a well-devoloped periderm at the surface of the bark. The bark was dominated by parenchyma spherical cells with intercellular spaces between them. The sieve elements of the phloem alternated along the radius with the ray parenchyma cells (Fig. 1A). Radially distributed zones of xylem parenchyma could be observed in the wood between the tracheal elements (Fig. 1B).

Numerous, large secretory canals, surrounded by a single-layered glandular epithelium, were found both in the parenchyma and in the phloem of the bark (Fig. 1A), while they were absent in the xylem of the wood (Fig. 1B).

The root anatomy of *P. saxifraga*. The root structure, position and number of secretory canals in the *P. saxifraga* root (Figs. 1C & D) were similar to those of *P. tragium*. In the root parenchyma of *P. saxifraga*, starch grains were more abundant than in the root of *P. tragium* (Fig. 1D). In the wood, vessels predominated over other xylem elements (Fig. 1C).

The stem anatomy of *P. tragium*. In cross-section, the stem was slightly ribbed. It had a primary structure with closed collateral vascular bundles (Fig. 2A). The epidermis was unilayered with a thickened outer cell wall and a thin-layered cuticle on its surface. The subepidermal part of the primary cortex consisted of collenchyma, chlorenchyma and parenchyma. Groups of collenchyma cells were found in the ribs, while single-layered collen-

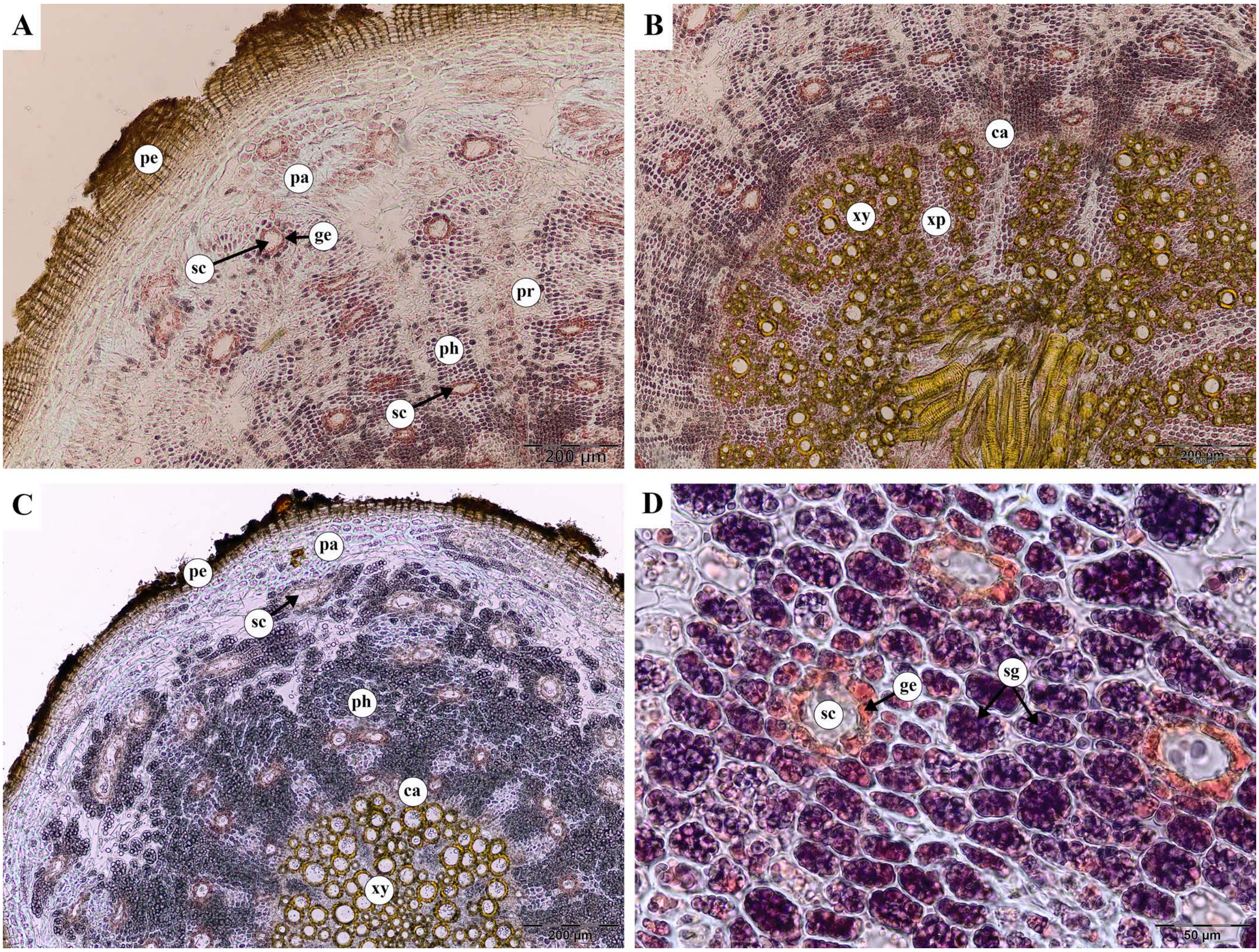

Fig. 1. Cross section of the root. A, B - *P. tragium*; C, D - *P. saxifraga*. Abbreviations: pe - periderm; pa - parenchyma; ph - phloem; pr - parenchyma rays; sc - secretory canal, ge - glandular epithelium, sg - starch grains; ca - cambium; xy - xylem; xp - xylem parenchyma.

chyma extended along the grooves of the stem. Chlorenchyma alternated with collenchyma cells in the ribs. The cells of the chlorenchyma were slightly elongated and placed perpendicular to the surface of the stem. The cortex terminated with multi-layered parenchyma whose cells were spherical in shape and larger than the cells of the chlorenchyma. Secretory canals surrounded by a single layer of glandular epithelium were present in the parenchyma of the cortex, mostly below the ribs (Fig. 2B).

The central cylinder started with a pericycle consisting of sclerenchyma and parenchyma. Isolated smaller or bigger groups of sclerenchyma fibers, connected only to the phloem (Fig. 2B) below the stem ribs, alternated with parenchyma along the stem grooves. Large closed collateral vascular bundles alternated with smaller ones forming a ring. The xylem parts of the bundles were connected by sclerenchyma fibers (Fig. 2A).

The central part of the stem was comprised of large, round parenchyma cells of pith (Fig. 2A). Secretory canals were present in the inner part of the primary cortex under the collenchyma (Fig. 2B) or chlorenchyma, as well as in the phloem of the vascular bundles (Fig. 2B) and in the pith of the central cylinder (Fig. 2A).

The stem anatomy of *P. saxifraga*. In cross-section, the stem was terrete, smooth (the population of Mt. Suva Planina, Fig. 2C) or slightly grooved (the population of Sićevo Gorge, Fig. 2D). It also had a primary structure with closed collateral vascular bundles.

As in the stem of *P. tragium*, the epidermis was unilayered with thickened outer cell walls and a thin cuticle layer on the surface. Rare, non-glandular, multicellular, uniseriate, cuneate trihomes were noted on the epidermis of the stem (Fig. 2D).

Collenchyma, chlorenchyma and parenchyma were present in the primary cortex. Groups of collenchyma cells formed weakly pronounced ribs, and chlorenchyma alternated with collenchyma (Fig. 2D). The chlorenchyma cells were spherical and small, while the multi-lay-

