

Diagnostic signs of aboveground and underground organs of *Ferula Tenuisecta* Korov. in natural conditions

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Abstract. This study presents a pioneering investigation into the anatomical composition of both above-ground and underground organs, including leaves, petioles, peduncles, pedicels, and roots of *Ferula tenuisecta*, within their natural ecological habitats. Notably, this research delves into the schizogenic type of tissue development found in the peduncle and root, elucidating its intricate connection to the robustness of the organ, which, in turn, aligns with the plant's distinct life form. The data gleaned from this study highlights distinctive and species-specific structural diagnostic attributes inherent in the above-ground and underground components of *Ferula tenuisecta*. These attributes carry significant potential for utilization in botanical systematics and the accurate identification of plant specimens within this species. Moreover, these findings have implications beyond taxonomy, as the revealed structural traits serve as valuable tools for detecting and assessing the presence of biologically active compounds within different organs and tissues. By shedding light on the intricate anatomical architecture of *Ferula tenuisecta*'s various organs, this study contributes to a deeper understanding of the plant's ecological adaptations and offers practical avenues for its systematic characterization and potential applications in the realm of biologically active substances research.

Keywords. Anatomy, diagnostic signs, leaf, petiole, peduncle and rhizome, *Ferula tenuisecta*.

1 Introduction

Family *Apiaceae* Lindl. - umbrella, distributed throughout the globe. Among the species of this family are valuable vegetable, fodder, spicy-aromatic, medicinal and technical plants [1]. There is information about the presence of biologically active substances in many species [2]. Representatives of this family in all their parts contain essential oils or resinous substances, coumarins, flavonoids, less often saponins with pharmacological activity [3]. Plants containing a high amount of flavonoids are used as choleric, juice-reducing, and capillary-

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strengthening agents [4]. Species of the genus *Ferula* are valuable essential oil (39), fodder (23), medicinal (17), melliferous (18), starchy (9) and food (8) [5, 6]. Many species of *Umbelliferae* are endemic, the study of these species is relevant due to the possibility of their extinction [7]. Species of the genus *Ferula* (family *Apiaceae*) occupy a special place not only among this family, but also in the entire flora of Uzbekistan. The genus *Ferula* L. belongs to the tribe *Peucedaneae* Dumort, subfamily *Apioidedeae* Drude, family *Umbelliferae* Juss. (*Apiaceae* Lindl.). By the number of species (170), the genus *Ferula* L. occupies a leading position in the family. On the territory of Central Asia and Kazakhstan, 105 species are found. There are 45 species on the territory of Uzbekistan, of which 8 are endemics [8, 9]. The issues of taxonomy and phylogeny of the Central Asian *Umbelliferae* are highlighted and compiled a new classification of *Ferula* L. species growing in the CIS [10, 11]. In 48 species of the genus *Ferula* L. under the conditions of Kazakhstan, issues related to synonymy, carpology, ecology, distribution, chemical composition, and economic importance were studied [12, 13]. The life forms on the basis of a detailed biomorphological analysis of ontogeny patterns in 14 Eastern European umbrella species, identified ecological groups, and showed possible deviations in the long-life cycle depending on various habitat conditions [15, 16].

There is information in the literature on the molecular biology of the *Umbelliferae* family, including some species of the genus *Ferula*, which confirmed the monophyletic nature of the genus [17-20].

The comparative ecological and anatomical structure of the vegetative organs of *Ferula* species was studied by [21-25]. The formation of various kinds of secretory receptacles is characteristic of the progressive direction of evolution, which is of great importance in phylogenetic systematics [26]. The structure of the secretory receptacles of *Ferula* is an important taxonomic feature [27, 28]. Secretory structures in the vegetative organs provide protection from ultraviolet radiation, increase the drought resistance of plants, and contain flavonoids, terpenoid-containing substances that are medicinal or poisonous [29].

Many species of *ferula* are forage plants, especially in deserts and semi-deserts; some are used as hay plants. For many species of *ferula*, in particular for *F. ferulaeoides* and *F. foetida*, people's experience of using them as fattening and milk-producing feeds has been described [30]. The fruits of umbrella plants are readily eaten by all animals. The nutritional value of the entire above-ground mass is 82.3 feed units [31]. In the desert, the core of the young inflorescence of *F. foetida* is used as a vegetable. In folk and official medicine, *F. mogoltavica* Lipsky ex. Korov., *F. tadshikorum*, *F. kuhistanica* Korov., *F. fedtschenkoana* K.-Pol., *F. sumbul*, *F. kirialovii* M. Pimen, *F. tenuisecta* Korov., *F. penninervis* Regel et Schmaih. [32-35].

Terpenoid coumarins and sesquiterpene lactones predominate in monocarpic species of the genus [36] developed a classification of terpenoid-containing plant structures. According to the type of structure of the secretory system (schizogenous receptacles), plants of the genus *Apiaceae* are direct descendants of the ancient *Araliaceae* or can be derived from some common ancestral group with them. Coumarins, sesquiterpenoids, sesquiterpene lactones, esters, glucosides, flavonoids (luteolins, cynarosides), carbohydrates, polysaccharides, pyrosine phosphates, etc. found in *Ferula* species [37-41]. L.D. A method for the qualitative determination of the amount of esters of terpenoid alcohols in the substance *Ferulen* was developed, on the basis of which the *Ferulen* preparation was created [42]. Resins have been and are used as hemostatic, spasmodic, tonic, wound healing, expectorant, stimulant, tonic, anthelmintic and abortifacient. The range of treatment of diseases with *ferula* resin is very wide [43, 44].

