



## Morphological-anatomic analysis of the medicinal plant raw material of the herb *Daucus carota*

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*Daucus carota* (wild carrot) is a wild plant with unlimited resources used in pharmacology. Its medicinal raw materials are fruits (*Fructus dauci*), but inflorescences and leaves have various biologically active substances. There is a need for their detailed study. The research study aims to describe in detail this biennial herb's anatomical and morphological characteristics, which are promising for phytotherapeutic use. The *D. carota* leaves are short-petiolate, deep green, oblong in outline, three times pinnately dissected, with incised-toothed lobes, pubescent with one-, rarely two-, three-celled sharp hairs. Up the shoot, the leaves are sessile, forming a sheath; their terminal lobes gradually become oblong. The epidermis on the lower side of the leaf has rectangular cells with slightly curved walls and a diacytic stomatal apparatus. Under it lies the collenchyma. Along the veins are pointed, one-celled, warty hairs. The epidermis on the upper side is formed from small polygonal cells; stomata are single. Columnar parenchyma is formed by 1–2 rows of elongated cells. The spongy tissue consists of wide, irregularly shaped cells that do not fit tightly together. There is a layer of special cells in which assimilates accumulate at the border between the spongy and palisade tissues. The conducting bundle is closed, bilateral, surrounded by sheath cells. The inflorescence is a compound umbel, each ray forming a secondary umbel. At the base of the compound umbel there is a spathe of pinnately dissected leaves. The marginal flowers of the outer umbels have petals of different sizes, incised into unequal lobes, with veins, yellowish-white with pink edges. In the inner flowers of the umbels and the inner umbels of the complex inflorescence, the flowers have five identical, broadly oval, white petals, incised in the middle, and curved upwards. Their epidermal cells are oval, large, with nuclei and papillary hairs. Since a high content of coumarins and pyrocatechins was found in the inflorescence of *D. carota*, the diagnostic features of its medicinal raw material (the herb) were described for further scientific research, writing a monograph for the European Pharmacopoeia and creating new herbal medicines based on it.

**Keywords:** *Daucus carota*; herb; plant raw materials; inflorescences; fruits; microscopy; macroscopy; epidermis; dialytic stomatal apparatus.

### Introduction

Wild medicinal herbs are of great interest, having large industrial reserves and having been used in folk medicine for a long time. One such plant is *Daucus carota* L., the medicinal plant raw material of which is considered to be the fruits collected and dried at the time of full maturity and used to manufacture medicines. *D. carota* seeds have long been used in folk medicine as a diuretic, carminative, antispasmodic, choleric, and antimicrobial agent. References to the use of *D. carota* date back to the first century AD in Afghanistan. From there, it spread to China in the 13th and 14th centuries and later reached Europe by the 15th century. It is widely used in Indian folk medicine and the countries of the Middle East. The plants were brought to North America by European settlers (Jansen & Wohlmuth, 2014). Traditionally, *D. carota* was utilized by the ancient Greeks and Romans for various medicinal purposes. Dioscorides, a Greek physician and botanist who lived in the 1st century AD, documented the uses of the plant in his renowned work “De Materia Medica”.

In pharmacology, *D. carota* seeds are used mainly as a source of various substances of primary synthesis and biologically active substances: phenolic compounds, flavonoids, and essential oils. Several scientists in Europe and Ukraine have been studying the beneficial properties of *D. carota*. The plant is known to possess antithetic, diuretic, carminative, antipyretic, analgesic, anti-inflammatory, antiseptic, antidiabetic properties (Mockute et al., 2004; Özcan & Chalchat, 2007; Dawid et al., 2015).

The pharmacological effect of plant extracts depends on the qualitative composition of essential oils. The microbiological activity of essential oils with monoterpene hydrocarbons was highest in mature umbels, then herbals, and lowest in flowers. The sensitivity of the studied bacterial strains to the oil decreased from gram-positive, *Can-*

*didia albicans*, to gram-negative microorganisms and *P. expansum* (Staniszewska et al., 2005). The fruit's essential oil had significant activity against *Cryptococcus neoformans* and dermatophytes, inhibited germ tube formation, and formed biofilms of *Candida albicans*, but it also had low antioxidant activity. Concentrations below 0.64 µL/mL were safe for various human cell types. This suggests the possibility of using the essential oil for therapeutic purposes as an anti-inflammatory agent (Acimovic et al., 2016; Alves-Silva et al., 2016).

The essential oil of *D. carota* was found to contain mainly sesquiterpenes. The hemotype of their derivatives has exhibited bactericidal activity against *Campylobacter* and other gram-negative food-spoiling bacteria that cause bacterial gastroenteritis in humans (Dedieu et al., 2010). They exhibited antimicrobial activity against *Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 43300, as well as clinical strains of *Candida albicans* and *C. tropicalis* (Marzouki et al., 2010). Essential oils from fruits of *D. carota* from the Montenegrin coast had a similar sesquiterpene composition. The CEOs ( carrot essential oils )isolated from mature umbels had better antioxidant activity than immature umbels (Stanojević et al., 2023). Essential oils with sesquiterpene chemotype of the fruits of *D. carota* growing in Turkey showed antidiabetic, anti-inflammatory, and antilipoxygenase activity but weak antibacterial activity against gram-negative and gram-positive bacteria and weak antioxidant activity (Servi et al., 2021).

A high content of fatty acids was found in all types of *D. carota* raw materials, of which 79–90% were saturated fatty acids; the lowest content was in the fruits. The saturated fatty acids, palmitic and stearic acids, dominated all analyzed objects. The dominant unsaturated fatty acids were linoleic, and the fruits contained oleic and linolenic acids (Kyslychenko et al., 2018). In the seeds of carrot *D. carota* from Konya, Turkey, more than half of the composition was petroselinic, and

the rest was linoleic, palmitic, and stearic acids. Carotol (30.6%), daucol, and copanolen were essential components of the edible oil of carrot seeds (Özcan & Chalchat, 2007).

Previous studies have shown that essential oils have high antimicrobial and anti-inflammatory properties but weaker antioxidant properties. Due to the presence of many phenols, polyphenols, flavonoids, and coumarins in various organs, *D. carota* exhibits antioxidant, antitumor, and gastroprotective activity, which is confirmed *in vitro* and *in vivo*. Also in 70% aqueous-alcoholic extract of *D. carota* fruits, rosmarinic, chlorogenic, caffeic acids, rutin, apigenin, luteolin, and hyperoside were identified, which had high antimicrobial activity against various strains of *Staphylococcus aureus*. *D. carota* seed waste, formed after industrial extraction with 96% ethanol, was re-extracted. The results showed that the 70% EtOH extract has a higher total phenol and flavonoid content and a higher antioxidant activity ( $86.9 \pm 3.0\%$ ) than the 40% EtOH extract ( $78.7 \pm 3.3\%$ ). The extracts also had antimicrobial activity (Pavlyuk et al., 2015; Pazyuk et al., 2017).

Thus, extracts rich in phenolic substances and flavonoids demonstrated significant DPPH antioxidant activity and high FRAP values. In experiments on mice, various essential oil fractions showed hepatoprotective properties. The sesquiterpene-rich fraction had the highest activity (Shebaby et al., 2015).

A monograph on wild carrot fruits is included in the Pharmacopoeia of the People's Republic of China, 2005. In the British Herbal Pharmacopoeia, wild carrot herba was included in the year 1983. Currently, the regulatory documentation for this raw material in Ukraine is the temporary FS 42-2817-91, which regulates the quality of *D. carota* fruits. The results of the study of the anatomical structure of *D. carota* fruits and *D. carota* subsp. *sativus* (Hoffm.) Arcang. fruits were used in the design of the "identification B" section of the national part of the "*Daucus carota* fruits" monograph (Kyslychenko et al., 2019). Ten complex drugs and biologically active additives which include wild carrot seeds are registered on the world pharmaceutical market: AromadetoX, Evamilk, Hepatalgina, Metiogen, NaturSel-C, Natur-Zin, Palatrobil Sciargo, Watershed, and two in Ukraine – "Urolesan" and "Uroholum" (Tkachuk et al., 2013).

