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Modern Aspects of Pharmacognostic Analysis of Wild Carrot Fruits (*Daucus carota* L.)

V.V. Artemyeva[1], G.M. Dandash[1], I.N. Zilfikarov[1, 2], D.I. Shishkalov[1], V.M. Ryzhov[3], T.K. Ryazanova[3], I.I. Bochkareva[1], Z.A. Guseynova[4], A.K. Arutyunov[1]

[1] Maykop State Technological University,
191 Pervomayskaya Str., Maykop, Russia, 385000,
[2] All-Russian Scientific Research Institute of Medicinal and Aromatic Plants,
7A Grin Str., Bldg. 1, Moscow, Russia, 117216
[3] Samara State Medical University,
89 Chapaevskaya Str., Samara, Russia, 443099
[4] Dagestan Federal Research Center, Russian Academy of Sciences,
75 Magomed Yaragsky Str., Makhachkala, Russia, 367030

E-mail: denis7radnet.ru@mail.ru

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The aim. Pharmacognostic study of wild carrot fruits: identification of the main microscopic features; chromatographic-mass spectrometric study of volatile compounds and determination of numerical commodity indicators of medicinal plant raw materials.

Materials and methods. Morphological and anatomical features of wild carrot root raw materials were studied by light microscopy; luminescence of tissues and working standard samples of substances were studied by luminescent microscopy; crystalline structures were studied by polarization microscopy; the profile of volatile compounds in raw materials was assessed using chromatographic-mass spectrometry. The preparation of microscopic slides, the determination of numerical commodity indicators of medicinal plant raw materials were carried out according to the requirements of the State Pharmacopoeia of the Russian Federation XV edition.

Results. Morphological and anatomical analysis established the characteristics of carrot fruits: oval shape of the fruit, tapering towards the apex, brown in color with seven veins; schizogenous containers of angular shape with non-cellular septa; endosperm parenchyma of isodiametric, oval cells; endocarp of parquet type from elongated, thick-walled, tightly closed cells; stellate druses in the tissues of the embryo with four symmetrical rays. Luminescence in the excitation range of 330–400 nm of the mesocarp — bright blue; epithelial cells — light yellow; resinous secretion — blue; chromatographic-mass spectrometric study of hexane and chloroform fractions of the extract from carrot fruits determined the profile of volatile compounds, with carotol being predominant (25.9% and 30.1%, respectively).

Conclusion. The identified microscopic features, the main biologically active substance, and numerical commodity indicators are of practical importance for inclusion in the draft pharmacopoeial monograph “Wild Carrot Fruits”.

Keywords: wild carrot fruits; light microscopy; luminescent microscopy; polarization microscopy; anatomical and morphological features; diagnostic features; chromato-mass spectrometry; carotol; numerical commodity indicators

Abbreviations: MPRMs — medical plant raw materials; BASs — biologically active substances; SPh RF — State Pharmacopoeia of the Russian Federation; BAAs — biologically active additives (food supplements); GPM — general pharmacopoeial monograph; RS — reference standard; PM — pharmacopoeial monograph; GC-MS — gas chromatography-mass spectrometry; UV light — ultraviolet light.

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Современные аспекты фармакогностического анализа плодов моркови дикой (*Daucus carota* L.)

В.В. Артемьева[1], Г.М. Дандаш[1], И.Н. Зилфикаров[1, 2], Д.И. Шишкалов[1], В.М. Рыжов[3], Т.К. Рязанова[3], И.И. Бочкарева[1], З.А. Гусейнова[4], А.К. Арутюнов[1]

[1] Федеральное государственное бюджетное образовательное учреждение высшего образования «Майкопский государственный технологический университет», Россия, 385000, г. Майкоп, ул. Первомайская, д. 191

[2] Федеральное государственное бюджетное научное учреждение «Всероссийский научно-исследовательский институт лекарственных и ароматических растений», Россия, 117216, г. Москва, ул. А. Грина, д. 7, стр. 1