The main losses of tefestrol and established the advantages and disadvantages of the existing technology in the production of the substance from the aerial parts of *Ferula tenuisecta* were identified [45, 46]. It was found that the final product obtained by the existing technology has a green color that does not meet the requirements of the pharmacopoeial

article. The stage of extraction of the sum of esters of sesquiterpene alcohols from the aerial part of *Ferula tenuisecta* and purification of the obtained extract is proposed, which subsequently makes it possible to obtain the substance of tefestrol without green color. It has been shown that the use of activated carbon makes it possible to remove the green color of the extract. To purify tefestrol from other esters of sesquiterpene alcohols and related substances, chromatographic separation on silica gel was proposed under the following conditions: the ratio of the amount introduced into the column and the sorbent was 1:20; the ratio of the height of the sorption layer to the diameter of the column is 4:1; elution rate, 35 L/(m² h). To obtain the pharmacopoeial substance of tefestrol, crystallization of a technical product from a mixture of ethyl acetate with hexane in a volume ratio of 1:3 was proposed. Based on the results obtained, a technology for obtaining the substance of tefestrol from the aerial part of *Ferula tenuisecta* was developed. According to this technology, 70.96% of the substance of tefestrol is obtained from the content in the raw material, according to the existing technology - 33.9%.

In this regard, of scientific interest is the study of the anatomical structure of the aboveground and underground organs of the raw material of medicinal plants *Ferula tenuisecta*, growing in the conditions of Uzbekistan, in order to determine the diagnostic features and localization of biologically active substances in organs and tissues.

2 Materials and methods

The objects of the study were *Ferula tenuisecta*, a perennial polycarpic medicinal plant with a thickened, fusiform root. The neck is densely wrapped with fibers of old dead leaves, many-headed. Stems 80-90 cm tall, strong, paniculate-branching in the upper part; the panicle is oblong-oval in outline, its branches are alternate below, several at the top are collected in whorls. The leaves are oval-rhombic in outline, rough from short, hard hairs, basal on long petioles; plate trifoliate, with 2 additional segments on the sides, repeatedly-(5)-dissected into numerous, 2-5 mm long, linear, grooved above, obtuse lobes; cauline with a reduced plate, their sheaths enclosing the stem at the base, leathery, oval-lanceolate, swollen; the upper ones are represented only by the sheaths. Umbrellas different, terminal 8-15-rayed, sessile or on short stalks, 6-8 cm wide, lateral arranged 2-5 on long stalks exceeding the terminal umbel. Umbrellas 10-flowered, with wrappers consisting of scaly, falling leaves. Cup without teeth. Petals yellow, oval, narrowed and curled at the top, 1.2 mm long. Fruits are oblong oval, convex, with thickened, pale edges, 9x5 mm long, width; ribs are convex; tubules in the furrows are single, wide, in the commissure there are 2-6 of them. Blooms in May; fruiting in June. *Ferula tenuisecta* grows on soft mountain slopes in the shrub belt. Distributed in the Tien Shan mountains at an altitude of 600-2500 m above sea level, where it occurs on rocky and grassy slopes, in steppes and bushes [47].

Samples of fresh plants were fixed in a 70% alcohol solution. Preparations prepared by hand were stained with methylene blue followed by gluing in glycerol-gelatin [48, 49]. Transverse sections of the leaf are made through the middle, petiole, peduncle, pedicel and rhizomes - the base. Descriptions of the main tissues and cells are given [50]. Microphotographs of the anatomical features of the aboveground and underground organs were taken with a computer microphotographic attachment with a Canon A123 digital camera under a Motic B1-220A-3 microscope.

3 Results and discussion

In the species *Ferula tenuisecta*, the leaves are oval-rhombic in outline, rough with short, stiff hairs, basal on long petioles; plate trifoliate, with 2 additional segments on the sides,

repeatedly (5) - dissected into numerous, linear, grooved above, obtuse lobes, almost bare above, shortly pubescent below, stem-like with a reduced plate, their sheaths enclosing the stem at the base, leathery, oval-lanceolate, swollen; the upper ones are represented only by the sheaths.

The anatomical structure of the leaf in the studied species *Ferula tenuisecta* is the structure of the leaf mesophyll of the isolateral-palisade type [51], which is represented by palisade, spongy cells and vascular bundles. Epidermal cells are single-row, oval, larger on the adaxial side of the leaf than on the abaxial side. Between the adaxial and abaxial epidermis is an assimilation tissue consisting of palisade and spongy cells. The palisade parenchyma is two-row, large-celled and elongated with the presence of large intercellular spaces. The spongy parenchyma is round, small-celled, consists of 4-5 rows with small cavities. Palisade and spongy parenchyma cells are chlorophyll-bearing (Figure 1).

The main leaf vein protrudes on the abaxial side. The rest of the vein is occupied by the main thin-walled round-oval cell parenchyma. In the main vein of the leaf there is one large vascular bundle of closed collateral type. In the studied species, in the main leaf vein under the abaxial epidermis, there are groups of multi-row lamellar collenchyma cells. Localizations of biologically active substances in the leaves of the schizogenous receptacle type were determined, which are located above each main and lateral vascular bundles on the abaxial side of the leaf mesophyll and between the collenchyma and vascular bundles in the main vein of the leaf (Figure 1).