Thus, wild carrots contain a variety of biologically active compounds: phenolic compounds, flavonoids, and essential oils in both the fruits, inflorescences, and above-ground parts. They have a variety of pharmacological effects: antimicrobial, antioxidant, and others. It has long been known that similar biologically active substances accumulate in all plant organs of the same species. In addition, based on the analysis of literary data, it has been established that *D. carota* herbs can be used as a medicinal raw material in addition to fruits. Therefore, our work aimed to determine the content of biologically active substances characteristic of *D. carota* fruits, herbs and flowers using qualitative reactions and to establish diagnostic signs of their raw materials. Based on a detailed description of the anatomical and morphological characteristics of this biennial plant, which is promising for phytotherapeutic use, we aim to establish diagnostic features of its medicinal raw materials – inflorescences and herbs. The results obtained can form the basis for further research and writing a monograph on the herbs, flowers, and fruits of this plant for the European Pharmacopoeia and creating new herbal medicines with a broad spectrum of action on its basis.

## Materials and research methods

The work aimed to prove the content of phenols and coumarins by qualitative reactions and to establish diagnostic signs of medicinal plant raw materials of wild carrot herb *Herba Daucus carota* using macro- and microscopic analysis.

The object of the study was wild carrot (*D. carota* ssp. *carota* L.). *Daucus carota* grows as a weed, on roadsides, in the fields (Flora of the Ukrainian SSR, 1955; Flora Europaea, 1968). Material for the study was collected in the territory of Lviv Oblast in the vicinity of the city of Lviv and the city of Kamyanka-Buska, Lviv region. For the research, generative shoots in the flowering phenophase and inflorescences in the fruiting phase were selected.

Macro- and microscopic studies of the raw material were performed according to the methods of State Pharmacopoeia of Ukraine, 2014 2.8.23 "Microscopic study of herbal raw material" and European Pharmacopoeia, 2011. To conduct macro- and microscopic analyses, we took generative herbaceous shoots in the phenophase of complete blossoming (3–5 shoots of 10 individuals). Using macroscopic analysis, we identified the morphological features: type of life form and shoot, peculiarities of the structure of the stem, leaves, flowers, their pubescences, presence of essential oil-bearing structures and type of inflorescence. For morphological studies, we used binocular microscope MBS 1.

In the microscopic study, we analyzed the internal structure of the stem, leaves and flower petals and their epidermis structure, pubescence and essential oil-bearing structures. On the transversal section of the leaf, we examined mesophyll structure, mechanical, vascular and secretory tissues. We also analyzed the structure of the upper and lower epidermis of the leaf, in particular: shape, sizes of cells and structure of their membranes, types of stroma apparatus; structure of cuticle, hairs and glandular structures. In the generative sphere, we studied: structure of calyx and corolla of flower and bracts, structure of the epidermis, presence of hairs. The microscopic studies of the raw material were performed using a Leica DM 500 microscope. Freshly collected raw material was fixed in the mixture of 96% ethanol P – glycerol P – purified water P (1:1:1). To study peculiarities of the anatomic structure of leaves and stems, we prepared from them 10–20  $\mu\text{m}$  thick transversal sections using a microtome and blade. We used glycerol to illuminate the temporary preparations prepared of fresh plants, and stained them using iodine solution in iodine potassium and acid fuchsin. Microimages were taken using a digital camera and Bresser camera eyepiece.

Work was carried out to determine the presence of phenols in the aqueous extract and coumarins in the 95% alcohol extract of leaves, inflorescences, and fruits of wild carrot (*D. carota*) according to the methods of the State Pharmacopoeia of Ukraine, 2014. In the extract, the detection of coumarins was carried out using qualitative reactions, namely the lactone test and the reaction with diazo reagent in an alkaline medium. Phenols were determined by the aqueous extract's response with a solution of iron (III) chloride.

## Results

*Phytochemical studies of the content of biologically active substances in various organs of D. carota.* We studied the content of phenols and coumarins in wild carrot leaves, flowers, and fruits. As a result of the studies conducted, natural compounds such as coumarins and phenols, particularly pyrocatechins, were established in the raw material of *D. carota*. The most significant amount of coumarins and phenols was present in the extract prepared from umbels, which gives grounds to speak about the feasibility of using not only fruits, but also inflorescences of *D. carota* for further scientific research and the creation of new herbal preparations with a broad spectrum of action on its basis. In addition, there is a need to determine the diagnostic features of medicinal plant raw materials, not only fruits, but also leaves and inflorescences of the studied species.

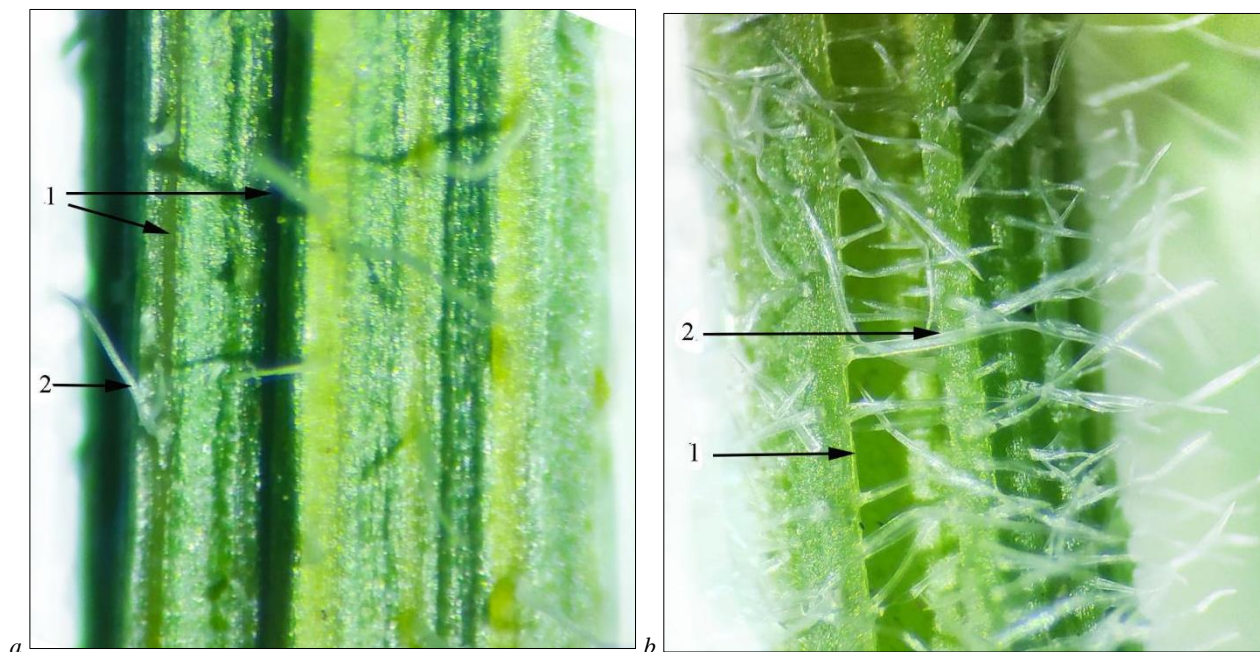
Therefore, the next stage of our work was to conduct a macro- and microscopic analysis of the above-ground part of this plant.

*Diagnostic signs of the herb D. carota.* Macroscopic and microscopic analysis of raw plant materials is one of the critical stages of the pharmacognostic study of medicinal plants, and it serves to establish parameters for the standardization of raw plant medicinal materials. In addition, *D. carota* is an outcrossing species that often hybridizes, significantly complicating the identification of *D. carota* subsp. *carota*.

*Macroscopic analysis of medicinal plant raw materials of D. carota* subsp. *carota*. According to the temporary pharmacopoeial article FS 42-2817-91, which regulates the quality, the medicinal raw materials of the wild biennial herbaceous plant wild carrot (*D. carota*) of the Celery family (Apiaceae) are collected in the period of full maturity. Kyslychenko et al., (2019) described the features of the anatomical structure of the fruits of wild carrot and field carrot, the difference between which was practically not detected. We conducted

morphological and anatomical studies of the above-ground part of *D. carota* subsp. *carota* L., to establish its diagnostic features, particularly the stem, leaves, and flowers. *D. carota* is a hard-fibered biennial, or winter annual, semi-rossette herbaceous plant. The plants develop a rosette shoot in the first year of life, 0.2–0.5 m tall at the mature vegetative stage. In the second year, an erect, branched generative shoot develops from its apical bud. Its is 1.2–1.5 m tall when flower-

ing. The stem is stiff and grooved (polyhedral), covered along the edges with thick, coarse, pointed, two-, three-celled hairs, between which there are unicellular hairs (Fig. 1a, 1b). The taproot is fleshy, straight, conical to cylindrical, 50 mm – 0.5 m long and 20–50 mm in diameter at the top, orange (most common), reddish-violet, yellow or white; the core (xylem) of mature roots is usually somewhat lighter in color than the phloem, and the top of the root is often green.