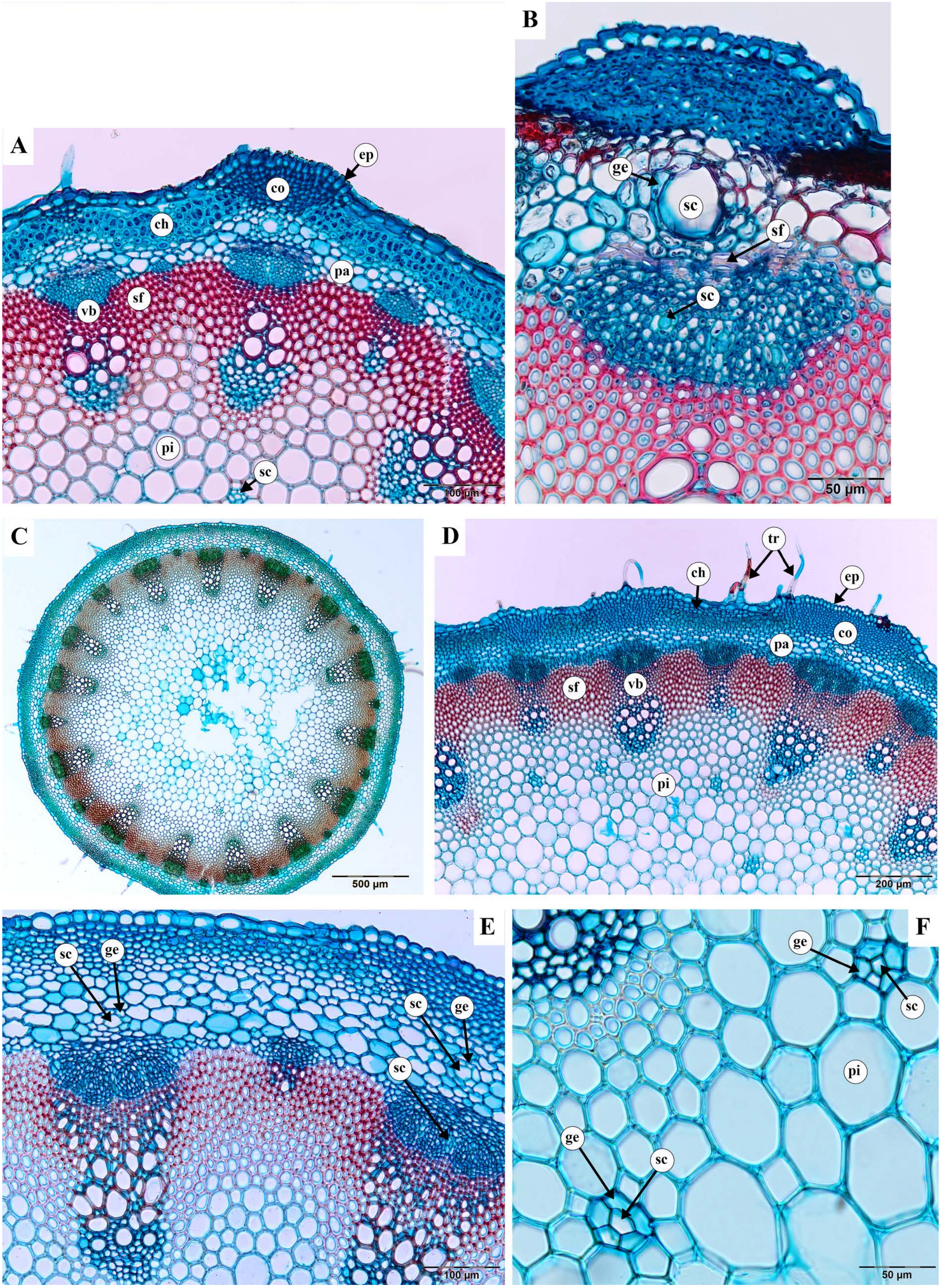

Fig. 2. Cross section of the stem. A, B - *P. tragium*; C-F - *P. saxifraga*. Abbreviations: ep - epidermis; co - colenchyma; ch - chlorenchyma: pa - parenchyma; vb - vascular bundle; sf - sclerenchyma fibers; pi - pith; sc - secretory canal; ge - glandular epithelium; tr - trichomes.

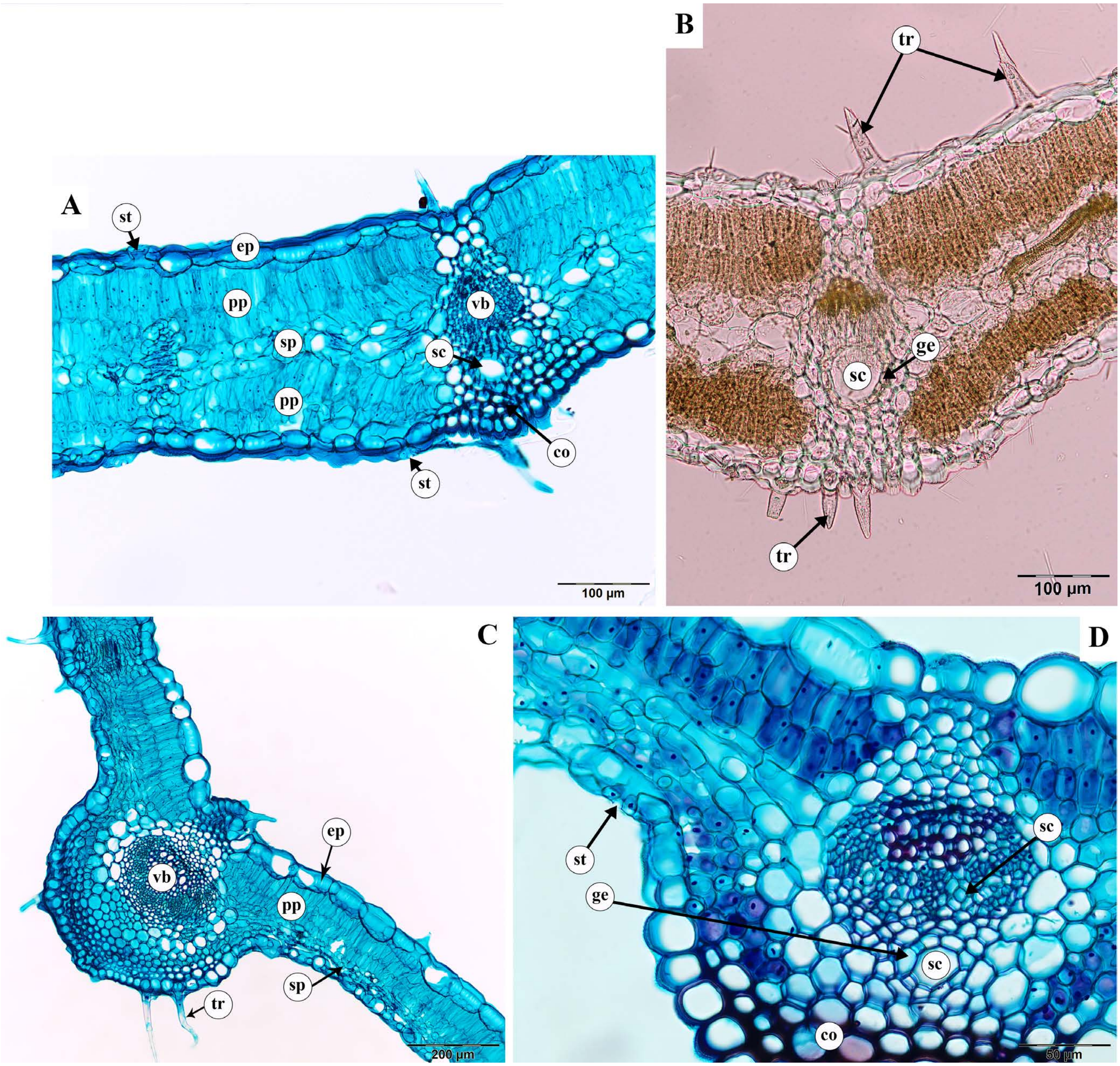

Fig. 3. Cross section of the leaf. A, B - *P. tragium*; C, D - *P. saxifraga*. Abbreviations: ep - epidermis; st - stomata; pp - palisade parenchyma; sp - spongy parenchyma; vb - vascular bundle; co - collenchyma; sc - secretory canal; ge - glandular epithelium; tr - trichomes.

ered parenchyma cells were spherical and larger than the cells of the other tissues of the cortex. Secretory canals surrounded by a single layer of glandular epithelium were observed in the cortical parenchyma (Fig. 2E).

The pericycle in the central cylinder was not clearly differentiated. The arrangement of the vascular bundles, sclerenchyma fibers and the secretory canals in the vascular bundles, phloem and pith (Figs. 2C-F) were the same as in the *P. tragium* stem. In the vascular bundles, phloem, tracheids, vessels, xylem parenchyma and libriform fibers were located at the same radius and alternated tangentially (Fig. 2E).

The leaf anatomy of *P. tragium*. The leaves were isobilateral (Fig. 3A). The epidermis, both adaxial and abaxial, was unilayered. The epidermal cells were isodiametric, approximately of the same size on both the adaxial and abaxial surfaces, with thickened outer cell walls and a thin cuticle layer. The leaves were amphistomatic. Non-glandular trichomes were rare, short, uni- or bicellular with a pointed top (Fig. 3B).

The leaf mesophyll consisted of two layers of palisade parenchyma on both the adaxial and abaxial sides. Between the palisade layers a two-layered spongy parenchyma made up of spherical cells was also present (Figs. 3A & B).

The leaf vascular system consisted of the dominant central vein and lateral, less developed veins. Along with the vascular bundle of the central vein and larger lateral vascular bundles, the collenchyma was visible under the upper and lower epidermis (Fig. 3A).