According to the literature data, the structure of *Ferula* secretory receptacles is an important taxonomic feature that provides protection from ultraviolet radiation in vegetative organs, increases drought resistance of plants, contains flavonoids, terpenoid-containing substances that are medicinal or poisonous.

The structure of the petiole. On the transverse section, the structure of the bundle-type petiole is oval-lanceolate with a swollen shape. In the studied species, epidermal cells are single-row, thin-walled, pubescent with simple, unicellular hairs. Under the epidermis are chlorophyll-bearing two-row subepidermal and 7-8 row parenchymal cells. The peripheral parts of the petiole are the most sclerified, due to the presence of a group of multirow lamellar collenchyma cells under the subepidermal cell, which alternate with 7-8 row chlorophyllon-bearing parenchyma. The localizations of biological active substances in petioles of the schizogenic type of receptacle, which are located above each peripheral vascular bundle and collenchymal cells, were determined. Conductive bundles of collateral type. Peripheral vascular bundles are represented by alternating large, medium and small numerous bundles, which are located in an annular shape, randomly in the central part of the petiole (Figure 2).

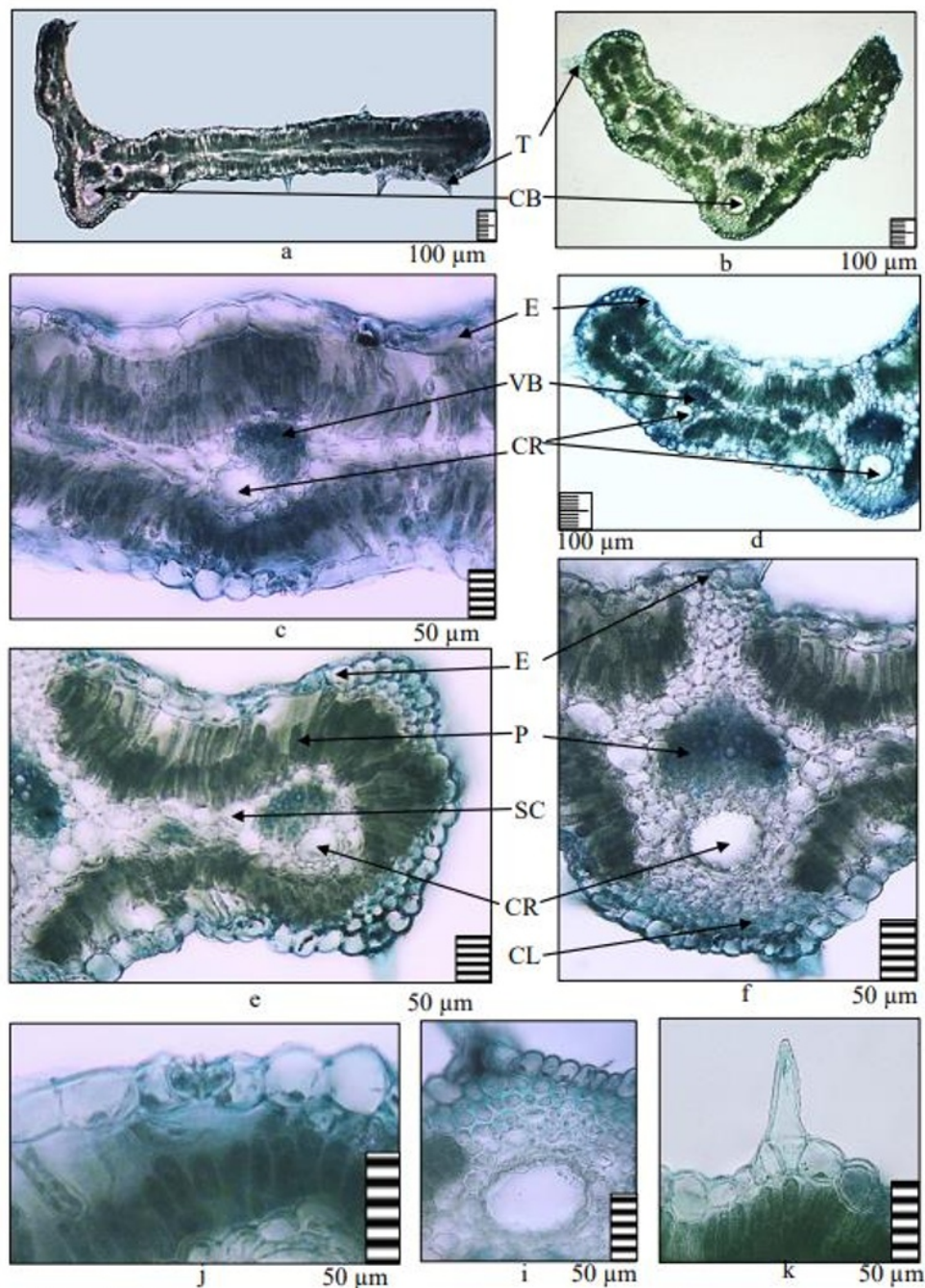


Figure 1. Anatomical structure of the leaf mesophyll of *Ferula tenuisecta*: a-b – general view of the leaf; c-e – detail of the leaf mesophyll; j – non-immersed stomata; f-i – schizogenic type secretory receptacles; k – single-celled simple trichomes. Legend: GP - spongy parenchyma; CL – collenchyma; P – palisade parenchyma; VB – vascular bundles; SR– secretory receptacles; T – trichome; E – epidermis.