**Fig. 1.** General view of the *D. carota* stem: in the lower part (a) and in the upper part (b): 1 – stem edges; 2 – stem pubescence with hairs

Rosette leaves are short-petiolate, 140–200 mm long and 40–60 mm wide. The blades are long, broadly ovate in outline, oddly three to four times pinnately dissected into oval-triangular form, incised-toothed, and pointed at the apex of the lobes. There are more than three pairs of first-order segments – often 5–6; their number decreases upwards along the shoot (Fig. 2a). The arrangement of the leaves on the stem is alternate; all stem leaves cover the stem with the base of the blade, forming a sheath, which is typical for species of the Umbelliferaeae family. The lower leaves gradually decrease upwards along the shoot, become sessile, oblong, three times pinnately dissected, with incised-toothed oblong or linear lobes; the upper ones are soft, bent downwards (Fig. 2c). Leaf blades are pubescent along the veins and at the base, especially on the lower side, with unicellular, rarely bicellular, tricellular, sharp hairs, sometimes compressed at the base. The petioles are also densely pubescent (Fig. 2b). The upper leaves are twice pinnately dissected into linear, pointed lobes, almost not pubescent (Fig. 2d). The flowers are densely collected in complex inflorescences – large umbels, in which the flower-bearing stems emerge from one point in rays. Each ray divides again, forming a secondary umbel and white flowers; the outer ones are irregular and larger than the others. Each complex umbel is formed from 25–40 simple, flat umbels with a diameter of up to 10 mm, carrying 30–50 flowers. The peduncles are rough from pubescence and have simple hairs. At the base of the complex umbel is a spathe of several leaves, 1–2-pinnately dissected into linear lobes, the same length as the rays, or shorter. The bracts of the outer individual umbels are 3-part, and the inner ones are simple, styloid, grayish on the outside from ciliate pubescence, and green inside (Fig. 3a, 3b).

The flowers are bisexual; the calyx is poorly developed, consisting of 5 small, inconspicuous teeth. Fruits are enlarged and hard. The corolla is formed of 5 free petals. In the outer flowers of the marginal umbels, the three upper petals are enlarged, white, slightly with a yellowish-pink tint, weakly zygomorphic, deeply cut, 2-lobed lobes; the upper lobe is much larger than the lower one, with a curved tip (Fig. 3a, 4a). In the inner flowers of the marginal umbels, as in all other inner umbels, the petals are the same, deeply cut from above into two larger and smaller lobes, and the edges are curved (Fig. 3b,

4b). The flowers are actinomorphic. An infertile flower on a long stalk is in the middle of the umbel. Stamens alternate with the petals; their filaments are curved inward. Pollination occurs immediately upon bud opening. Fruit formation continues in the flowering stage. Therefore, the stamens of flowers in which fruit is formed fall off.

The inflorescence is concave, densely pubescent with single-celled, sharp hairs arranged in rows (Fig. 4c). The gynoecium is coenocarpous; the pistil is formed due to the two carpels' growth along the abdominal suture. The ovary is inferior, two-nodular. The two carpels in the lower part of the ovary are fused to the abdominal suture, which separates during fruiting into a column – a carpophore. The upper part of the carpels is transformed into a nectary, which usually develops in the form of a subcolumn or stylode, which is cup-shaped (Fig. 4c). There are two columns, and they emerge from the glandular expansion of the ovarian apex. The receptacle is capitular. In the ovary of the pistil, four seed primordia are laid at the base of the subcolumn, two of them do not develop, and in each nest, one hanging seed primordium with one cover remains. The seed usually fuses with the fruit.

When the fruits are ripe, the rays of the umbel come together and the umbels close. The fruit is a dry dicotyledon of an ovoid shape, 3 mm long and 1.5 mm wide, which splits into separate mericarps with long, straight, thin, sharp spines, which are almost not fused at the base and are equal to the width of the fruit (Fig. 5a, 5b). The fruit of a dicot is formed from two carpels that separate. The outer side of the achene is more or less convex, called the dorsum; the inner side, by which the carpels adjoin each other, is the commissure, mostly flat, less often concave. On the dorsum, there are four main, or primary, ribs, two of which lie on the lateral edges of the hemicarps and are called marginal, and the other two are dorsal (Fig. 5c). The central ribs are slightly protruding, covered with bristles and long hairs. Three secondary ribs are visible between them, with a row of long, expanded spines at the base, clearly visible under a magnifying glass. In the spaces between the ribs, there are grooves. Secondary ribs develop above the grooves, and then primary ribs. On the ventral, slightly concave side, two ribs with two rows of hairs slightly protrude (Fig. 5a, 5b). In the middle of the hemi-fruits, secretory tubules contain essential oils. The primary and secondary ribs are transformed

into hooked, tenacious spines and bristles. The color of the fruit is

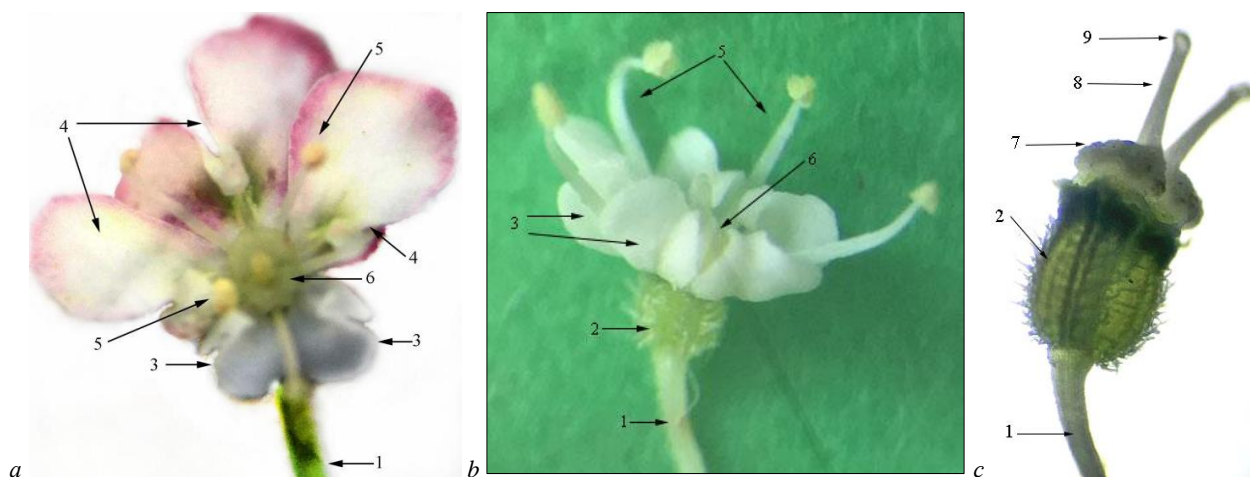
light brown. The ribs, hairs, and spines have a grayish tint.



**Fig. 2.** Appearance of *D. carota* leaf: rosette leaf (a); part of a dissected leaf from the lower side of the blade (b); stem leaf with sheath (c); upper leaf (d): 1 – stem; 2 – sheath; 3 – pinnately dissected leaf; 4 – lobe of the upper leaf blade; 5 – lobe of the lower leaf blade; 6 – petiole; 7 – pubescence of the lower side of the leaf blade



**Fig. 3.** Appearance of *D. carota* inflorescences: separate marginal umbel (a); separate inner umbel (b):  
 1 – marginal flowers of outer umbels; 2 – inner flowers of outer and inner umbels, 3 – bracts; 4 – peduncles



**Fig. 4.** Structure of *D. carota* flower: marginal umbel flower (a), inner umbel flower (b) and gynoecium (c);  
 1 – pedicel; 2 – hairy receptacle with an inferior ovary; receptacle; 3 – lower petals; 4 – upper petals; 5 – stamens;  
 6 – gynoecium; 7 – upper part of the pistil ovary with nectaries; 8 – style; 9 – pistil stigma