Secretory canals were present in the parenchyma and phloem of the central and prominent lateral veins (Figs. 3A & B) and sporadically in the spongy parenchyma.

The leaf anatomy of *P. saxifraga*. The leaves were dorsiventral (Fig. 3C). The epidermis, both adaxial and abaxial, was unilayered, consisting of isodiametric cells, larger on the adaxial than on the abaxial side, with thickened outer cell walls and a thin cuticle layer on both sides. The leaves were hypoamphistomatic. The non-glandular trichomes were uniseriate, uni- or bicellular with a pointed top, and were more numerous in the zones of more prominent veins (Fig. 3C).

The leaf mesophyll was differentiated on two layers of palisade parenchyma on the adaxial surface and two layers of spongy parenchyma on the abaxial surface. The palisade parenchyma cells were cylindrical with narrow intercellular spaces, while those of the spongy parenchyma were small and spherical with larger intercellular spaces. The ratio of palisade and spongy parenchyma in the leaf was 2:1 (Fig. 3C).

The leaf vascular system consisted of the dominant central vein and lateral, less developed veins. The collenchyma could be observed under the epidermis in the zone of the central and large lateral veins (Fig. 3D).

Secretory canals were rare, localised in the parenchyma and phloem of the central vein (Fig. 3D).

Based on the differentiation of the mesophyll, the leaves of both species were xeromorphic. The leaf epidermis of both species had thickened outer cell walls. The mesophyll of the *P. tragium* leaves consisted of four compact layers of palisade parenchyma subdivided by a two-layered spongy parenchyma. Such a structure of the leaves is an ecological adaptation to the environmental conditions, i.e. it ensures the survival of the plants in open, warm mountain pastures, limestone rocky areas and screes. Yurtseva & Tikhomirov (1998) obtained similar results in their study on different ecotypes of the *P. tragium* species. In the dorsiventral leaves of *P. saxifraga*, the palisade tissue was more strongly developed compared to the spongy one, which represents another type of xeromorphosis which may be associated with the ecological conditions in open sunny meadows, pastures, scrub and rocky places.

The petiole anatomy of *P. tragium*. The petiole was cordatum, ribbed with an adaxial groove, sparsely covered with short uni- or bicellular non-glandular trichomes with a pointed top (Fig. 4A). The epidermis was uni-layered with thickened outer cell walls and covered with a thin cuticle layer (Fig. 4B).

In the subepidermal part of the cortex, collenchyma strands were present in the ribs, and multi-layered chlorenchyma along the grooves. Multi-layered parenchyma were observed on the inner side of the chlorenchyma (Fig. 4B). The five marginal closed collateral vascular bundles were arranged in an arc; each in the zone of the prominent ribs (Fig. 4A). Along with the phloem and xylem of the vascular bundles, sclerenchyma fibers, bast and libriform fibers were also present (Fig. 4C). The central tissue of the petal was comprised of parenchyma.

Secretory canals were present in the cortex, below the collenchyma, and in the phloem of the vascular bundles (Figs. 4B & C).

The petiole anatomy of *P. saxifraga*. The petiole of *P. saxifraga* was also cordatum, ribbed with an adaxial groove, and sparsely covered with short uni- or bicellular and long uniseriate, multicellular, non-glandular trichomes with a pointed top (Fig. 4D).

The structure of the epidermis, the arrangement of tissues in the cortex (Fig. 4E) as well as the number and position of vascular bundles (Fig. 4D) were identical to those of *P. tragium* petiole.

Secretory canals were present below the collenchyma, in the phloem of the vascular bundles and also in the central parenchyma closer to the xylem (Figs. 4E & F).

The fruit anatomy of *P. tragium*. The mericarps were homomorphic, semicircular, not compressed (terete) and pubescent (Fig. 5A). The non-glandular trichomes were numerous, multicellular, uniseriate, straight or curved, gradually acuminate (Fig. 5B). The dorsal rib and lateral ribs were slightly prominent (obsolete). The marginal ribs were broader than the dorsal and lateral ones (Fig. 5A). The unilayered exocarp consisted of isodiametric small cells with thin walls. The mesocarp was multi-layered, comprising larger, non-lignified parenchymatous cells (Fig. 5B), with five vascular bundles. Four vallecular vittae were present in each furrow, and were generally larger than the vascular bundles. Four commissural vittae were located between the commissural bundles (Fig. 5A). The endocarp was unilayered, and composed of non-lignified, thin-walled parenchyma cells (Fig. 5B).

The fruit anatomy of *P. saxifraga*. The mericarps were homomorphic, almost round, not compressed (terete) and glabrous (Fig. 5C). The shape of the dorsal rib, lateral and marginal ribs (Fig. 5C), as well as the structure of the exocarp, mesocarp and endocarp (Fig. 5D) were very similar, and the number of commissural vittae and vascular bundles (Fig. 5C) were the same as in the fruit of *P. tragium*. Three vallecular vittae, which were larger than the vascular bundles, were present in each furrow (Fig 5C). Parenchyma cells with fatty oil drops were clearly visible in the endosperm of the seed (Fig. 5E).

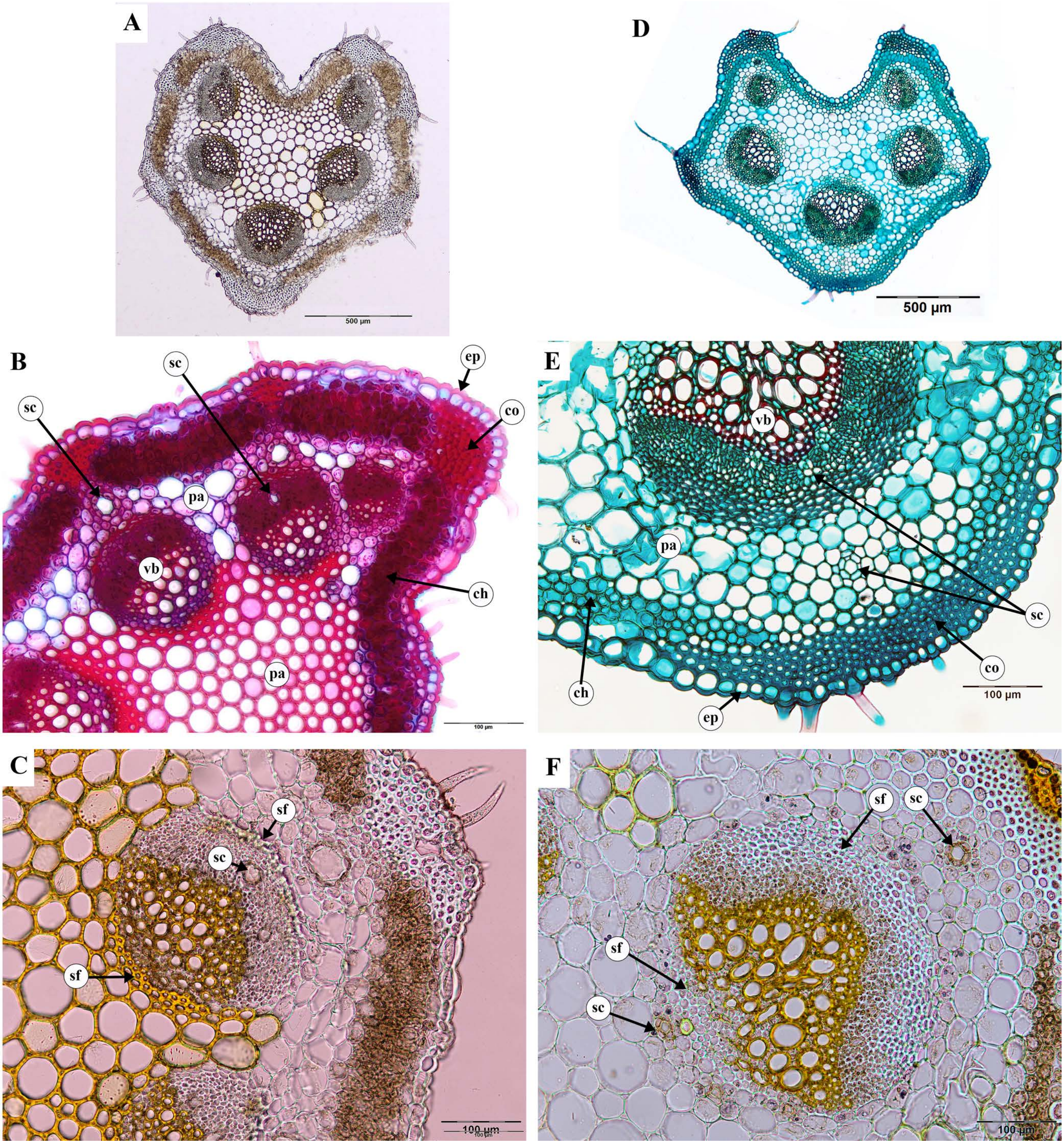

Fig. 4. Cross section of the petiole. A-C - *P. tragium*; D-F - *P. saxifraga*. Abbreviations: ep - epidermis; co - collenchyma; ch - chlorenchyma; pa - parenchyma; vb - vascular bundle; sf - sclerenchyma fibers; sc - secretory canal.