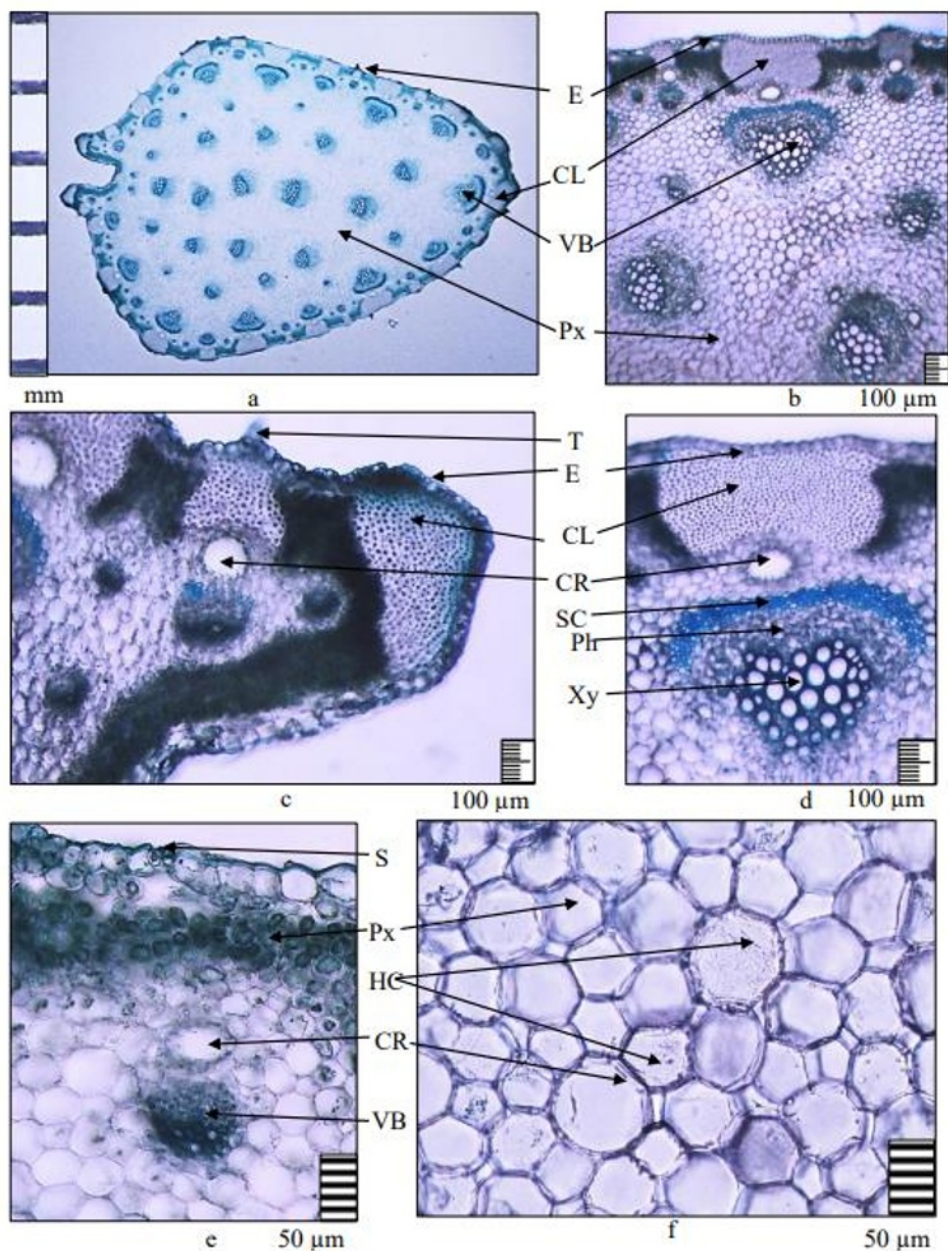


Figure 2. Anatomical structure of the petiole of the leaf of *Ferula tenuisecta*: a - general view of the petiole of the leaf; b-c - detail of the petiole of the leaf; d - epidermis, collenchyma, vascular bundles and secretory receptacles; e - non-immersed stomata, chlorophyllbearing parenchymal cells; vascular bundles and secretory receptacles; f - parenchymal and hydrocytic cells. Legend: HD - hydrocytic cells; CL—collenchyma, Xy—xylem, VB— vascular bundles, Px—parenchymal cells, CR— secretory receptacles, SC—sclerenchyma; T - trichome; Ph - phloem, E - epidermis.

The vascular bundles are most sclerified due to the bast fibers (sclerenchyma cells), which form multilayer strands in the form of caps over the phloem of the bundles, the central bundles are less sparsely layered. Above the peripheral vascular bundles, there is one

secretory receptacle of the schizogenic type, which consists of 12-14 epithelial cells. Large peripheral vascular bundles from the side of the xylem have 2 secretory receptacles. In the central conducting bundles, there are three secretory receptacles of the schizogenic type, which consist of 9-10 epithelial cells. Parenchymal cells are thin-walled, large, round-oval. Among the parenchymal cells, there are hydrocytic cells (Figure 2).