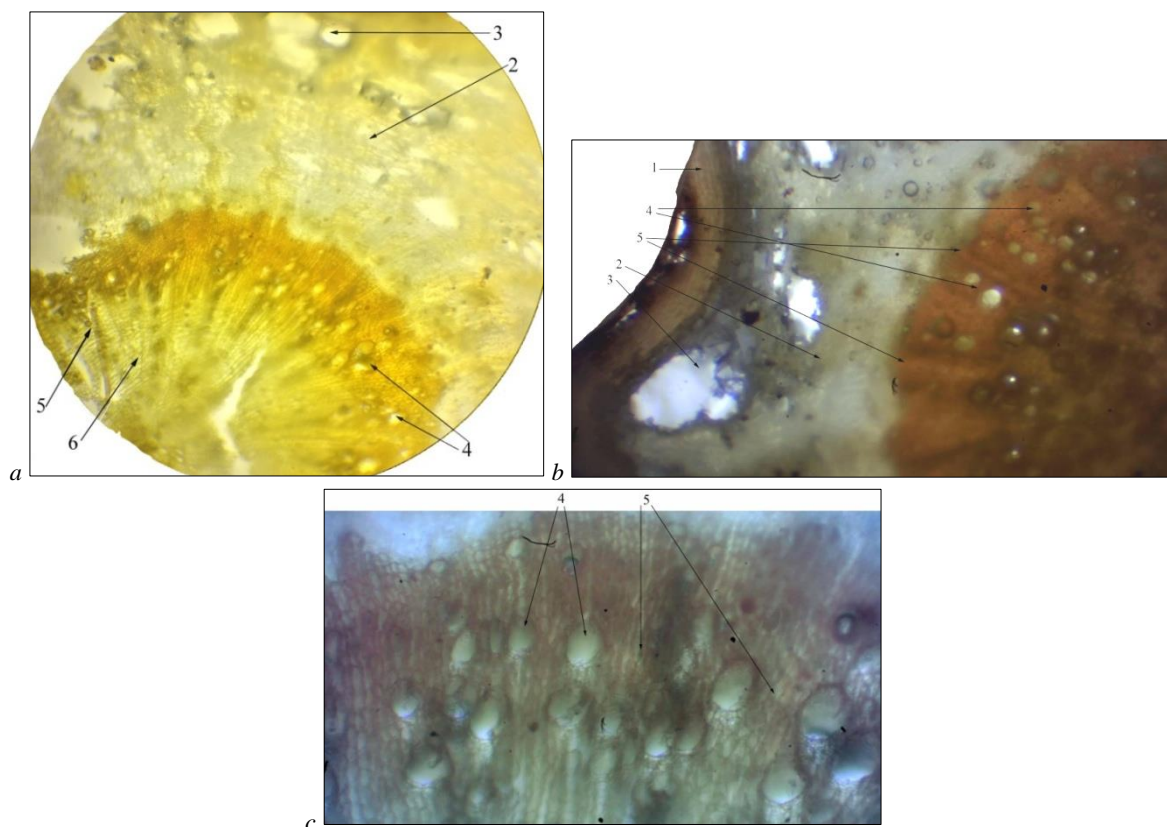
**Fig. 5.** Structure of the fruit of *D. carota*: carpel (a), cross section through carpel (b), rib with long hairs (c): 1 – pedicel; 2 – mericarps; 3 – stylopodium (stylodes); 4 – style; 5 – grooves; 6 – dorsal ribs; 7 – hairs; 8 – marginal ribs; 9 – carpophore; 10 – cross-section through mericarps; 11 – secondary ribs with hooks

The seeds of *D. carota* can be dispersed by wind, usually less than 3 m from the source plant (Quagliotti, 1967). However, the umbel curls into a ball, hence one of its common names, bird's nest, which acts as a seed disperser when detached from the host plant ('tumbleweed' effect). The most standard means of dispersal is attachment to animal fur or human clothing. Shinohara (1984) showed that *D. carota* seeds adhering to wool could be transported 400 km by transhumant sheep flocks, with 7% remaining attached for up to 6 months. Finally, *D. carota* seeds can be transmitted attached to people's clothing or through mud stuck to the underside of vehicles (Stein & Nothnagel, 1995). They can also be transmitted as contaminants of seeds and grain.

*Microscopic analysis of D. carota subsp. carota plant organs.* We conducted an anatomical and histological (microscopic analysis) of the roots, stem, petiole, leaves, flowers, and fruits of *D. carota* to establish diagnostic features of medicinal plant raw materials and recognize its subspecies. The root shows clearly distinguished bark, central cylinder, and pith. The bark is formed from a thin periderm and bast. The periderm has up to 5 layers of cells (Fig. 6b). Nutrients are stored in the bast parenchyma, and schizogenic receptacles develop. Sieve tubes are

in a thin layer above the cambium (Fig. 6a, 6b). The central cylinder is bundle-free, with pronounced vessels in rows between the pith rays (Fig. 6c). The pith is formed from rectangular cells of the primary tissue (Fig. 6a).

The stem in cross-section is rounded, weakly ribbed, and formed from the bark, central cylinder, and pith. The bark contains periderm and ground tissue (Fig. 7.1a, 7.1b). In the primary tissue of the bark, opposite the large conductive bundle, which coincides with the stem rib, is a schizogenic essential oil reservoir (Fig. 7.1d). The rib is formed due to the development of collenchyma, which alternates with chromal-bearing tissue (Fig. 7.1a, 7.1c). Open bilateral conductive bundles are located in the central cylinder. They are formed from secondary vessels, sieve tubes, and remnants of the primary xylem, which is woody (Fig. 7.2e, 7.2i). Over time, the inter-bundle cambium develops additional conductive bundles that are smaller (Fig. 7.2d). At the end of the growing season, there is sclerification of the primary cortex tissue and interfascicular parenchyma, which merges with the rugged bast of the bundle and forms a continuous mechanical ring (Fig. 7.2a). Inside, the stem is filled with large-celled parenchyma.



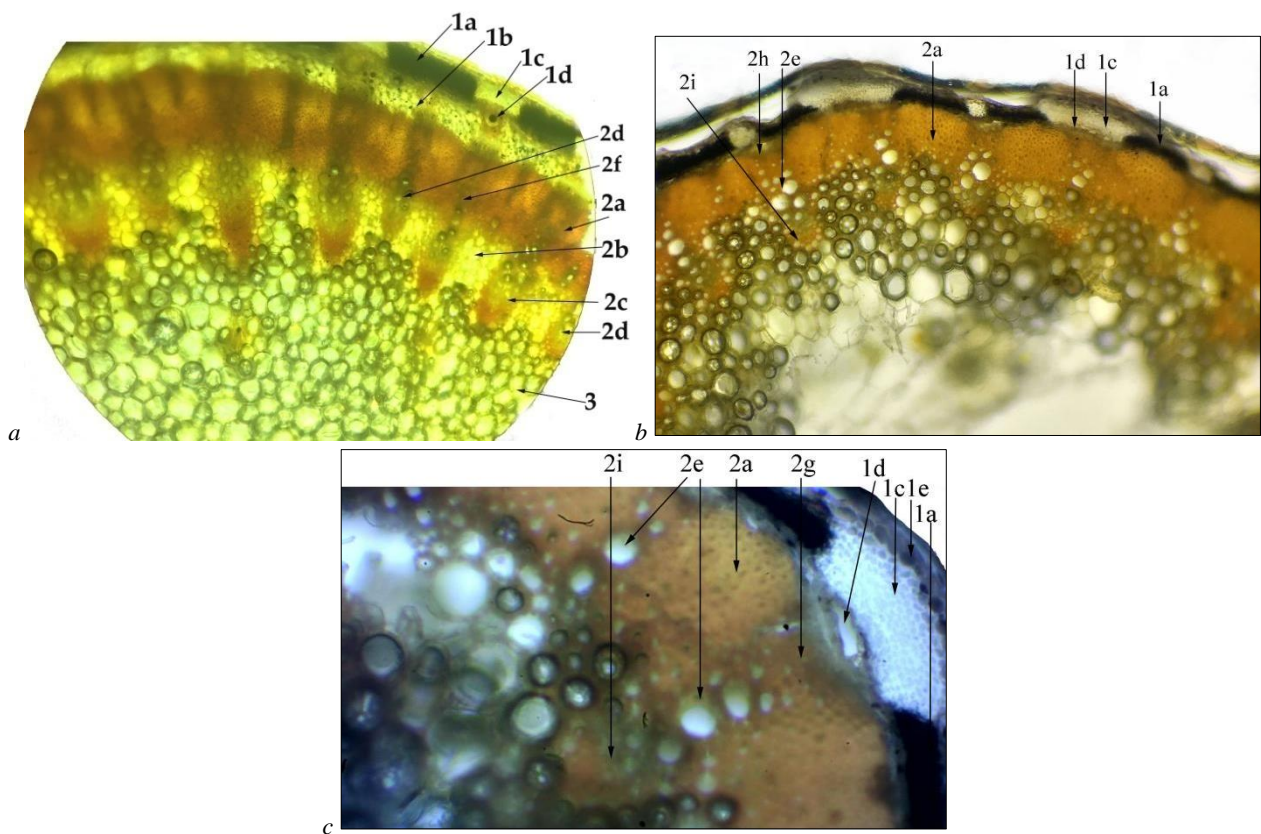
**Fig. 6.** Anatomical structure of the root of *D. carota*: cross-section (a), fragment of cross-section (b), root xylem vessels (c):  
 1 – periderm; 2 – bark parenchyma; 3 – schizogenic essential oil vessels; 4 – xylem vessels; 5 – pith ray; 6 – parenchyma of central cylinder

On the cross-section of the petiole, seven ribs are visible, which are filled with collenchyma. There are two-celled hairs on the ribs and between them (Fig. 8). In the cortex under each rib, there is a schizogenic essential oil reservoir, between which, under the epidermis, a thin starch layer (endoderm) is visible. In the parenchyma of the central cylinder of the petiole, there are open collateral vascular-fibrous conductive bundles. In the middle of the petiole, the most extensive bundle (central vein) develops, on both sides of which there are small ones between the large bundles. In the bundle above the conductive tissues (vessels and sieve tubes), sclerenchyma develops. A thin layer of sclerenchyma develops on the ventral side of the bundle. Above the veins are pointed, simple hairs.