The mericarps of both species were similar in shape and structure. Significant differences were observed in the number of vallecular vittae and the presence or absence of trichomes. The mericarps of *P. tragium* were pubescent with numerous multicellular, uniseriate, straight or curved, gradually acuminate non-glandular trichomes. In contrast, the mericarps of *P. saxifraga* were glabrous. This is one of the main features on the basis of which species of the genus *Pimpinella* are classified into sections. *Pimpinella tragium* is a member of section *Tragium* and *P. saxifraga* of section *Tragoselinum*.

The results of this research pertaining to the anatomy of the vegetative organs and fruits of *P. tragium* and *P. saxifraga* are in accordance with previously pub-

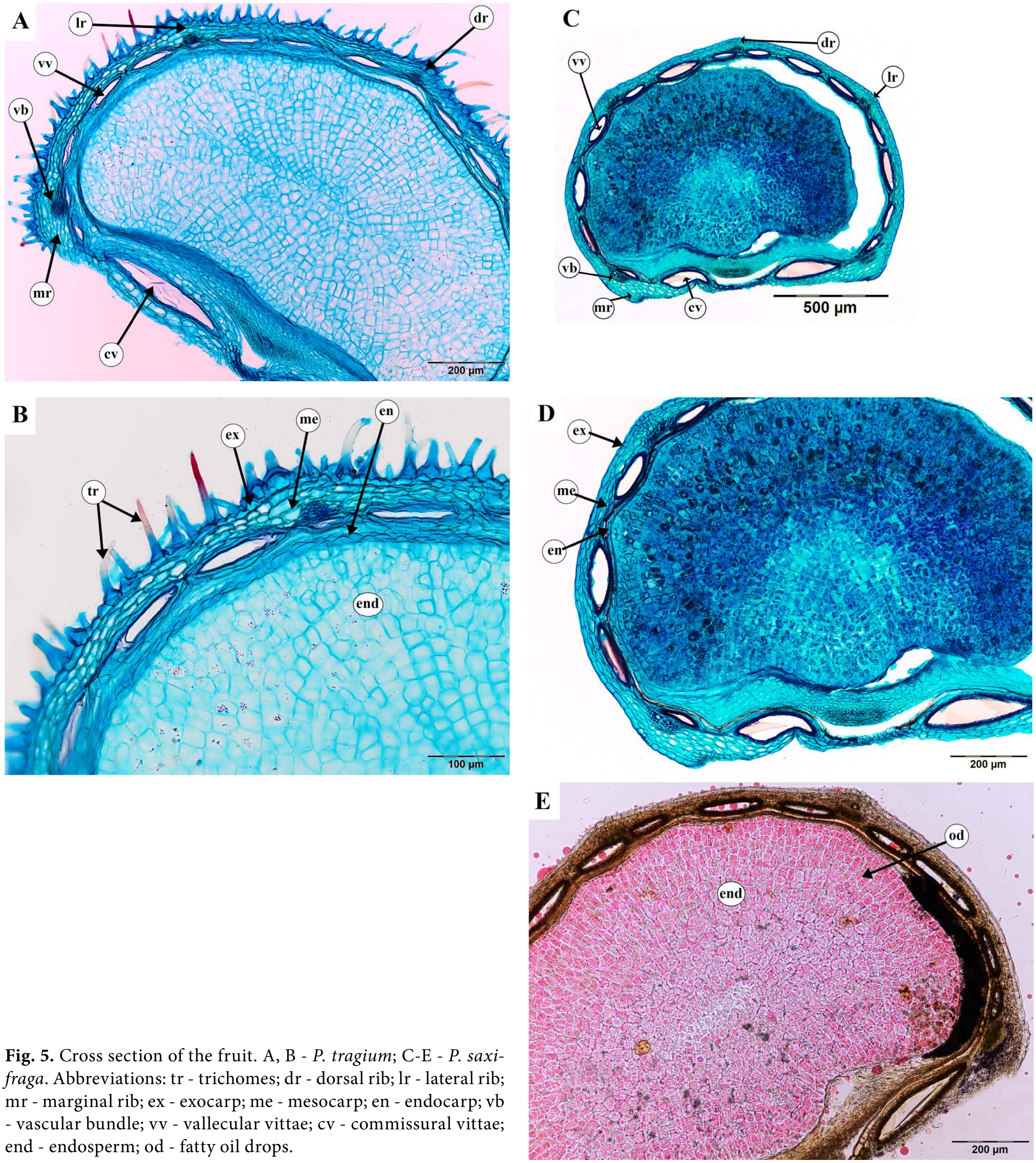

Fig. 5. Cross section of the fruit. A, B - *P. tragium*; C-E - *P. saxifraga*. Abbreviations: tr - trichomes; dr - dorsal rib; lr - lateral rib; mr - marginal rib; ex - exocarp; me - mesocarp; en - endocarp; vb - vascular bundle; vv - vallecular vittae; cv - commissural vittae; end - endosperm; od - fatty oil drops.

lished data related to the general structure of plants of the genus *Pimpinella* (Abebe 1992; Khajepiri *et al.* 2010; Akalin *et al.* 2016; Nasir & Yabalak 2021), as well as with those related to individual species (Yurtseva & Tikhomirov 1998; Marchyshyn *et al.* 2015).

Essential oil yield and chemical composition. The essential oil yields (w/w) of *P. tragium* ranged from 0.2–0.6% in the flowering aerial parts, 0.3–1.1% in the roots in the flowering stage, and 0.8–0.9% in the fruits and 0.8–1.0% in the roots from the fruiting stage, whereas for *P. saxi-*

fraga the highest essential oil content was determined in the fruits (1.5%), followed by the roots from the fruiting period (0.5–0.7%), while the roots from the flowering period (0.2–0.4%) and the aerial flowering parts contained smaller amounts (0.1–0.2%) of essential oil (Table 1). The essential oils from the roots in both stages of both species were deep blue, whereas those from the flowering aerial parts and fruits were bluegreen or various shades of green, with the exception of the fruit oil of *P. tragium* from Bosilegrad, which was light yellow and the yellow coloured fruit oil of *P. saxifraga* from Sićevo Gorge.

The results of the qualitative and quantitative analysis of the essential oils are given in Table 2. In total, 76 compounds were identified constituting 89.2–99.8% of the total oils.

In the *P. tragium* essential oils, C-12 norsesquiterpenes (trinorsesquiterpenes) (44.9–88.5%) accounted for the predominant class of compounds except for the fruit essential oil from one locality (Bosilegrad), in which the fraction of sesquiterpene hydrocarbons (59.7%), almost entirely composed of β-bisabolene (57.2%), was the most represented.

The essential oils of the *P. tragium* roots from both stages, collected from both localities showed a similar qualitative and quantitative composition in terms of the main constituents. The main components of these oils, as well as of those from the flowering aerial parts and the fruits from Mt. Galičica were C-12 norsesquiterpenes pregeijerene (29.0–56.2%) and its thermal rearrangement product geijerene (14.1–22.9%). Although the essential oils of the aerial parts and fruits from Bosilegrad also contained pregeijerene (8.6–12.8%) and geijerene (3.3–7.5%), the most dominant compounds in these oils were β-bisabolene (19.1–57.2%) and the phenylpropanoid, epoxy-pseudoisoeugenyl-2-methylbutyrate (17.4–22.2%). Phenylpropanoids with the pseudoisoeugenol skeleton (mainly represented by epoxy-pseudoisoeugenyl-2-methylbutyrate) were also present in prominent amounts in the essential oils of the roots (6.9–11.2%). In addition, the essential oils of the roots from Mt. Galičica contained remarkable amounts of azulenes in both stages of development, 4,10-dihydro-1,4-dimethylazulene (10.4–15.4%) and 1,4-dimethylazulene (5.9–6.3%), whereas in the essential oils from the roots from Bosilegrad they were also present but in lower amounts.