Morphological and anatomical structure of the peduncle. The species *Ferula tenuisecta* is a perennial polycarpic plant, peduncle below 80-90 cm in height, strong, paniculate-branching in the upper part; the panicle is oblong-oval in outline, its branches are alternate below, several at the top are collected in whorls. In the studied species, the cross section of the base of the peduncle has a parenchymal-beam type of structure and an oval shape. The epidermis is single-row with a thick cuticle, the outer and inner walls of which are thickened. Under the epidermis there is a single-row hypodermis and 4-5 row chlorophyll-bearing cells of the core parenchyma, which alternate with areas of lamellar collenchyma (Figure 3). Under the collenchyma there are large secretory receptacles of the schizogenic type, which consist of 11-12 epithelial cells. The primary cortex is separated from the central cylinder by a ring of sclerenchyma. The thickness of this ring and the degree of lignification of the cells in some respects reflect the evolutionary advancement of the species. Sclerenchyma is thick-walled, consists of 4-5 rows of cells (Figure 3).

The central cylinder is extensive, parenchymal cells are thin-walled, large, and round-oval. Numerous vascular bundles are located among the thin-walled parenchymal cells of the central cylinder. Among the parenchymal cells of the central cylinder, hydrocytic cells are found. Peripheral conductive bundles are open and form a discontinuous circle, large conductive bundles alternate with small ones. The vascular bundles are most sclerified, due to the bast fibers (sclerenchyma cells), which form multilayer strands in the form of caps over the phloem of the bundles. Xylem is represented by large, numerous vessels located in groups and chains. In the core, near each conducting bundle, there are numerous large and small secretory receptacles of the schizogenic type. Medullary vascular bundles vary in shape, size, and orientation. They are located in the core radially and in groups, randomly and are represented by collateral and bicollateral types (Figure 3).

The anatomical structure of the pedicel in a transverse section of the parenchymal-fascicular type of structure and rounded shape. The epidermis is single-row with a thin-walled cuticle. Under the epidermis there is a single-row hypodermis and 4-5 row chlorophyll-bearing cells of the bovine parenchyma, which alternate with areas of lamellar collenchyma. Under the collenchyma there are large secretory receptacles of the schizogenic type, which consist of 11-12 epithelial cells. The primary cortex is separated from the central cylinder by a ring of sclerenchyma. The thickness of this ring and the degree of lignification of the cells in some respects reflect the evolutionary advancement of the species and perform the functions of power and support. Sclerenchyma is thick-walled, consists of 4-5 rows of cells. The central cylinder is extensive, parenchymal cells are thick-walled, large, and round-oval. Among the parenchymal cells of the central cylinder, there are hydrocytic cells (Figure 4).

Peripheral conductive bundles are open and form a discontinuous circle, large conductive bundles alternate with small ones. The vascular bundles are of the collateral type, the xylem of the bundles is represented by large, numerous vessels located in groups and a chain. Under the peripheral vascular bundles there are large numerous secretory receptacles of the schizogenic type, the central vascular bundles are small, few secretory receptacles (Figure 4).