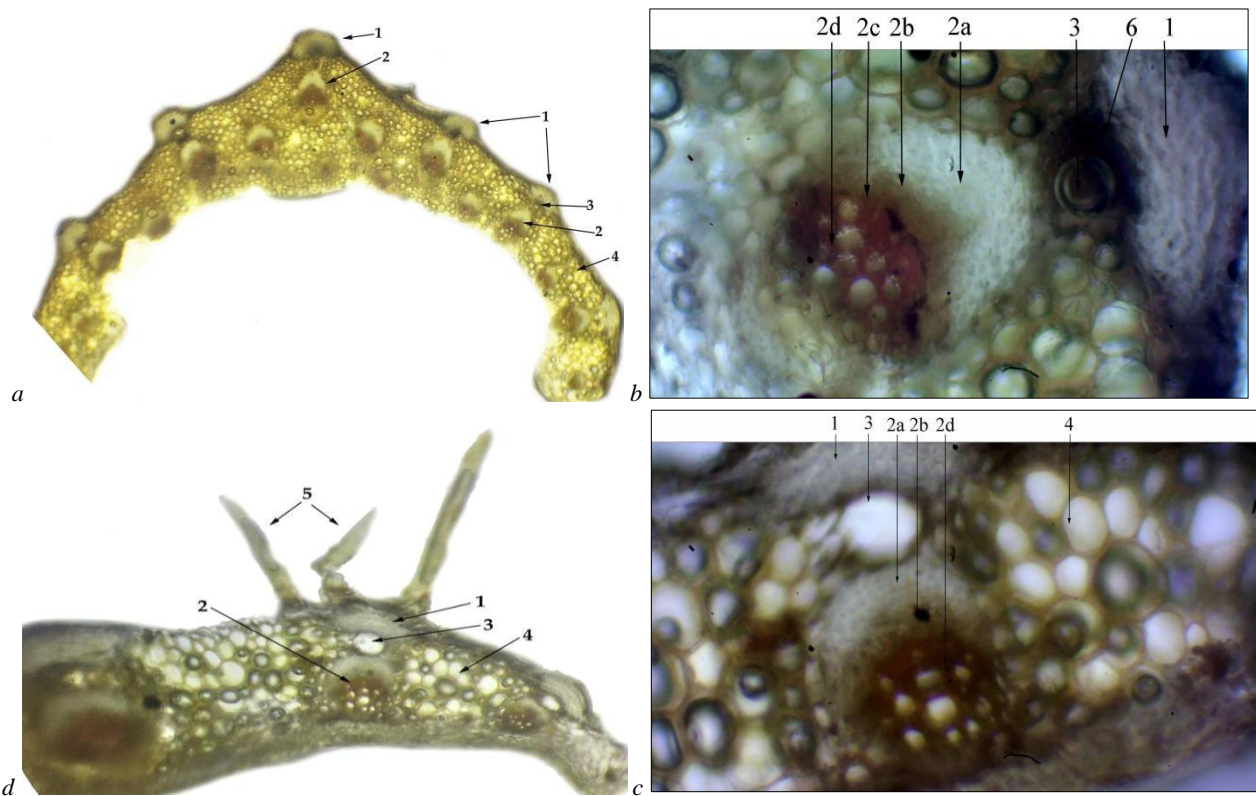
The leaf is simple, pinnately dissected. In the upper part, it becomes dissected, pubescent on the lower side along the midribs with two- and one-celled simple hairs. The cross-section of the leaf was carried out in the area of its lower lobe (Fig. 9). In the center, one thick vein surrounded by covering cells is visible, a vascular-fibrous conductive bundle. The leaf is covered with a colorless single-layer epidermis from above and below. Under the epidermis on the upper side of the leaf, there is columnar parenchyma, formed by two rows of cells, elongated perpendicular to the epidermis. The cells are narrow, with thin cellulose walls and a large number of chloroplasts. This tissue provides photosynthesis. Under the epidermis, on the lower side of the leaf, there is a tissue consisting of wide, irregularly shaped cells that do not fit tightly to each other. This is a spongy parenchyma; its numerous, thin-walled cells of irregular shape have few chloroplasts, and between them, there are pretty wide intercellular spaces. On the border between the spongy and columnar tissues is a layer of special accumulating cells, where the products of assimilation are

collected. These cells contain few chloroplasts (Fig. 9c, 4a, 4b). In the center of the leaf blade, there is a closed (without cambium) collateral conducting bundle (leaf vein) surrounded by sheath cells (Fig. 9.1). Large veins stand out slightly above the upper surface of the leaf, very firmly on the lower, due to the location under the epidermis of the angular collenchyma (Fig. 9.5). The veins are surrounded by sheath cells (Fig. 9.3). These cells do not contain chlorophyll grains, are rounded, adjacent to each other, the intercellular spaces between them are insignificant, and have a lot of cell sap. A layer of sclerenchyma surrounds the bundle. On the lower side of the bundle, the mechanical tissue is thick; above it, the phloem develops, then small vessels (Fig. 9.1d, 1e, 1f). In the veins, the xylem is turned to the upper side of the leaf. The bast is turned to the lower part of the stem, from there the conductive tissues pass into the leaf: the wood lying in the stem from the inside should be at the top of the leaf, and the bast lying in the stem to the outside, in the leaf, is located under the wood. The conductive bundles in the leaf in both monocotyledonous and dicotyledonous plants are closed, and there is no secondary growth in thickness in them.

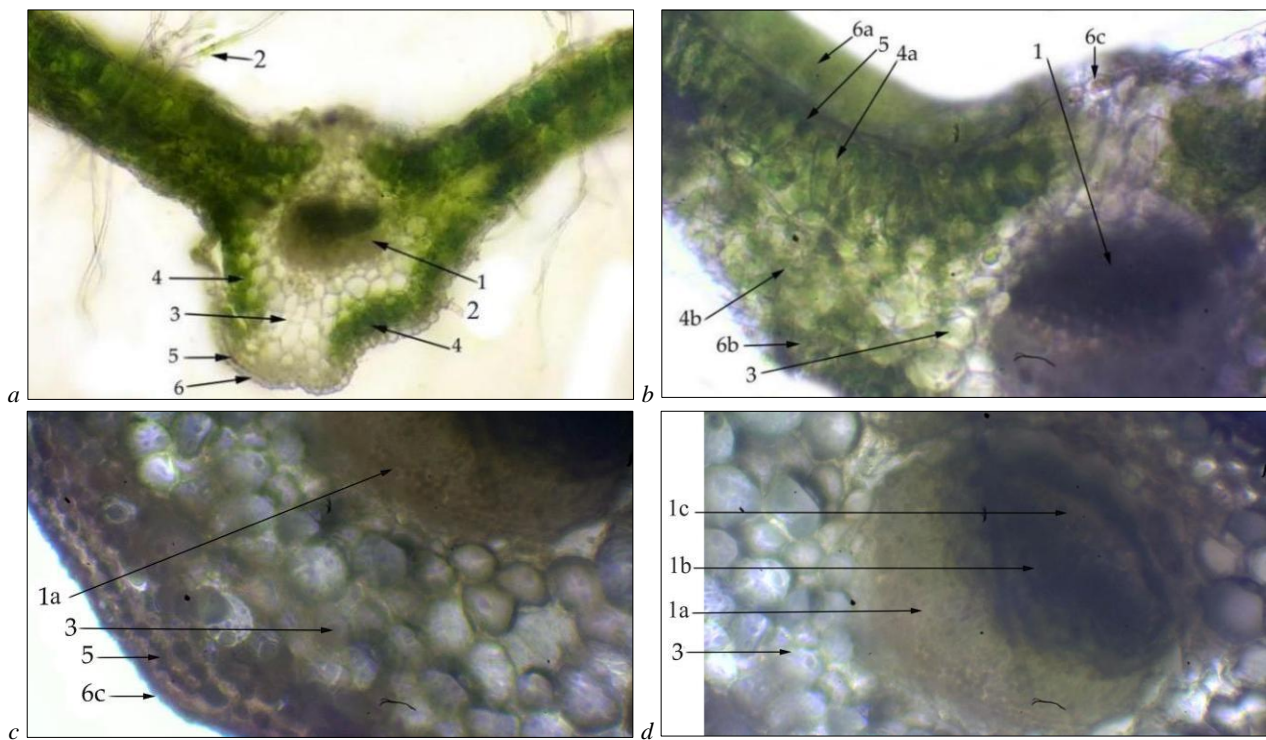
When diagnosing the raw materials of a medicinal plant leaf, the epidermis structure is of great importance. The section shows the leaf covered above and below with a colorless single-layer epidermis (Fig. 10), consisting of thin-walled, chlorophyll-free cells with a cuticle. The cells are alive, with fine-grained, vacuolated protoplasm and a nucleus; on the upper side, they are larger than on the lower. The stomata are very small and located mainly on the lower side of the leaf. The epidermis on the lower side of the leaf blade is formed from rectangular cells with slightly curved walls (Fig. 10a, 10b).