[3] Федеральное государственное бюджетное образовательное учреждение высшего образования «Самарский государственный медицинский университет» Минздрава России, Россия, 443099, г. Самара, ул. Чапаевская, д. 89

[4] Федеральное государственное бюджетное учреждение науки «Дагестанский федеральный исследовательский центр» Российской академии наук, Россия, 367030, г. Махачкала, ул. Магомеда Ярагского, д. 75

E-mail: denis7radnet.ru@mail.ru

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Цель. Фармакогностическое изучение плодов моркови дикой: выявление основных микроскопических признаков; хромато-масс-спектрометрическое исследование летучих соединений и определение числовых товароведческих показателей лекарственного растительного сырья.

Материалы и методы. Морфолого-анатомические признаки сырья плодов моркови дикой исследовали световой микроскопией; люминесценцию тканей и рабочих стандартных образцов субстанций изучали люминесцентной микроскопией; кристаллические структуры исследовали поляризационной микроскопией; профиль летучих соединений в сырье оценивали с помощью хромато-масс-спектрометрии. Изготовление микропрепаратов, определение числовых товароведческих показателей лекарственного растительного сырья осуществляли по требованиям Государственной Фармакопеи Российской Федерации XV издания.

Результаты. С помощью морфолого-анатомического анализа установили особенности плодов моркови: овальная форма плода, зауженная к верхушке, коричневого цвета с семью жилками; схизогенные вместилища угловатой формы с перегородками неклеточного строения; паренхима эндосперма из изодиаметрических, овальных клеток; эндокарпий паркетного типа из вытянутых, толстостенных, плотно сомкнутых клеток; звёздчатые друзы в тканях зародыша с четырьмя симметричными лучами. Люминесценция в диапазоне возбуждения 330–400 нм мезокарпия — ярко-голубая; клеток эпителия — светло-желтая; смолянистого секрета — голубая. Хромато-масс-спектрометрическое исследование гексановой и хлороформной фракции извлечения из плодов моркови определило профиль летучих соединений, преобладающим является каротол (25,9 и 30,1%, соответственно).

Заключение. Выявленные микроскопические признаки, основное биологически активное вещество и числовые товароведческие показатели имеют практическое значение для включения в проект фармакопейной статьи «Моркови дикой плоды».

Ключевые слова: плоды моркови дикой; световая микроскопия; люминесцентная микроскопия; поляризационная микроскопия; анатомо-морфологические признаки; диагностические признаки; хромато-масс-спектрометрия; каротол; числовые товароведческие показатели

Список сокращений: ЛРС — лекарственное растительное сырье; БАВ — биологически активные вещества; ГФ РФ — Государственная фармакопея Российской Федерации; БАД — биологически активные добавки к пище; ОФС — общая фармакопейная статья; СО — стандартный образец; ФС — фармакопейная статья; ГХ-МС — газовая хроматография с масс-спектрометрией; УФ-свет — ультрафиолетовый свет.

INTRODUCTION

The fruits of wild carrot (also known as common carrot) represent a medicinal plant raw material (MPRM) and a promising source of biologically active compounds (BACs) of phenolic and terpenoid nature. The interest in wild carrot fruits has been demonstrated by numerous domestic and international researchers. This raw material is known to exhibit antibacterial, antifungal [1, 2], and anti-inflammatory activities [3–5]. In particular, studies have reported its diuretic and choleretic effects in both wild and cultivated carrot fruit extracts [6–8]. An aqueous-alcohol extract of wild carrot fruits is an official substance used in the pharmaceutical preparations “Urolesan” (ART-PHARM LLC, Russia) and “Urokhol” (VIFITEH CJSC, Russia). Extractive substances from this MPRM are also included in the dietary supplement “Urolit” (VITAUKT-PROM LLC, Republic of Adygea,Russia). However,

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despite the phytotherapeutic significance of this plant species, no pharmacopoeial monograph for this raw material is included in the current edition of the State Pharmacopoeia of the Russian Federation (SPh RF).