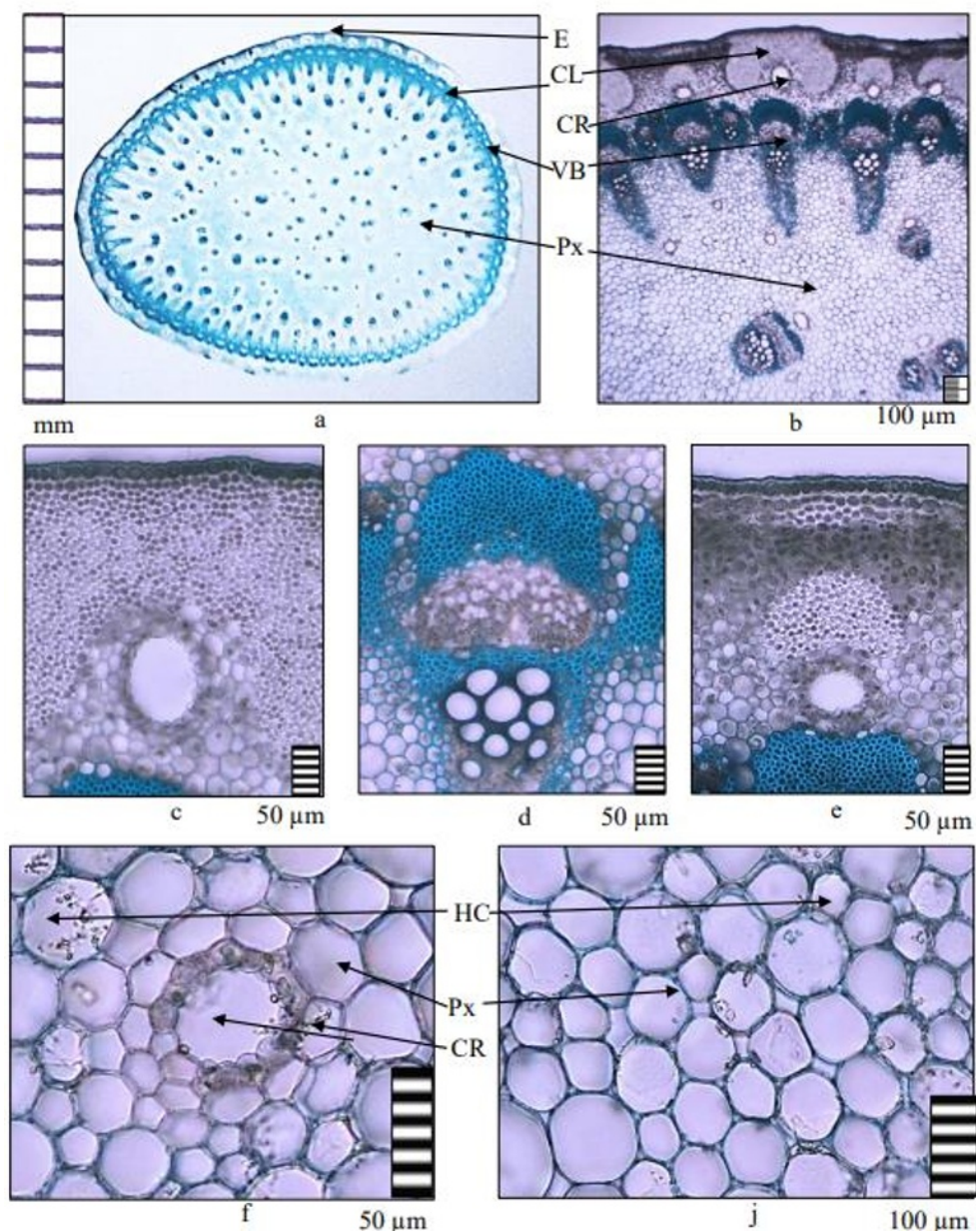


Figure 3. Anatomical structure of the peduncle of *Ferula tenuisecta*: a - general view of the peduncle; b - detail of the peduncle; d – conductive bundles; c, e – epidermis and collenchyma, large and small secretory receptacles; f - secretory receptacles; j – parenchymal and hydrolytic cells. Legend: HD - hydrocytic cells; CL – collenchyma; VB – vascular bundle; Px – parenchymal cells; SR – secretory receptacles; Ph – phloem; E – epidermis; EC – epithelial cells.