**Fig. 7.** Anatomical structure of the stem of *D. carota*: cross-section through the stem (a), fragment of the cross-section (b), cross-section through the stem in the area of the secretory receptacles (c): 1 – bark: a – starch layer, b – parenchyma of the primary bark, c – rib formed from collenchyma, d – schizogenic essential oil reservoir, e – epidermis; 2 – central cylinder: a – lignified inter-bundle parenchyma, b – core rays, c – conductive bundle, d – additional conductive bundles, e – vessels of the secondary xylem, f – cambial ring, g – secondary phloem, h – bundle sclerenchyma, and – remnants of the primary xylem; 3 – pith



**Fig. 8.** Anatomical structure of the leaf petiole of *D. carota*: cross-section (a), cross-section through the leaf's vascular bundle near the midrib (b), cross-section in the lateral section of the leaf (c), structure of the conductive bundle in the lateral section (d): 1 – rib; 2 – conductive bundle: a – bundle sclerenchyma; b – phloem, c – cambial ring, d – xylem vessels; 3 – essential oil reservoir; 4 – filling parenchyma; 5 – simple hairs; 6 – endoderm



**Fig. 9.** Cross-section of the leaf of *D. carota*: *a* – in the center of the leaf blade; *b* – in the area of the vascular bundle: 1 – central vein (vascular bundle): *a* – sclerenchyma, *b* – phloem, *c* – xylem; 2 – hairs; 3 – sheathing cells of the vascular bundle; 4 – chlorophyll-bearing tissue: *a* – columnar, *b* – spongy parenchyma; 5 – collenchyma; 6 – epidermis: *a* – from the upper, *b* – lower side of the leaf blade, *c* – stomata; *c*, *d* – cross-section through the vascular bundle

It has densely located diacytic-type stomatal apparatuses. At the same time, the upper cell, situated above the stomata, is much larger than the lower one. The peristomatal cells are perpendicular to the stomatal slit. Along the midribs on the lower side, rarely on the upper side, the leaf is pubescent with simple, pointed, unicellular, warty hairs. The epidermis cells above the veins are elongated-rectangular (Fig. 10a). The epidermis on the upper side of the leaf blade is formed from small, polygonal, irregularly shaped cells. The stomata are single; groups of cells with dense cytoplasmic content are observed (Fig. 10c).

Umbels of *D. carota* are also used as a raw material for preparing herbal teas in folk medicine. The marginal flowers of the outer umbels have different petals, unevenly incised into large and small lobes (Fig. 11a, 11b). The upper three are larger; the lower two are smaller in size. The petals are different, have clearly defined veins, and are white or yellowish-white with pink edges. The inner flowers of these umbels and the inner umbels of a complex inflorescence have five identical, broadly oval, white petals, incised in the middle and curved upwards. The epidermal cells are oval, large, with nuclei, and have papillary hairs (Fig. 11c).

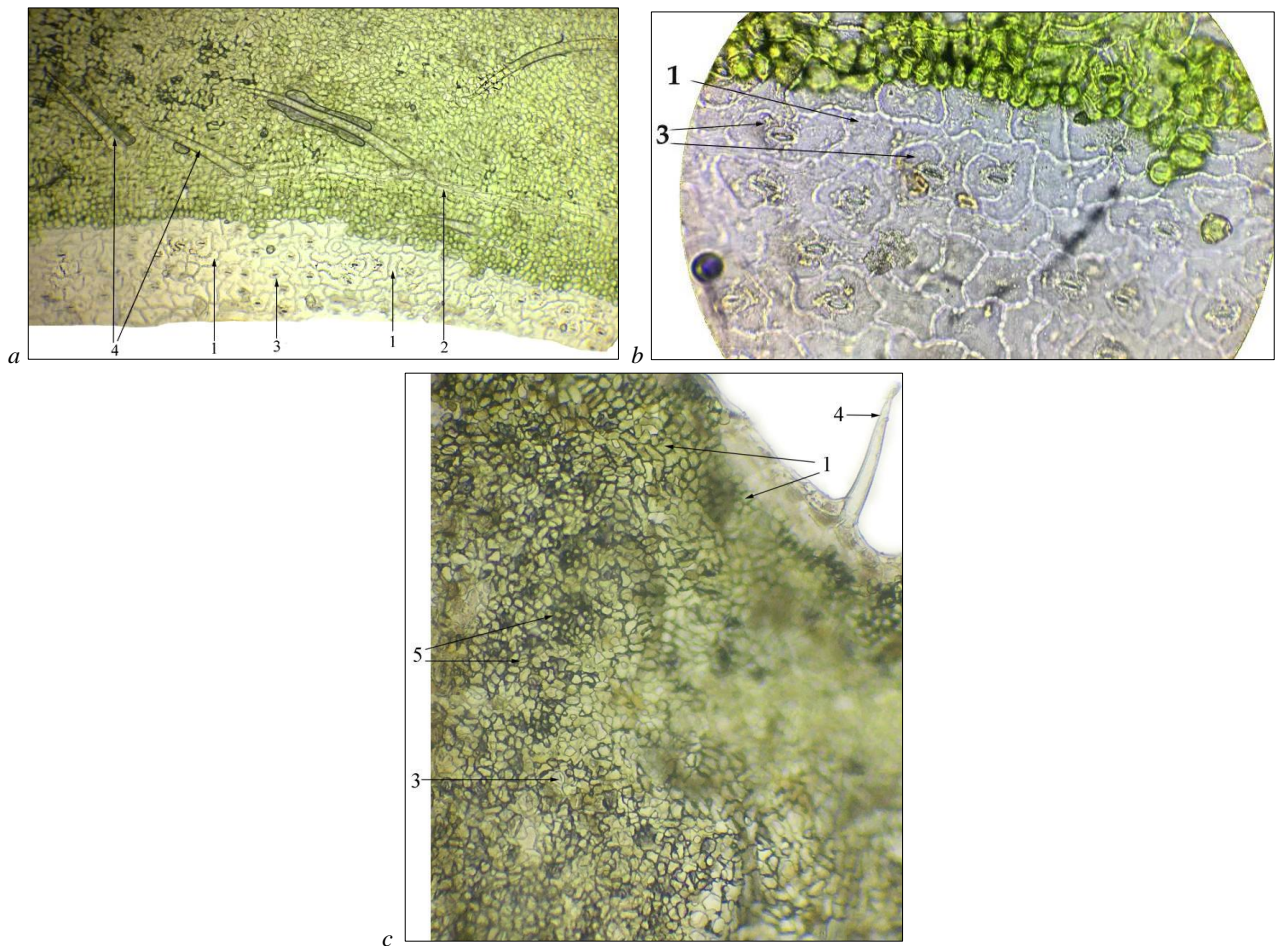
## Discussion

Wild carrot (*Daucus carota* L.) has a cosmopolitan range, distributed from the north of Southern Scandinavia, in Atlantic, Central and Eastern Europe, to the Mediterranean, Central Asia – in the south. As an adventitious plant, it grows in North and South America, Australia, and New Zealand. *D. carota* is a biennial weed that settles in ruderal (changed by man) habitats, for example: in thickets, on degraded pastures or meadows, on areas along railways and roadsides, old pastures, abandoned fields, along fences, wastelands, garbage dumps. It is often found in the herbaceous stage of old field successions, but rarely grows in cultivated areas. Periodic vegetation disturbances are necessary to maintain the population of *D. carota*. The invasive potential of this plant is quite difficult to control because it produces many seeds (Holm et al., 1979). In Ukraine, it grows wild throughout the territory except for the high-mountainous regions of the Carpathians (Flora of the Ukrainian SSR, 1955; Flora Europaea, 1968).