Key stages of the standardization and subsequent quality control of MPRM include the establishment of identity through external (morphological) and microscopic (anatomical) features, detection of major BACs, and assessment of raw material quality by the determination of numerical quality indicators. Previously, researchers identified taxonomic and phylogenetic relationships between representatives of the genus *Daucus* L. [9], and studied the main anatomical and diagnostic features of pharmacopoeial MPRM [10]. Nevertheless, in the course of updating the regulatory documentation for medicinal products from wild carrot fruits, we are implementing the task of updating the pharmacopoeial monograph on this type of MPRM for the new edition of the SPh RF, taking into account better practices and updated requirements for microscopic examination. Modern chromatographic analysis methods make it possible to better characterize the component composition of the low-polar volatile fraction of secondary metabolites, thereby providing a more accurate identification and reliable assessment of the goodness of MPRM containing essential oils.

THE AIM of the study is a pharmacognostic study of wild carrot fruits: identification of the main morphological and anatomical diagnostic features, taking into account modern requirements for microscopy techniques; chromato-mass spectrometric study of the profile of volatile organic compounds and determination of numerical commodity indicators of the quality of medicinal plant raw materials.

MATERIALS AND METHODS

he objects of study

The object of study was industrial samples of wild carrot fruits provided by VITAUKT-PROM LLC (Republic of Adygea, Russian Federation). The raw materials were harvested in 2023 in the Krasnodar Territory (Ust-Labinsky district) and comply with the requirements of GOST 32592-2013 “Seeds of vegetable, melon crops, fodder root crops and fodder cabbage. Varietal and sowing qualities. General technical conditions” for the category of reproductive seeds.

Macroscopic, microscopic, microchemical and histochemical analyses

The external features of MPRM were examined visually and using a Motic DM 39 stereomicroscope (Motic Xiamen, China), based on the requirements of the general Pharmacopoeia monograph GPhM.1.5.1.0007 “Fruits”[1] and GPhM.1.5.1.0008 “Seeds”[2].

Anatomical (microscopic) signs of raw materials were examined using light microscopy in transmitted and reflected light on a Motic DM1802 light microscope (Motic Xiamen, China). Micro-preparations were prepared according to GPhM.1.5.3.0003 “Microscopic and microchemical analysis of medicinal plant raw materials and medicinal products of plant origin”[3] and GPhM.1.5.3.0003.15 “Technique of microscopic and microchemical examination of medicinal plant raw materials and medicinal herbal preparations”[4]. Reagents for microchemical and histochemical analysis were prepared in accordance with GPhM.1.3.0001 “Reagents. Indicators”[5].

The analysis of the luminescence of fruit tissues, as well as working standard samples of substances and plant raw materials, was carried out using an Altami LUM-2 luminescent microscope (Altami LLC, Russian Federation) with a special configuration and using blue and yellow light filters of 32 mm. The light source was a high–voltage mercury lamp (HBO 100W); luminescence excitation spectral range: blue excitation filter — 420–550 nm; UV-filter — 330–400 nm. The standard samples (SS) Quercetin and SS Coumarin provided by the Pharmacy Research Center of the Federal State Budgetary Educational Institution of Higher Medical Education of the Ministry of Health of the Russian Federation were used as working standard samples of phenolic nature. The luminescence of SS substances was evaluated directly in air–dry form by irradiating crystals of substances on a slide under an Altami LUM-2 microscope at various magnifications. The luminescence of crystals of SS substances in the form of a suspension in purified water on a slide covered with a cover glass was also evaluated.