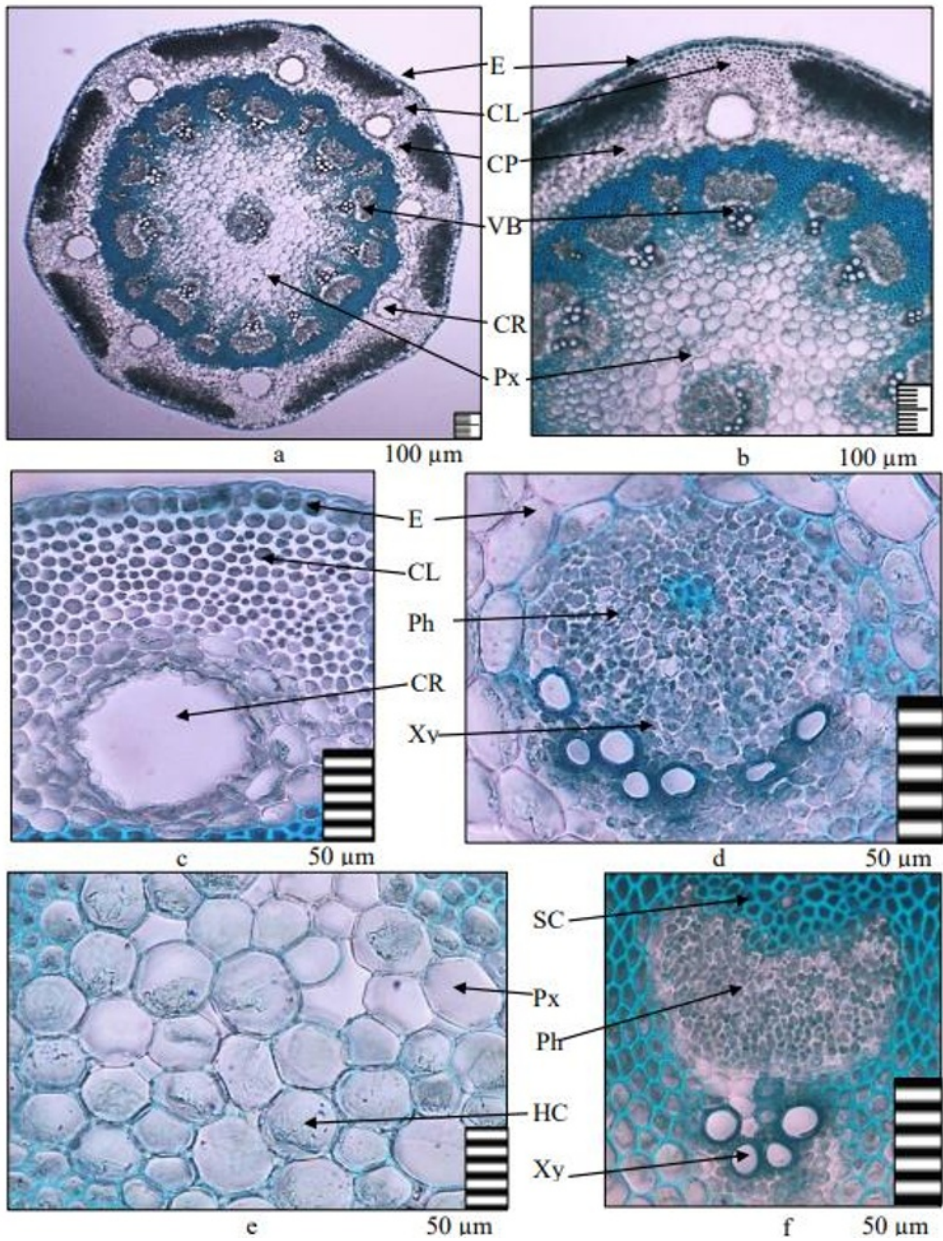


Figure 4. Anatomical structure of the peduncle of the *Ferula tenuisecta*: a – general view of the peduncle; b – detail of the peduncle; c – epidermis, collenchyma and secretory receptacles; d, f – vascular bundles; e – parenchyma and hydrocytic cells. Legend: HD - hydrocytic cells; KL - collenchyma, CP - cortical parenchyma, Xy - xylem, VB - vascular bundle, Px - parenchymal cells, SR - secretory receptacles, SC - sclerenchyma, Ph - phloem, E - epidermis.

Anatomical structure of the underground organs of *Ferula tenuisecta*. The root of *Ferula* is thick, with an upright rhizome. The caudex is simple. The anatomical structure of the plant shows a deep connection with the origin of the species, a comparison of these structures with other members of the family reveals the same type or heterogeneity of the structure, interconnected with adaptation to environmental conditions. The study of the secretory system of various organs and the characterization of terpenoid-containing receptacles are important for elucidating the localization of some biologically active compounds.

The morpho-anatomical structure of the underground organs of *Ferula* has been little studied. The underground stem part of perennial herbaceous plants, located on the basal part of the tap root, has received different names in the literature: many-headed root, many-headed caudex, and many headed rhizome. Authors of the Atlas of Descriptive Morphology of Higher Plants. Stem and root” suggest calling the underground stem part of herbaceous perennials the stem root. It consists of lignified perennial shoots, connected at the bases with a lignified hypocotyl, turning into a woody taproot, usually with contractility.

The morphological features of underground organs of 15 *Ferula* species, including *F. varia* and *F. angreni*. Two main groups have been distinguished according to the structure of underground organs: deep-rooted species with a well-defined branched stem root and species with a turnip-like thickened main root and an indistinct stem root. There is information in the literature on the study of the structure of the axial and assimilation organs of some species of the umbrella family.

The root of *Ferula tenuisecta* is thickened, fusiform. The neck is densely wrapped with fibers of old dead leaves, many-headed. In the studied species, the root bark is wrinkled, easily separated, and darker brown in color. Older areas are covered with a thick layer of cracking and flaking cork. The cork is uneven, multi-row, the cells are flattened-oblong. Parenchymal cells of the peripheral part of the root cortex are severely destroyed. Numerous secretory receptacles are located in the cortex parenchyma. Groups of parenchymal cells untouched by destruction are preserved only around secretory receptacles, which are relatively little subject to destruction.

The phloem is multi-rowed and thin-walled. In the studied species, the cambium is multi-rowed, the xylem part is parenchymal. In the studied species, parenchymal cells and radial rays are filled with starch grains. Secretory receptacles located in the phloem part of the secondary cortex can be divided into 3 groups: young small receptacles directly on the border with the cambium, well-developed receptacles in the inner part of the cortex, and larger aging receptacles in its outer part. *Ferula tenuisecta* the structure of the root is similar, polycentric anomalous structure, the vascular bundles are grouped into concentric circles of different orientations, the radial rays are wide, in places they branch and bend. Secretory receptacles are numerous, the lumen of secretory receptacles is the largest, epithelial cells are numerous. The root of *Ferula tenuisecta* performs the function of storage for the formation of a more or less powerful peduncle in a short time, which determines its structure (Figure 5).