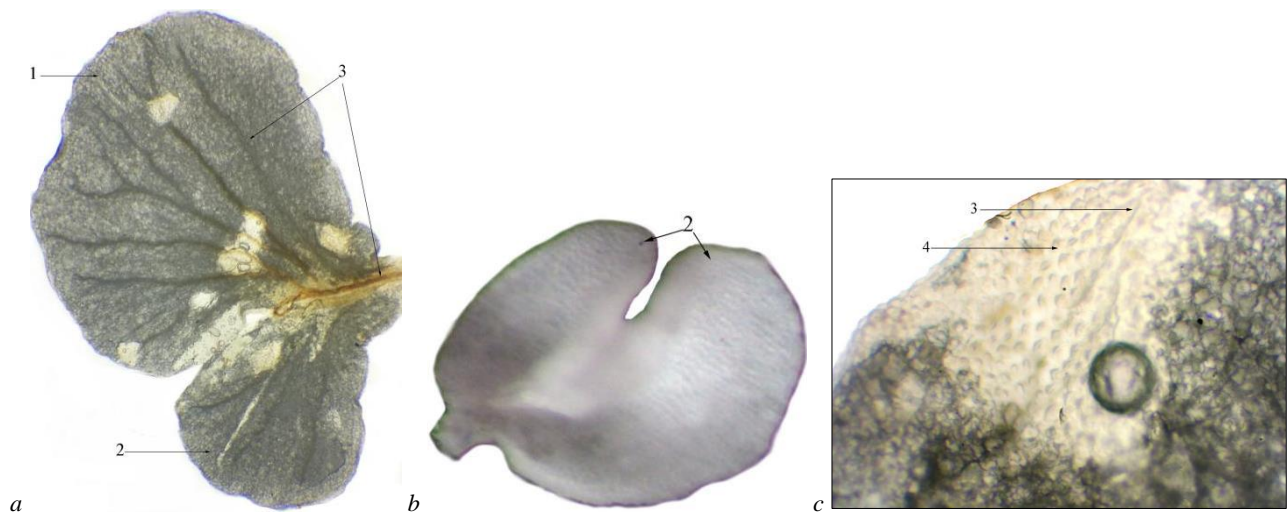
*D. carota* belongs to the family Apiaceae, which includes spicy-aromatic, edible, and poisonous species. According to the Flora of Europe (1968) *Daucus carota* L. (including *D. gingidium* L.) is a polymorphic species. It contains twelve subspecies, which often hybridize with each other, which complicates their identification. Thus, *D. carota* ssp. *sativus* (Hoffm.) Arcang. (the most famous variety) and *D. carota* ssp. *boissieri* (Schweinf.) H. A. Hosni (red carrot) are edible cultivated varieties. The wild carrot subspecies subsp. *carota* with a set of chromosomes  $2n = 18$  has the largest distribution area. Macroscopic analysis can be used to identify closely related subspecies of *D. carota*. According to Heywood (1983), the geographical origin of the plant sample, the plant organs, and its stage of development may affect its chemical composition. Determining the correct plant subspecies of wild carrot in individual localities (biotopes) and its occurrence is significant. A detailed description of macroscopic and microscopic characteristics is fundamental, supplemented by several figures.

Traditionally, wild carrot fruits are used in medicine. A draft national monograph “Wild Carrots Fruits N” was developed, which was included in the State Federal University 2.4. The draft national monograph “Wild Carrots Fruits N” (*Dauci carotae fructus*) indicates that medicinal plant raw materials are dried mature unpolished or polished fruits of the wild biennial herbaceous plant *Daucus carota* L. The main morphological and anatomical diagnostic features of carrot fruits were the presence of spines on the central ribs of the mericarp and simple unicellular hairs on the filamentous ribs or their remains, the presence of round-triangular essential oil tubules and large oval tubules close together on the ventral side of the mericarp (Pavlyuk et al., 2015; Kyslychenko et al., 2019).

In Nigeria (Ayeni et al., 2017), pharmacognostic standards for *D. carota* leaves were established, including moisture content (5.56%), alcohol extractive capacity (15.60%), water extractive capacity (21.80%), total ash (11.60%), acid-insoluble ash (1.00%), and water-soluble ash (5.67%). Diagnostic features of the leaves: alternate arrangement, leaf covering the stem, tripinnate blades, parallel venation with delicate veins, pointed apex, toothed leaf blade. The leaf has a finely pubescent surface, greenish color, minty odor, and pleasant taste. Microscopic features revealed wavy epidermal cells and several diacytic stomata on the lower and upper surfaces.



**Fig. 10.** Structure of the epidermis of *D. carota* leaf: epidermis from the lower side of the leaf blade (magnification 140 times) (a), epidermis from the lower side of the leaf blade (b), epidermis from the upper side of the leaf blade (magnification 270 times) (c): 1 – epidermal cells; 2 – epidermal cells above the large veins; 3 – stomatal apparatus; 4 – unicellular pointed hairs; 5 – groups of cells with dense cytoplasmic content



**Fig. 11.** Flower structure of *D. carota*: large petal of the marginal flower (a), petal of the inner flower (b), appearance of the petal under a microscope (c): 1 – large leaf lobe; 2 – small leaf lobe; 3 – midrib; 4 – papillae

The content of essential oil in wild carrot fruits should be at least 12 mL/kg. The amount of flavonoids in terms of luteolin-7-glucoside is at least 0.20%. Loss in mass upon drying is no more than 13.00%. 1,000 g of powdered raw material (500) is dried at a temperature of 105 °C; total ash is no more than 11.00%; ash insoluble in hydrochloric acid is no more than 1.00%; extractive substances when extracted with 50% ethanol – not less than 12.00% (Ayeni et al., 2017).

The quantitative leaf microscopy of the lower epidermal layer revealed that the stomata number was  $14.80 \pm 0.97$ , stomata index  $31.22 \pm 3.09\%$  and palisade ratio  $6.20 \pm 0.37$ . In contrast, examinati-

on of the upper epidermal layer revealed that the stomata number was  $10.60 \pm 0.87$ , stomata index  $32.55 \pm 1.25\%$ , vein islet number  $12.80 \pm 1.98$ , and veinlet termination  $31.80 \pm 2.33$  (Kyslychenko, 2018).

Over the past two decades, studies have established the significant antioxidant, anti-inflammatory, antitumor, gastroprotective, antibacterial, and antifungal activity of *D. carota*, which has been confirmed *in vitro* and *in vivo*. First, a wide range of pharmacological action is due to the quantitative content and qualitative composition of essential oils, phenols, and flavonoids in plant organs. Essential oils found in schizogenic mericarps of *D. carota* are the most studied of this type

of raw material. Our study revealed a large number of essential oil tubules along the veins of the leaf and along the ribs of the stem, which suggests that wild carrot herba contains a large number of essential oils. In Ukraine it was found that the total content of volatile components in *D. carota* fruits was the highest – 3,850.36 mg/kg, and the main compounds were sesquiterpenes: caryophyllene oxide, carotol, caryophyllene, germacrene,  $\alpha$ -bisabolol, and  $\beta$ -bisabolene. In herba and root crops, the content of EO was 3 times lower than in fruits, and in the qualitative composition, carotol prevailed, less caryophyllene, caryophyllene oxide,  $\alpha$ -bisabolol and  $\beta$ -bisabolene (Pazyuk et al., 2017). In the leaves of *D. carota*, starch, tannins, lignin, cellulose, starch grains, aleurones, mucus, suberin, and cutin were found, but calcium oxalate crystals were not found (Ayeni et al., 2017).