As for *P. saxifraga*, it was noted that the essential oils of the same organs from different localities have a similar qualitative composition with minor quantitative differences. In terms of the dominant class of compounds, norsesquiterpenes prevailed in the essential oils of the *P. saxifraga* roots from both phases and both localities (57.5–68.2%), as was found in the essential oils of the *P. tragium* roots. The essential oils of the flowering aerial parts and fruits of *P. saxifraga* from both localities were characterised by a higher content of sesquiterpene hydrocarbons (31.5–79.5%). The most common compounds in the essential oils of the roots, in both phases, were azulenes 4,10-dihydro-1,4-dimethylazulene (15.2–25.2%) and 1,4-dimethylazulene (10.5–16.2%) as well as the norsesquiterpene pregeijerene (13.7–18.8%). In the essential oils of the flowering aerial parts and fruits, the most dominant constituent was β-bisabolene, which was present in a significantly higher amount in the essential oils of the fruits (73.0–76.0%) compared to the essential oils of the aerial parts (28.9–29.1%). In addition, a significant amount of epoxy-pseudoisoeugenyl-2-methylbutyrate was present in all the *P. saxifraga* essential oils: 20.8–21.8% in the essential oils of the flowering aerial parts, 10.2–16.0% in the essential oils of the roots and 7.7–16.9% in those of the fruits.

Pseudoisoeugenol (2-hydroxy-5-methoxy-1-(E)-propenylbenzene) type phenylpropanoids are found to be unique to *Pimpinella* species and trinorsequiterpenes, pregeijerene and geijerene were also marked as characteristic compounds of these plants (Tabanca *et al.* 2005; Maggio *et al.* 2013).

The essential oils from all the roots were deep blue in colour due to the presence of azulenes, but it is also possible that pregeijerene as a bluegreen compound also contributes to their colour (Sadgrove *et al.* 2022). Geijerene and cogijerene, as green-coloured compounds (Sadgrove *et al.* 2022), together with pregeijerene are probably responsible for the different green colours of the *Pimipinella* essential oils, but considering that some of the analysed green oils have a low content of geijerenes, it can be assumed that some other compounds also affect the formation of the colour of the oils.

There are a number of reports in the literature on the chemical composition of the essential oils of *P. tragium* (Askari & Sefidkon 2005; Tabanca *et al.* 2006; Maggio *et al.* 2013; Hosseini *et al.* 2014) and *P. saxifraga* (Kubezcka *et al.* 1989; Tabanca *et al.* 2006; Massoudi *et al.* 2009; Ksouda *et al.* 2019) (Table 3).

C12-norsesquiterpenes were also present in significant amounts in some previously studied essential oils from various organs of *P. tragium* of different origin, with geijerene as the most dominant (Tabanca *et al.* 2006; Maggio *et al.* 2013; Hosseini *et al.* 2014), while in the oils examined in this study pregeijerene predominated, and geijerene was present in prominent amounts.

In contrast to our samples, certain reports on the essential oils of *P. tragium* record the main constituents as monoterpenes (α- or β-pinene) (Askari & Sefidkon 2005) or sesquiterpenes such as (*Z*)-β-farnesene (Tabanca *et al.* 2006) (Table 3).

In terms of the previously analysed essential oils of *P. saxifraga* (Table 3), the essential oils of the roots of *P. saxifraga* subsp. *nigra* (Miller) Gaudin from Germany and Italy (Kubezcka *et al.* 1989) were dominated by azulenes and pregeijerene or trinoranastreptene, which is similar to our samples.

Table 2. The chemical composition of *P. tragium* and *P. saxifraga* essential oils (%)[a]

		P. tragium								***P. saxifraga***							
		Bosilegrad				Mt. Galičica				Mt. Suva Planina				Sićevo Gorge			
Compound	**RI_{exp}**[b]	Root (fl)[c]	Root (fr)[d]	Aerial parts	Fruit	Root (fl)	Root (fr)	Aerial parts	Fruit	Root (fl)	Root (fr)	Aerial parts	Fruit	Root (fl)	Root (fr)	Aerial parts	Fruit
α-Pinene	933	-[e]	tr	0.2	tr[f]	tr	tr	0.1	tr	-	-	tr	tr	-	tr	tr	-
Sabinene	974	-	tr	tr	tr	tr	tr	tr	tr	-	-	tr	-	-	-	tr	tr
β-Pinene	978	-	-	tr	-	tr	tr	tr	-	-	tr	0.3	0.3	-	-	tr	tr
6-Methyl-5-hepten-2-one	991	-	-	tr	-	-	-	-	-	-	tr	tr	tr	-	-	tr	-
2-Pentylfuran	992	0.3	0.2	0.3	-	tr	tr	tr	tr	0.2	-	0.3	tr	0.2	tr	0.2	tr
n-Octanal	1005	0.4	0.1	tr	-	tr	tr	-	-	tr	tr	-	-	tr	tr	-	tr
p-Cymene	1025	0.3	0.4	tr	tr	0.3	0.4	0.1	0.1	tr	tr	tr	tr	tr	tr	tr	tr
Limonene	1029	tr	tr	1.6	0.1	-	-	0.1	0.4	-	-	tr	tr	-	-	tr	tr
(*Z*)-*β*-Ocimene	1036	tr	0.2	tr	0.1	0.1	0.1	tr	tr	-	tr	-	-	-	tr	-	tr
(*E*)-*β*-Ocimene	1047	tr	0.2	tr	0.3	0.2	0.5	0.1	0.1	tr	tr	tr	tr	tr	tr	tr	tr
γ-Terpinene	1059	0.5	0.5	tr	0.1	0.5	0.7	tr	0.2	tr	tr	tr	tr	tr	tr	tr	tr
Linalool	1099	-	-	0.5	-	-	-	0.2	tr	-	-	-	-	-	-	-	-
n-Undecane	1100	tr	0.2	-	tr	0.4	0.9	-	-	0.9	0.9	6.2	2.1	0.7	1.5	4.4	1.2
n-Nonanal	1103	0.8	0.2	tr	tr	-	-	-	-	tr	-	-	-	tr	tr	tr	-
Geijerene isomer	1137	1.0	1.3	0.5	0.3	1.1	1.2	1.9	1.9	0.4	0.6	tr	tr	0.4	0.6	tr	tr
Geijerene	1144	16.3	19.0	7.5	3.3	14.1	14.9	21.8	22.9	5.3	7.1	0.5	0.4	5.3	7.3	0.4	0.1
(2*E*)-Nonen-1-al	1161	tr	tr	-	-	tr	tr	tr	tr	tr	tr	tr	-	tr	0.1	tr	-
Trinoranastreptene	1205	3.2	3.2	2.8	0.7	5.0	5.0	0.6	0.2	6.5	5.3	12.9	5.5	3.2	6.3	14.2	1.3
Thymol methyl ether	1231	1.1	0.9	tr	-	1.0	1.0	-	-	tr	tr	-	tr	tr	tr	-	tr
Carvacrol methyl ether	1236	1.2	1.0	tr	-	1.0	1.3	-	-	tr	tr	-	tr	tr	0.1	-	tr
Isogeijerene C	1251	0.3	0.1	0.8	0.1	tr	tr	0.5	0.4	tr	tr	-	tr	tr	tr	-	
(2*E*)-Decenal	1265	0.9	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cogeijerene	1287	0.4	0.3	0.7	0.1	0.3	0.2	0.5	0.4	tr	tr	tr	tr	tr	tr	tr	-
Pregeijerene	1290	29.0	37.1	12.8	8.6	33.6	33.1	56.2	52.0	13.7	18.8	1.0	1.0	13.7	16.9	0.9	0.1
Tridecane	1300	tr	tr	-	-	-	-	-	-	-	-	tr	tr	-	tr	tr	tr
(2*E*,4*E*)-Decadienal	1320	1.0	0.7	tr	-	-	tr	-	-	0.9	-	tr	-	0.7	tr	tr	tr
δ-Elemene	1340	-	-	-	-	-	-	-	-	tr	0.1	tr	tr	tr	0.2	tr	tr
3,10-Dihydro-1,4-Dimethylazulene	1354	0.3	0.3	0.3	-	0.7	0.7	0.5	-	0.4	0.4	tr	tr	0.3	0.4	tr	-
α-Longipinene	1355	-	-	-	-	tr	tr	-	-	-	-	-	-	-	-	-	-
4,10-Dihydro-1,4-Dimethylazulene	1358	3.5	4.2	0.5	-	10.4	15.4	-	-	16.8	25.2	0.5	tr	15.2	21.9	0.4	-
(2*E*)-Undecenal	1361	0.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-Copaene	1378	tr	0.1	tr	tr	tr	tr	0.2	0.1	tr	tr	tr	tr	tr	tr	tr	tr
8-*epi*-Dictamnol	1383	2.8	0.7	7.6	0.4	0.3	0.2	2.0	1.4	2.0	0.2	1.9	0.1	1.6	0.2	1.5	0.1
β-Elemene	1394	tr	0.1	-	tr	0.1	0.1	tr	tr	0.3	0.2	tr	tr	0.5	0.5	tr	tr
Dodecanal	1412	-	-	-	-	-	-	-	-	-	-	tr	0.1	-	-	tr	0.1
(*E*)-Caryophyllene	1422	0.6	0.8	2.1	1.3	1.1	0.8	4.4	5.1	1.3	1.2	0.3	0.1	1.4	1.3	0.3	0.1
2,5-Dimetohoxy-*p*-Cymene	1429	tr	0.2	-	-	0.1	0.1	-	-	-	-	-	-	-	-	-	-
Dictamnol	1432	6.2	1.4	10.9	1.4	0.5	0.2	4.2	2.3	2.4	0.1	1.1	0.1	1.6	0.7	1.0	0.1
α-*trans*-Bergamotene	1436	-	-	-	tr	-	-	-	-	tr	tr	tr	0.1	tr	tr	tr	-