[1] GPhM.1.5.1.0007 “Fruits”. State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/
izdanie-15/1/1-5/1-5-2/plody/

[2] GPhM.1.5.1.0008 “Seeds”. State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/ izdanie-15/1/1-5/1-5-2/semena/

[3] GPhM.1.5.3.0003 “Microscopic and microchemical analysis of medicinal plant raw materials and medicinal products of plant origin”. State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/ izdanie-15/1/1-5/1-5-1/mikroskopicheskiy-i-mikrokhimicheskiy-analiz-lekarstvennogo-rastitelnogo-syrya-i-lekarstvennykh-sred/

[4] GPhM.1.5.3.0003.15 “Technique of microscopic and microchemical examination of medicinal plant raw materials and medicinal herbal preparations”. State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia. regmed.ru/pharmacopoeia/izdanie-13/1/1-5/1-5-3/1-5-3-3/ tekhnika-mikroskopicheskogo-i-mikro

[5] GPhM.1.3.0001 “Reagents. Indicators”. State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/ pharmacopoeia/izdanie-15/1/1-3/reaktivy-indikatory

If necessary, for contrasting, the method of polarization microscopy on a PLM 213 polarization microscope (LOMO JSC, Russia) was used in the study of crystalline structures. Cross-sections of fruits were prepared manually (for luminescence of dry raw materials), as well as on an HM 325 Thermo FS microtome (Thermo Fisher Scientific, USA).

Sample preparation for GC-MS analysis

To analyze the volatile organic compound (VOC) profile via gas chromatography–mass spectrometry (GC–MS), aqueous-ethanol extracts were prepared using 60% ethanol as the extractant by modified fractional maceration in three extractors (raw material-to-solvent ratio of 1:5), followed by a final thermal stage — heating for 30 minutes at 70°C — as described in the invention patent by V.A. Kurkin [11]. The resulting extract was kept for 48 hours at 8 °C in a light-protected environment, then filtered through "Red Ribbon" ashless filter paper into a dark glass bottle and sealed with polyethylene stoppers and screw caps.

Further, the hexane and chloroform fractions of the amounts of substances that were analyzed were obtained from alcohol-water extraction. The choice of fractions is determined by the literature data that volatile organic compounds, which may be part of the studied plants, are soluble in these extractants [12–14].

For this, 10.0 mL of the aqueous-ethanol extract was placed in a separatory funnel (30–50 mL volume), and 5 mL of *n*-hexane (or chloroform) was added. The mixture was shaken for 2 minutes and then left to separate completely. The upper hexane (or lower chloroform) phase was filtered through "Red Ribbon" filter paper into a 25 mL volumetric flask. The extraction was repeated two more times with 5 mL portions, filtering each into the same volumetric flask. The final volume was adjusted to the mark with *n*-hexane (or chloroform), and the solution was mixed.

GC-MS analysis

The analysis was performed under the conditions described earlier [15, 16]. The chromatographic conditions of the analysis are presented in Table 1.

Component identification was performed by calculating linear retention indices and comparing both the results and full mass spectra with reference databases (NIST 2.4 mass spectral library) and published literature. Only components with identification probabilities above 90% were considered. The relative content of each component was calculated by internal normalization using peak area percentages on the total ion current chromatogram [17, 18].

Numerical Quality Indicators

Numerical quality indicators of wild carrot fruits characterize their good quality, compliance with the rules of harvesting and primary processing, transportation and storage conditions, as well as the accumulation of certain secondary metabolites, in particular essential oil, concentration of macro- and microelements, heavy metals, etc. One of the stages in the formation of regulatory documentation on quality and the justification of the MPRM specification is the experimental establishment of normalized indicators and ranges of acceptable values.

Wild carrot fruits are processed both in whole and crushed form, and can also be included in the composition of medicinal products as an active component in powder form, which served as the basis for establishing most of the standardized numerical indicators. The analysis was carried out in accordance with the GPhM.1.5.1.0007 "Fruits", GPhM.1.5.3.0010.15 "Determination of essential oil content in medicinal plant raw materials and medicinal herbal preparations"[6], GPhM.1.5.3.0006 "Determination of the content of extractive substances in medicinal plant raw materials and medicinal herbal preparations"[7], GPhM.1.2.2.2.0013 "Total ash"[8], GPhM.1.5.3.0005.15 "Ash insoluble in hydrochloric acid"[9], GPhM.1.2.2.2.0014 "Sulphate ash"[10], GPhM.1.2.2.2.0012 "Heavy metals"[11], GPhM.1.2.1.0010 "Loss in weight during drying"[12] of SPh RF.