The qualitative composition of essential oil depends on many factors, including growth conditions, climate, place of collection, degree of maturity of fruits, part of the plant, and pollution of the environment (Kryvtsova et al., 2022). Thus, in Lithuania, *D. carota* plants growing near roads contained 83.4–95.1% monoterpenoids and 3.4–10.5% sesquiterpenoids in their essential oil. Plants growing far from roads had a sesquiterpenoid chemotype. This is apparently due to different types of pollution in the growing sites (Mockute & Nivinskienė, 2004). In plants growing in the urban area of Vienna, the quantitative and qualitative content of essential oil of *D. carota* subsp. *carota* also varies. The essential oil yield was – 0.2% to 1.6% for roots, leaves, and fruits. The root oil was dominated by monoterpenes:  $\alpha$ -terpinolene. The leaves contained  $\alpha$ -pinene and sabinene, less myrcene and limonene. The EOs  $\alpha$ -pinene, sabinene geranyl-acetate dominated in the fruits (Chizzola, 2010).

As is known from the literature, the sesquiterpene chemotype (CEO) dominates in *D. carota*, but it is more common in the Mediterranean, while the monoterpene chemotype tends to prevail in temperate climates. Thus, essential oils of *D. carota* subsp. *carota* from Poland, Portugal and Serbia had a high content of essential oil (1.67%), but its composition was dominated by monoterpene hydrocarbons (50.0–71.9%): sabinene,  $\alpha$ -pinene, geranyl acetate, and 11 $\alpha$ H-himachal-4-en-1 $\beta$ -ol, oxygenated monoterpenes (Staniszewska et al., 2005; Acimovic et al., 2016; Alves-Silva et al., 2016). The essential oil of *D. carota* from subtropical Corsica was dominated by aromatic compounds. Further studies have shown that the essential oil of *D. carota* and its purified and chemically synthesized molecules, (E)-methylisoeugenol, elemicin, and eugenol, exhibited antibacterial effects (Gonny et al., 2004; Dedieu et al., 2020). The main components of the essential oil of flowering and mature umbels with fruits of *D. carota* subsp. *carota* from Sejnane were sesquiterpenes and their derivatives: eudesm-7(11)-en-4-ol, carotol, sabinene,  $\alpha$ -selinene, and 11- $\alpha$ -H-himachal-4-en-1-beta-ol. The sesquiterpene chemotype (carotol, elemicin, limonene) is also characteristic of the fruits of *D. carota* growing in Turkey, Tunisia and Montenegrin coast (Özcan & Chalchat, 2007; Marzouki et al., 2010; Servi et al., 2021; Stanojević et al., 2023).

Phenolic substances accumulate in various parts of wild carrot plants. In the roots of *D. carota*, 270.45 mg/kg of the total phenolic compounds were accumulated, 92% of which was neochlorogenic acid; the rest was caffeic acid and flavonoid – luteolin. The content of phenolic compounds in the herbs was 99.64 mg/kg, of which one third was hydroxycinnamic acids: chlorogenic, ferulic acid, and a little caffeic acid. Among the phenolcarboxylic acids, salicylic and gallic acids prevailed. In the herb, 27.50 mg/kg of flavonoids, including rutin and cynaroside, accumulated. The most significant amount of phenols, 700.26 mg/kg, was identified in the fruits of *D. carota*, of which acyclic quinic acid accounted for 76%. The rest was composed of chlorogenic acid and 10 flavonoids (125.72 mg/kg), including: cynaroside (65–70% of the total flavonoid content), rutin, hyperoside, scolymoside. *D. carota* has been found to have a high content of coumarins: in fruits (0.85%), herb 0.40–0.44%, traces of coumarins have been found in root crops (Pazyuk et al., 2017).

The content and composition of flavonoids in the plant raw materials of *D. carota* fluctuates and depends on the growing conditions. Thus, in industrial batches it is 0.103–0.267%, in wild samples from Western Ukraine – 0.080–0.124% in terms of apigenin (Chubka et al., 2013). The composition and ratio of flavonoids in terms of luteolin in

samples of raw materials from the Western region of Ukraine ranged from 0.114% to 0.792%, the main components were ferulic acid, rutin, luteolin, and apigenin. From Crimea, the content of flavonoids was 0.071–0.080% in terms of hyperoside, which dominated, and quercetin, kaempferol, chlorogenic, caffeic, chicory, and rosmarinic acids was also identified (Smalyukh et al., 2013; Stadnytska et al., 2014).

As can be seen from the analysis of literary sources, a high content of various biologically active substances is present not only in the fruits, but also in the herba and umbels of *D. carota*, which can also be raw materials for the manufacture of various drugs. For this purpose, we studied the content of phenols and coumarins in the leaves and inflorescences of wild carrot. We established their morphological and anatomical features for identifying medicinal raw materials in the flowering phenophase. Given the significant content of various biologically active substances: phenols, flavonoids, essential oils not only present in the fruits, but also in other parts of the plant – inflorescences, leaves (herb) of wild carrot, the natural reserves of which are large, it is advisable to conduct further scientific research. Based on a detailed pharmacognostic analysis, we intend to write a pharmacopoeial monograph and propose the raw herb for use in the manufacture of medicinal products.

## Conclusions

As a result of the conducted research, a significant content of phenolic glycosides (pyrocatechin) was established in the extract from the raw material of *D. carota* flowers, less in the leaves, which gives grounds to speak about the expediency of using *D. carota* herbs for further scientific research.

As a result of the conducted macro- and microscopic pharmacognostic analysis, diagnostic signs of medicinal plant raw materials of wild carrot (*Daucus carota* L.) – herbs (*Herba dauci*) were identified. It was established that the rosette leaves of *D. carota* are short-petiolate, rich green, oblong in outline, twice-, thrice-pinnately dissected, with incised-toothed lobes. The leaves become sessile up the shoot, forming a sheath; their terminal lobes gradually become oblong or linear. The leaves are pubescent along the veins and at the base, especially on the underside, with unicellular, less often bicellular, tricellular sharp hairs, sometimes appressed.

The inflorescence is a complex umbel; each ray forms a secondary umbel, and white flowers. At the complex umbel's base is a spathe of several whole pinnately dissected leaflets. The leaflets of the spathes grow together to the middle or above, forming a continuous coverlet. The involuclers are made of 3-partite or whole linear pointed leaflets. The flowers are bisexual, regular. The calyx consists of 5 small teeth. The five petals are white, with a slightly yellowish-pink tint, notched, two-lobed, and the extreme ones have a tip bent inward. In the outer flowers of the umbel, three petals are enlarged.

The epidermal cells flower petals are oval and large, with papillary hairs. There are five stamens. The peduncle is concave, densely pubescent with single-celled sharp hairs. The gynoecium is coenocarpic; the pistil is formed due to the growth of two carpels along the ventral suture. The ovary is inferior, two-nodular. There are two columns, and they emerge from the glandular expansion of the ovary apex. The stigma is capitate. The fruit composed of two small achenes (mericarps) fused to a central filament (carpophore); the pericarp bears ridges, usually with oil-canals (vittae) between the main ridges.

Microscopic analysis has established that the epidermis on the underside of the leaf blade is formed of rectangular cells with weakly curved walls. The stomata are of the diacetic type; the upper cell above the stomata is larger than the stomata. The cells of the epidermis above the veins are elongated-rectangular. Along the veins are pointed, unicellular, warty hairs. The epidermis on the upper side of the leaf blade is formed from small polygonal, irregularly shaped cells; it lacks stomata. Under the epidermis on the upper side of the leaf is columnar parenchyma, formed by 1–2 rows of cells, elongated perpendicular to the epidermis. The cells are narrow, with thin cellulose walls and a large number of chloroplasts. The spongy tissue consists of wide, irregularly shaped cells that do not fit tightly together. There is a layer of special, accumulating cells on the border between the spon-

gy and palisade tissues. In the center of the leaf blade is a closed collateral conductive bundle surrounded by sheath cells. Large veins stand out slightly above the upper surface of the leaf and very strongly on the lower, due to the presence of subepidermal collenchyma. The veins are surrounded by sheath cells without chlorophyll grains, rounded, adjacent, and have a lot of cell sap. A layer of sclerenchyma surrounds the bundle. It is thick on the lower side of the bundle; under it are vessels and sieve tubes.

*Daucus carota* umbels are also used as raw materials. The marginal flowers of the outer umbels have different petals, unevenly incised into large and small lobes. The upper three are larger, the lower two are smaller in size. The petals are different, have clearly defined veins, and are white or yellowish-white with pink edges. The inner flowers of these umbels and the inner umbels of a complex inflorescence have five identical, broadly oval, white petals, incised in the middle, and curved upwards. The epidermal cells are oval and large, with nuclei and papillary hairs.

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