γ-Elemene	1438	tr	0.2	-	-	0.1	0.1	-	-	-	0.1	-	0.1	-	-	-	0.1
β-Barbatene	1444	tr	tr	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-
(Z)-β-Farnesene	1444	-	-	-	tr	-	-	0.5	-	-	-	-	tr	-	-	-	tr
1-methoxy-Naphthalene	1445	tr	tr	-	tr	tr	tr	-	-	tr	0.1	0.3	0.1	tr	tr	0.4	tr
α-Himachalene	1452	-	tr	-	-	0.1	tr	tr	tr	-	-	-	-	-	-	-	-
α-Humulene	1456	0.1	0.1	0.6	0.2	0.1	0.1	0.5	0.5	0.1	0.2	-	-	0.1	0.3	-	-
(*E*)-β-Farnesene	1458	tr	0.1	-	0.2	0.1	-	-	tr		0.1	0.7	0.7			0.9	0.6
γ-Muurolene	1483	-	-	-	-	-	-	tr	-	0.2	0.1	tr	0.1	0.5	0.3	tr	0.1
γ-Himachalene	1485	tr	0.4	tr	-	1.1	0.3	tr	0.2	-	-	-	-	-	-	-	-
Germacrene D	1488	0.3	0.5	0.4	0.3	1.6	1.4	0.5	0.4	0.9	0.8	0.4	0.5	1.2	1.3	0.4	0.1
α-Zingiberene	1497	-	-	-	-	1.5	1.2	tr	tr	0.7	0.8	tr	0.2	tr	0.3	tr	tr
4-*epi*-*cis*-Dihydroagarofuran	1503	0.5	0.4	-	-	0.2	0.1	-	-	tr	0.1	-	-	0.2	0.2	-	-
Bicyclogermacrene	1503	0.5	0.9	0.2	0.1	0.1	0.1	0.2	tr	-	-	0.1	0.2	-	-	0.3	0.1
β-Himachalene	1503	-	-	-	-	0.1	-	tr	tr	-	-	-	-	-	-	-	-
trans-β-Guaiene	1504	0.4	0.5	-	-	-	tr	-	-	6.6	4.4	0.7	1.0	10.4	10.8	0.7	0.4
β-Bisabolene	1510	6.8	5.4	19.1	57.2	0.5	0.4	0.5	3.1	3.5	3.8	28.9	76.0	5.9	3.3	29.1	73.0
β-Sesquiphellandrene	1526	-	-	-	0.1	0.3	0.3	-	-	0.5	0.5	tr	0.4	-	0.3	tr	0.3
δ-Cadinene	1526	tr	tr	tr	-	-	tr	0.2	0.1	-	-	-	-	0.3	-	tr	-
Kessane	1531	0.6	0.6	tr	-	0.3	0.1	tr	tr	0.3	0.2	-	-	0.3	0.2	-	-
β-Vetivenene	1557	-	-	-	-	-	-	-	-	tr	tr	tr	tr	0.7	tr	tr	tr
1,4-Dimethylazulene	1558	3.3	3.2	0.5	-	5.9	6.3	0.3	0.2	14.7	10.5	0.5	tr	16.2	11.5	0.4	-
Germacrene B	1563	2.6	3.6	1.4	0.3	3.7	3.1	0.6	0.3	0.7	2.6	0.4	0.1	0.3	0.4	tr	tr
Spathulenol	1580	0.6	0.1	0.8	0.1	-	-	tr	-	-	-	0.5	tr	-	-	0.8	0.1
Caryophyllene oxide	1586	0.4	tr	2.3	0.1	tr	tr	0.4	0.4	0.5	-	1.2	tr	0.5	-	0.9	0.1
Guaiol	1603	-	-	-	-	-	-	-	-	0.7	0.3	tr	0.1	1.0	0.6	tr	tr
β-Atlantol	1612	tr	-	0.4	0.1	-	-	-	tr	-	-	5.1	0.2	-	-	5.5	0.6
β-Himachalene oxide	1613	-	-	-	-	-	-	-	-	-	-	-	-	0.5	0.3	-	-
Foeniculin	1680	-	-	-	tr	-	-	-	-	0.3	0.2	0.8	0.3	0.2	0.1	0.5	0.2
Helifolenol D	1685	-	-	-	-	-	-	-	-	-	-	1.5	tr	-	-	1.5	0.2
α-Bisabolol	1688	-	-	tr	tr	-	-	0.1	0.3	-	-	0.2	tr	-	-	tr	tr
Cryptomerione	1727	-	-	0.6	0.1	-	-	-	tr	tr	-	0.7	tr	tr	-	1.0	0.2
epi-Cyclocolorenone	1778	-	-	-	-	-	-	-	-	0.2	tr	0.3	tr	0.3	0.2	0.3	0.1
(*E*)-Pseudoisoeugenyl-2-methylbutyrate	1844	0.3	0.2	0.8	0.8	0.2	0.1	tr	0.1	0.2	0.1	1.2	0.7	0.5	0.1	1.4	1.3
Epoxy-pseudoisoeugenyl-2-methylbutyrate	1900	11.2	8.8	17.4	22.2	10.6	6.9	0.8	2.0	16.0	10.2	20.8	7.7	13.6	10.4	21.8	16.9
Pseudoisoeugenyl angelate	1930	-	-	-	tr	tr	tr	tr	0.3	-	-	-	-	-	-	-	tr
Hexadecanoic acid	1965	-	-	-	-	-		-	-	-	2.8	-	-	-	0.4	-	-
Epoxypseudoisoeugenyl tiglate	1972	-	tr	-	-	2.4	2.5	0.7	2.7	-	-	-	-	-	tr	-	tr
Identified		98.6	98.7	93.6	98.6	99.8	99.8	98.7	98.1	97.2	98.0	89.3	98.2	97.5	99.0	89.2	97.5
Grouped components																	
Monoterpene hydrocarbons		0.3	0.8	1.8	0.5	0.6	1.0	0.4	0.6	tr	tr	0.3	0.3	tr	tr	tr	tr
Oxygenated monoterpenes		2.8	2.6	0.5	0.1	2.6	3.1	0.2	0.2	tr	tr	tr	tr	tr	0.1	tr	tr

Sesquiterpene hydrocarbons	11.3	12.7	23.8	**59.7**[g]	10.6	7.9	7.6	9.8	14.8	14.9	**31.5**	**79.5**	21.3	19.0	**31.7**	**74.8**
Oxygenated sesquiterpenes	2.1	1.1	4.1	0.4	0.5	0.2	0.5	0.7	1.7	0.6	9.5	0.3	2.8	1.5	10.0	1.3
Nor-sesquiterpenes	**66.3**	**70.8**	**44.9**	14.9	**71.9**	**77.2**	**88.5**	**81.7**	**62.2**	**68.2**	18.4	7.1	**57.5**	**65.8**	18.8	1.7
Phenylpropanes	11.5	9.0	18.2	23.0	13.2	9.5	1.5	5.1	16.2	10.3	22.0	8.4	14.1	10.5	23.2	18.2
Others	4.3	1.7	0.3	tr	0.4	0.9	tr	tr	2.3	4.0	7.6	2.6	1.8	2.1	5.5	1.5

[a] Relative area percentage; [b] Retention indices relative to C_8-C_{40} *n*-alkanes experimentally determined on the HP-5MS column; [c] Collected during the flowering period; [d] Collected during the fruiting period; [e] Not detected; [f] Trace (< 0.1%); [g] Dominant group of constituents

Table 3. The most abundant components of previously analysed *P. tragium* and *P. saxifraga* essential oils.