[6] GPhM.1.5.3.0010.15 "Determination of essential oil content in medicinal plant raw materials and medicinal herbal preparations". State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/ izdanie-14/1/1-5/1-5-3/opredelenie-soderzhaniya-efirnogo-masla-v-lekarstvennom-rastitelnom-syre-i-lekarstvennykh-rastitelny/

[7] GPhM.1.5.3.0006 "Determination of the content of extractive substances in medicinal plant raw materials and medicinal herbal preparations". State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-5/1-5-1/opredelenie-soderzhaniya-ekstraktivnykh-veshchestv-v-lekarstvennom-rastitelnom-syre-i-lekarstvennykh/

[8] GPhM.1.2.2.2.0013 "Total ash". State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/ izdanie-15/1/1-2/1-2-2/1-2-2-2/obshchaya-zola/?sphrase_id=1143165

[9] GPhM.1.5.3.0005.15 "Ash insoluble in hydrochloric acid". Available from: https:// pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-14/1/1-5/1-5-3/ zola-nerastvorimaya-v-khloristovodorodnoy-kislote/

[10] GPhM.1.2.2.2.0014 "Sulphate ash". State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/ pharmacopoeia/izdanie-15/1/1-2/1-2-2/1-2-2-2/sulfatnaya-zola/

[11] GPhM.1.2.2.2.0012 "Heavy metals". State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-2/1-2-2/1-2-2-2/tyazhyelye-metally/

[12] GPhM.1.2.1.0010 "Loss in weight during drying". State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia. regmed.ru/pharmacopoeia/izdanie-15/1/1-2/1-2-1/poterya-v-masse-pri-vysushivanii/

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RESULTS

Macroscopic, microscopic, microchemical and histochemical analyses

During the analysis of raw materials, a direct correspondence was established with the morphological characteristics described earlier in the literature [9]. Mericarps of fruits are oval or oblong in shape, tapering towards the apex. The color of the fruits is brown or light brown (Fig. 1, A). Highly protruding veins of different sizes are localized on the surface of each mericarp. The number of veins is stable and amounts to 7 on each mericarp (Fig. 1, B–G). Their sizes differ. Thus, it is customary to distinguish furrowed veins in the number of 2; lateral primary veins in the number of 2; dorsal secondary veins in the number of 2 and the middle primary vein. In addition, from the side of the carpophore, it is customary to distinguish the commissural (connecting) vein, as well as two marginal primary veins (Fig. 1, D 1–6). However, they are hardly noticeable visually in the structure of the mericarp. Because of this, they are not used as a morphological feature. It is obvious that the number and nature of the expression of the ribs is a sign of raw materials and, as scientists interpret it, a systematic feature in the *Apiaceae* L. family [9].

The dorsal secondary and commissural primary ridges are anatomically similar. In cross-section, they appear irregularly triangular. The surface is covered with a thin-walled exocarp layer. The mesocarp, consisting of hollow cells, forms the base of the ridge wall. Staining with 1% ethanol solution of Sudan III revealed pink to dark brown coloration of the exo- and mesocarp cell walls, indicating suberization. The dorsal and commissural ridges are hollow, with a schizogenous duct occupying the cavity and mirroring the angular shape of the ridge. This cavity is lined with small epithelial cells. The protoplasts of these epithelial cells appear dark brown due to wall staining and amorphous protoplasm. Under UV light excitation (330–400 nm), epithelial cells show pale brown fluorescence. The resinous secretions within the ducts exhibit pale yellow to blue fluorescence (Fig. 2A–F).

When dissecting the fruit and preparing crushed preparations, a combination of schizogenous receptacles can be observed from the surface. These structures are not easily visible under classical light microscopy; therefore, fluorescence microscopy was used. Due to their darker fluorescence relative to surrounding tissues, the ducts can be readily identified within the exocarp (Fig. 2G–I).

On surface view, septa within the ducts are also observable, serving as an additional diagnostic trait for this species[13]. These septa lack cellular structure and resemble cell wall composition. Under UV excitation at 330–400 nm, they fluoresce blue, and at 420–550 nm, pale yellow (Fig. 2J–L).