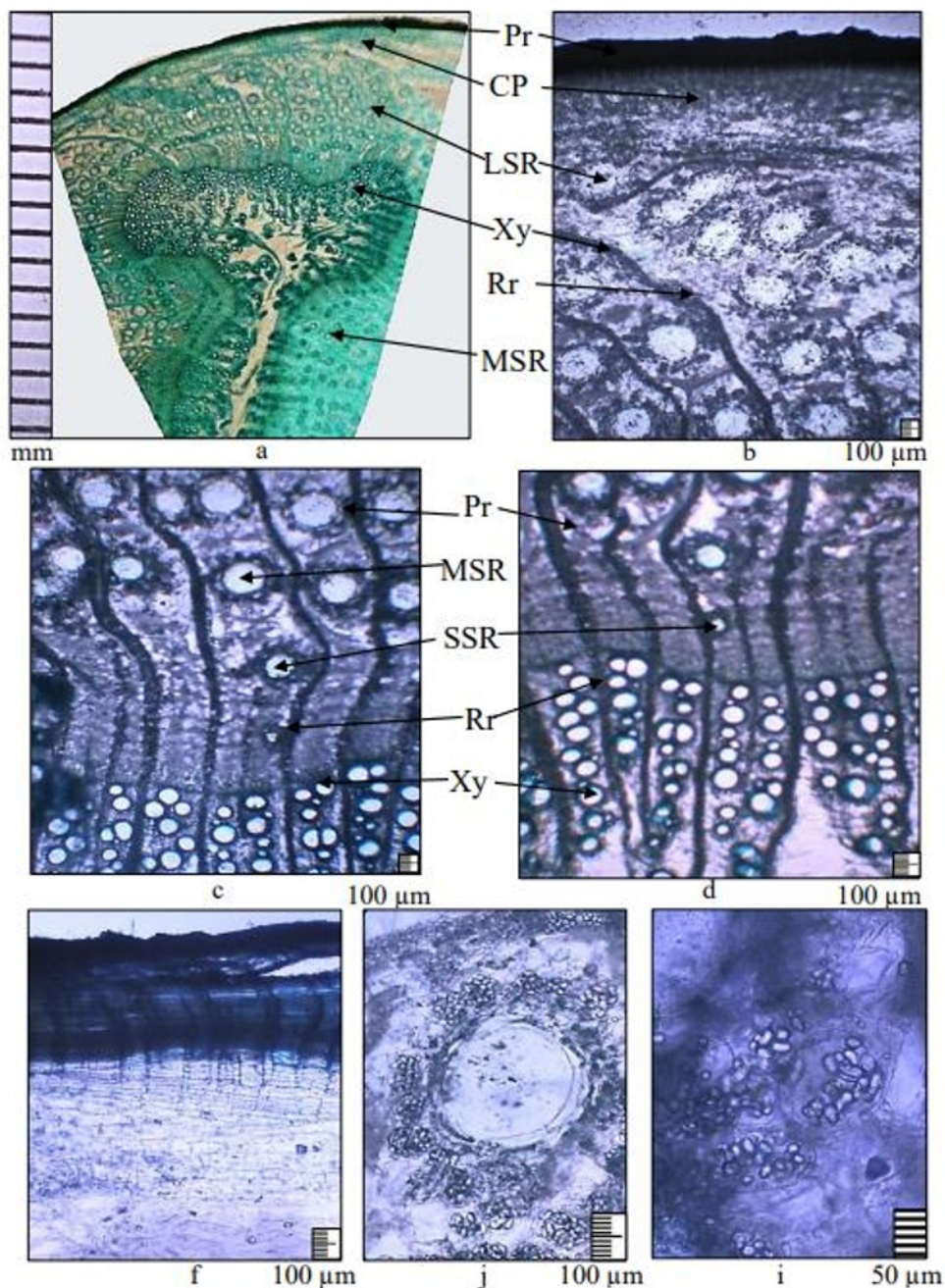


Figure 5. Anatomical structure of the root of *Ferula tenuisecta*: a - detail of the root; b – cortical parenchyma and larger aging secretory receptacles; c, d – vascular bundles and secretory receptacles; e - the root neck is covered with numerous fibers of dead leaves; f - secretory receptacles of schizogenic type; g - wood parenchyma and starch grains. Legend: CP – cortical parenchyma; Sg – starch grains; Xy - xylem, LSR – large secretory receptacles; SSR – small secretory receptacles; Pr – periderm; Rr – radial rays; MCR – mean secretory receptacle.

4 Conclusions

Thus, when studying the structural features of the aboveground and underground organs (leaf, petiole, peduncle, peduncle and root) of *Ferula tenuisecta* in natural environmental habitats, the following characteristic diagnostic, adaptive features were identified and the presence of localization of biological active substances was determined: the structure of the mesophyll of an isolateral-palisade leaf type; the structure of the petiole of the beam type; epidermis thin-walled, pubescent with simple, unicellular hairs; under the epidermis are chlorophyll-bearing subepidermal and parenchymal cells; the petioles are most sclerified, due to the lamellar type of collenchyma cells; the presence of large and small secretory receptacles of the schizogenic type; of the collateral type, the peripheral conducting bundles are most sclerified, due to the formation of bast fibers (sclerenchyma cells); among thin-walled and large parenchymal cells, hydrocytic cells are found; parenchymal-beam type structure of the peduncle and pedicel; the presence of a high and thick peduncle, a thick outer wall of epidermal cells, large-celled cortex and core parenchymal cells, which is due, on the one hand, to the xeric habitat, and on the other, to an earlier completion of the growing season; the vascular bundles are most sclerified due to bast fibers (sclerenchyma cells), which form multilayer strands in the form of caps over the phloem of the bundles; the presence of localization of biological active substances, determined in large and small numerous secretory receptacles of the schizogenic type in the peduncle, is associated with the power of the organ, which correlates with the life form. The study of the secretory system of various organs and the characterization of terpenoid-containing receptacles are important for elucidating the localization of some biologically active compounds (Safina, 2012).

Thickened turnip root type. Caudex differs from the main root by the presence of a pith. In the underground organs, the xylem part is larger than the phloem part, which is associated with a conductive function. Secretory receptacles are localized only in the phloem part and in the cortical parenchyma. The lumen of the secretory receptacles is the largest, the epithelial cells are numerous, in the xylem part the woody parenchyma is developed, which is dominated by parenchymatization. The data and identified structural diagnostic features of aboveground organs are species-specific and can be used in taxonomy and in the identification of plant materials for a given species and the determination of biologically active substances in organs and tissues.

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