Taxon	Origin	Plant part	Main components	Reference
P. tragium	Iran	Fruits	geijerene (33.5%)	Hosseini *et al.* 2014
P. tragium	Iran	Seeds *	β-pinene (25.3%)	Askari & Sefidkon 2005
		Stems with leaves	germacrene D (34.7%)	
		Inflorescences	β-pinene (23.8%)	
P. tragium subsp. *lithophila* (Schischkin) Tutin	Turkey	Fruits	β-bisabolene (29.8%) geijerene (22.9%)	Tabanca *et al.* 2006
		Stems with leaves	geijerene (32.2%)	
		Roots	geijerene (26.7%) 4,10-dihydro-1,4-dimethylazulene (13.5%)	
P. tragium subsp. *polyclada* (Boiss. & Heldr.) Tutin		Fruits	(*Z*)-β-farnesene (57.3%) epoxy-pseudoisoeugenyl-2-methyl butyrate (20.0%)	
		Stems with leaves	(*Z*)-β-farnesene (22.6%) epoxy-pseudoisoeugenyl-2-methyl butyrate (20.0%) α-pinene (12.2%)	
		Roots	4-methoxy-2-(3-methyloxiranyl)-phenyl angelate (39.9%) epoxy-pseudoisoeugenyl-2-methyl butyrate (16.0%)	
P. tragium subsp. *pseudotragium* (DC.) Matthews		Fruits	α-pinene (15.7%) β-bisabolene (18.8%)	
		Stems with leaves	α-pinene (30.5%) β-bisabolene (5.7%) epoxy-pseudoisoeugenyl-2-methyl butyrate (5.7%)	
		Roots	4-methoxy-2-(3-methyloxiranyl)-phenyl angelate (30.7%) epoxy-pseudoisoeugenyl-2-methyl butyrate (18.6%)	
P. tragium subsp. *glauca* (C. Presl.) C. Brullo & Brullo	Italy (Sicily)	Leaves	geijerene (28.9%) *trans*-dictamnol (15.4%) pregeijerene (10.5%)	Maggio *et al.* 2013
		Stems	geijerene (49.3%) pregeijerene (19.1%)	
		Inflorescences	geijerene (48.4%) pregeijerene (18.6%)	

Pimpinella saxifraga subsp. *eusaxifraga* Thellung (with segmented leaves)	Germany	Roots	*trans*-epoxy-pseudoisoeugenyl-2-methyl butyrate (61.39%) germacrone (16.92%)	Kubeczka *et al.* 1989
Pimpinella saxifraga subsp. *eusaxifraga* (with dissected leaves)	Germany	Roots	*trans*-epoxy-pseudoisoeugenyl-2-methyl butyrate (56.43%) *trans*-ß-farnesene (10.91%)	
Pimpinella saxifraga subsp. *alpestris* (Sprengel) Vollman	Italy	Roots	*trans*-epoxy-pseudoisoeugenyl-2-methyl butyrate (23.97%) pregeijerene (19.05%) β-bisabolene (17.53%) geijerene (12.48%)	
Pimpinella saxifraga subsp. *nigra*	Germany (Botanical Garden)	Roots	trinoranastreptene (17.71%) 1,4-dimethylazulene (16.10%) 3,10-dihydro-1,4-dimethylazulene (15.10%)	
Pimpinella saxifraga subsp. *nigra*	Italy	Roots	3,10-dihydro-1,4-dimethylazulene (43.56%) pregeijerene (13.95%) peijerene (11.96%)	
P. saxifraga	Iran	Flowering aerial parts	*trans*-α-bergamotene (20.1%) β-sesquiphellandrene (10.8%) β-bisabolene (10.1%)	Masoudi *et al.* 2009
P. saxifraga	Turkey	Fruits	sabinene (40.7%) β-pinene (20.6%) myrcene (14.1%)	Tabanca *et al.* 2006
		Stems and leaves	β-pinene (28.4%) myrcene (18.9%) sabinene (14.3%)	
		Root	epoxy-pseudoisoeugenyl-2 methyl butyrate (66.6%)	
P. saxifraga	Tunisia	Seeds*	anethole (59.47%) pseudoisoeugenol (20.15%)	Ksouda *et al.* 2019

* although in the referenced work the term seeds is used, it refers to the fruit

In the essential oils of the roots of *P. saxifraga* from Turkey (Tabanca *et al.* 2006), *P. saxifraga* subsp. *eusaxifraga* from Germany and *P. saxifraga* subsp. *alpestris* from Italy (Kubezcka *et al.* 1989) the main compound was phenylpropanoid epoxy-pseudoisoeugenyl-2-methylbutyrate (23.97–66.6%), which was also present in significant amounts in our samples of the essential oils of the roots (10.4–16.0%). On the other hand, essential oils of the fruits and stems with leaves from Turkey (Tabanca *et al.* 2006) were characterised by the presence of high amounts of monoterpenes, while in our samples of the fruits and flowering aerial parts monoterpenes were present in very low amounts (tr-0.3%). Also, in the essential oil of fruits originating from Tunisia (Ksouda *et al.* 2019) the most dominant was phenylpropanoid anethole (59.74%), which was not detected in any of the analysed fruit essential oils, nor in other previously reported fruit oils of *P. saxifraga*.

CONCLUSION

Based on the results of the analysis of the anatomy of the different organs of *P. tragium* and *P. saxifraga*, it can be concluded that although they were very similar in terms of the structure of the roots, stems and petioles, there were differences in the anatomy of the leaves and the indumentum of the fruits. The xeromorphic structure of the leaves represents an important adaptation to the ecological factors of the environment, while the presence or absence of trichomes on the fruits is of great importance for the classification of these two species into different sections.

The results of the anatomical analysis of *P. tragium* and *P. saxifraga* were in accordance with previously published data related to the general structure of plants of the genus *Pimpinella*.

Even though the *P. tragium* and *P. saxifraga* essential oils analysed in this study showed certain differences in qualitative and quantitative patterns, all of the oils were characterised by the presence of phenylpropanoid derivative of pseudoisoeugenol type, i.e. epoxy-pseudoisoeugenyl-2-methylbutyrate, as well as by that of trinorsesquiterpenes geijerene, pregeijerene and trinoranastreptene. In addition, azulenes were present in all the essential oils of the roots, while in most of the oils of the fruits and flowering aerial parts, the common compound was the sesquiterpene β-bisabolene.

The occurrence of pseudoisoeugenol-type phenylpropanoids and geijerenes is important from a chemotaxonomic point of view and confirms them as chemical markers of the *Pimpinella* species analysed so far.

In conclusion, the presence of biologically active compounds such as phenylpropanoids and azulenes in investigated *Pimpinella* essential oils indicates the possibility of their further research and potential application in medicine and pharmacy.

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REFERENCES

Abebe D. 1992. Systematic studies in the genus *Pimpinella* L. (Umbelliferae) from tropical Africa. *Botanical Journal of the Linnean Society* **110**: 327–372.

Adams RP. 2007. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th ed. Allured Publishing Corporation, Illinois, USA.

Akalin E, Yeşil Y & Akpulat A. 2016. Fruit anatomy of the Turkish *Pimpinella* species. *Flora* **223**: 62–73.

Askari F & Sefidkon F. 2005. Volatile components of *Pimpinella tragium* Vill. from Iran. *Iranian Journal of Pharmaceutical Research* **2**: 117–120.

Bohn I, Kubeczka KH & Schultze W. 1989. The essential root oil of *Pimpinella major*. *Planta Medica* **55**: 489–490.

Diklić N & Nikolić V. 1986. *Pimpinella tragium* Vill. In: Sarić M (ed.), *Flora SR Srbije* **10** (Dodatak 2), p. 149, Srpska Akademija Nauka i Umetnosti, Beograd.

EMA/HMPC/321184/2012. *Community herbal monograph on Pimpinella anisum L., fructus*. European Medicines Agency, Commitee on Herbal Medicinal Products, London.

EMA/HMPC/321185/2012. *Community herbal monograph on Pimpinella anisum L., aetheroleum*. European Medicines Agency, Commitee on Herbal Medicinal Products, London.

Fernández Prieto JA, Sanna M, Bueno Sánchez A, Molero-Mesa J, Llorens García L & Cires E. 2018. Polyphyletic origin in *Pimpinella* (Apiaceae): evidence in Western Europe. *Journal of Plant Research* **131**: 747–758.

Hosseini N, Arjmand HS & Ghorbanpour M. 2014. Phytochemical composition of the essential oils from two *Pimpinella* L. species (*P. deverroides* Boiss. and *P. tragium* Vill.) growing wild in Iran. *Journal of Medicinal Plants and By-products* **1**: 47–51.

Jančić R & Lakušić B. 2017. *Botanika farmaceutika*. Lento, Beograd.

Jordan M, Rolfs CH & Barz W. 1986. Characterization of volatile constituents from heterotrophic cell suspension cultures of *Ruta graveolens*. *Zeitschrift für Naturforschung C* **41**: 809–812.

Khajepiri M, Ghahremaninejad F & Mozaffarian V. 2010. Fruit anatomy of the genus *Pimpinella* L. (Apiaceae) in Iran. *Flora* **205**: 344–356.

Kljuykov VE, Zakharova AE, Ostroumova AT & Tilney MP. 2021. Most important carpological anatomical characters in the taxonomy of Apiaceae. *Botanical Journal of the Linnean Society* **195**: 532–544.

Ksouda G, Sellimi S, Merlier F, Falcimaigne-cordin A, Thomasset B, Nasri M & Hajji M. 2019. Composition, antibacterial and antioxidant activities of *Pimpinella saxifraga* essential oil and application to cheese preservation as coating additive. *Food Chemistry* **288**: 47–56.

Kubeczka KH, Bohn I & Schultze W. 1989. The compositions of the essential root oils from *Pimpinella saxifraga* s.l. and chemotaxonomic implications. *Zeitschrift für Naturforschung C* **44**: 177–182.

Kundaković TD, Drobac MM, Petrović SD, Maksimović ZA & Marčetić MD. 2017. *Makroskopska i mikroskopska analiza biljnih droga. Priručnik za praktičnu nastavu iz predmeta Farmakognozija*. Univerzitet u Beogradu, Farmaceutski fakultet, Beograd.

Liu RM, Plunkett MG, Lowry II PP, Wyk Van BE & Tilney MP. 2006. The taxonomic value of fruit wing types in the order Apiales. *American Journal of Botany* **93**: 1357–1368.

Maggio A, Bruno M, Spadaro V, Scialaba A, Senatore F & Oliviero F. 2013. Chemical composition, antimicrobial and antioxidant activity of the essential oils from *Pimpinella tragium* Vill. subsp. *glauca* (C. Presl.) C. Brullo & Brullo (Apiaceae) growing wild in Sicily. *Natural Product Research* **27**(24): 2338–2346.

Marchyshyn MS, Hontova MT & Panasiuk AE. 2015. Morphological-anatomical investigation of burnet saxifrage (*Pimpinella saxifraga* L.). *Pharmaceutical Review* **2**: 9–16.

Masoudi S, Rustaiyan A & Mazloomifar H. 2009. Composition of the essential oils of *Pimpinella anisactis* Rech.f. and *Pimpinella saxifraga* L. from Iran. *Journal of Essential Oil Research* **21**: 146–148.

Matevski V. 2005. *Pimpinella* L. In: Micevski K (ed.), *The flora of the Republic of Macedonia* **1**(6), pp. 1564–1568, Macedonian Academy of Science and Arts, Skopje.

Nasir A & Yabalak E. 2021. Investigation of antioxidant, antibacterial, antiviral, chemical composition, and traditional medicinal properties of the extracts and essential oils of the *Pimpinella* species from a broad perspective: a review. *Journal of Essential Oil Research* **33**: 411–426.

Nikolić V. 1973. *Pimpinella* L. In: Josifović M (ed.), *Flora SR Srbije* **5**, pp. 229–233, Srpska akademija nauka i umetnosti, Beograd.

Rohlena J. 1942. *Conspectus florae Montenegrinae*. Prague.

Sadgrove NJ, Padilla-González GF & Phumthum M. 2022. Fundamental chemistry of essential oils and volatile organic compounds, methods of analysis and authentication. *Plants* **11**: 789.

Sihoglu Tepe A & Tepe B. 2015. Traditional use, biological activity potential and toxicity of *Pimpinella* species. *Industrial Crops and Products* **69**: 153–166.

Tabanca N, Bedir E, Kirimer N, Husnu Can Baser K, Khan SI, Jacob MR & Khan IA. 2003. Antimicrobial compounds from *Pimpinella* species growing in Turkey. *Planta Medica* **69**: 933–938.

Tabanca N, Demirci B, Ozek T, Kirimer N, Baser KHC, Bedir E, Khan IA & Wedge DE. 2006. Gas chromatographic–mass spectrometric analysis of essential oils from *Pimpinella* species gathered from Central and Northern Turkey. *Journal of Chromatography A* **1117**: 194–205.

Tabanca N, Douglas AW, Bedir E, Dayan FE, Kirimer N, Baser KHC, Aytac Z, Khan IA & Scheffler BE. 2005. Patterns of essential oil relationships in *Pimpinella* (Umbelliferae) based on phylogenetic relationships using nuclear and chloroplast sequences. *Plant Genetic Resources* **3**: 149-169.
Tutin TG. 1968. *Pimpinella* L. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM & Webb DA (eds.), *Flora Europaea*, vol **2**, pp. 331-333, Cambridge University Press, Cambridge.
Yurtseva VO & Tikhomirov NV. 1998. Morphological diversity and taxonomy of the *Pimpinella tragium* VILL. group (Umbelliferae – Apioideae) in the Mediterranean. *Feddes Repertorium* **109**: 479-500.

REZIME

Botanica SERBICA

Anatomska građa i sastav etarskih ulja različitih organa vrsta *Pimpinella tragium* i *P. saxifraga* (Apiaceae)

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Cilj ovog istraživanja bio je analiza anatomske građe i hemijskog sastava etarskih ulja različitih organa dve vrste roda *Pimpinella*, *P. tragium* i *P. saxifraga*, poreklom iz centralnog dela Balkanskog poluostrva. Na osnovu rezultata analize unutrašnje građe različitih organa *P. tragium* i *P. saxifraga* može se zaključiti da postoje velike sličnosti u građi korena, stabla i lisne drške. Koren obe vrste bio je sekundarne građe, stablo primarne građe sa zatvorenim kolateralnim sprovodnim snopićima, a lisne drške srcolike, rebraste sa adaksijalnim žlebom, sporadično prekrivene kratkim jednoćelijskim ili dvoćelijskim, na vrhu zašiljenim nežlezdanim trihomama (obe vrste) ili dugim uniserijatnim, višećelijskim nežlezdanim trihomama (*P. saxifraga*). Uočene su razlike u anatomiji listova i indumentumu plodova ove dve vrste. Listovi *P. tragium* bili su izobilateralni, a *P. saxifraga* dorziventralni. Plodovi *P. tragium* bili su maljavi, prekriveni brojnim, višećelijskim, uniserijatnim, uspravnim ili zakrivljenim, postepeno zašiljenim nežlezdanim trihomama, dok su plodovi *P. saxifraga* bili goli. Ove razlike u anatomskoj građi su od ekološkog i taksonomskog značaja. U svim organima obe ispitivane vrste uočeno je prisustvo sekretornih kanala. Etarska ulja su izolovana postupkom destilacije vodenom parom iz različitih organa obe vrste, nadzemnog dela sa cvastima, korena prikupljenog u fazi cvetanja i u fazi plodonošenja i ploda i analizirana GC-FID/MS metodom. Iako su ispitivana etarska ulja *P. tragium* i *P. saxifraga* bila donekle različitog kvalitativnog i kvantitativnog sastava, za sva etarska ulja bilo je karakteristično prisustvo fenilpropanoida pseudizoeugenol tipa epoksi-pseudoizoeugenil-2-metilbutirata, kao i trinorseskviterpena pregeijerena, geijerena i trinoranastreptena. Pored toga, u svim etarskim uljima dobijenim iz korena bili su prisutni azuleni, a u većini etarskih ulja plodova i nadzemnih delova u cvetu karakterističan sastojak bio je sekviterpen **β**-bisabolen. Prisustvo fenilpropanoida pseudoizoeugenol tipa i trinorsekviterpena tipa geijerena značajno je sa hemotaksonomskog aspekta i potvrđuje njihovu ulogu hemijskih markera do sada ispitivanih *Pimpinella* vrsta.

Ključne reči: anatomija vegetativnih organa i ploda, sekretorni kanali, trihome, GC-FID/MS analiza