It is worth noting that the majority of monoterpenes are not detected in this spectral range, suggesting that the observed fluorescence may be associated with secondary metabolites of phenolic nature, possibly flavonoids.

The fluorescence of the mesocarp tissue appears bright blue, attributed to simple phenolic compounds of the C_6–C_1 and C_6–C_2 series. According to some literature, coumarin derivatives have been reported in *Daucus* species [14, 15].

To compare fluorescence patterns, the fluorescence of a coumarin reference standard (RS) was investigated. Under UV light at 330–400 nm, coumarin exhibits pale blue fluorescence (Fig. 3A–D). This similarity suggests the presence of structurally related compounds in the pericarp of wild carrot fruits. A working reference standard of quercetin was also used as an example of a flavonoid secondary metabolite. Under UV excitation at 330–400 nm quercetin fluoresces bright orange, resembling the fluorescence of epithelial cells surrounding the resin ducts (Fig. 3E–H). This fluorescence pattern indirectly indicates the presence of flavonol-class flavonoids in these cells.

The bast fibers are lignified, as confirmed by yellow staining with 10% sulfuric aniline solution. Closer to the center, phloem cells and pairs of xylem vessels are located, forming a collateral vascular bundle. Two schizogenous ducts are located on the dorsal side of the mericarp. A cavity is also present between the endocarp and the seed coat (Fig. 4D–F). The commissural ridge is often lost during dissection.

In the seed tissue, a well-developed parenchymatous endosperm is typically visible, characteristic of the *Apiaceae* Lindl. family[14]. The parenchymal cells of the endosperm are isodiametric, sometimes slightly elongated and oval. Cell walls are noticeably thickened and composed of cellulose. The protoplast is amorphous and unpigmented.

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[14] Ostroumova TA, et al. Analysis of Carpological Features of the *Umbelliferae* Family in the Flora of Russia. In: Systematic and Floristic Studies of Northern Eurasia; 2019. Vol. 2. 168 p. Russian

Table 1 – Chromatographic Conditions for Determining the Composition of Volatile Organic Compounds by GC–MS

Chromatograph	Gas chromatograph "MAESTRO 7820"
Column	Capillary quartz column HP-5, 30 m × 0.25 mm × 0.25 µm (stationary phase: 5 %-diphenyl-95 %-dimethylsiloxane)
Detector	Mass spectrometer Agilent 5975
Injection mode	Auto-injector
Carrier Gas	Helium
Flow rate	1 mL/min
Programming the temperature of the column thermostat	Isothermal at 40°C for 5 min; ramp to 80°C at 3°C/min; to 180°C at 4°C/min; to 280°C at 8°C/min; isothermal at 280°C for 15 min; injection without split
Injector temperature	270°C
Ion source temperature	150°C
Quadrupole temperature	230°C
Transfer line temperature	280°C
Injection volume	1 µL

Table 2 – Component composition of volatile organic compounds in hexane and chloroform fractions of aqueous-ethanol extract from Wild Carrot Fruits

Retention Time, M ± SD, min	Compound	Relative Content, M ± SD, %	
		Hexane	Chloroform
11.725 ± 0.012	2-Thujene	0.09 ± 0.03	0.07 ± 0.02
12.106 ± 0.042	α-Pinene	16.62 ± 2.49	3.66 ± 0.55
12.939 ± 0.013	Camphene	1.22 ± 0.18	0.37 ± 0.11
14.238 ± 0.012	Sabinen	4.98 ± 0.75	2.03 ± 0.30
14.426 ± 0.006	β-Pinene	1.34 ± 0.20	0.52 ± 0.16
15.189 ± 0.01	β-Myrcene	2.51 ± 0.38	1.15 ± 0.17
17.108 ± 0.009	o-Cymene	2.15 ± 0.32	2.02 ± 0.30
17.254 ± 0.007	D-Limonene	1.66 ± 0.25	0.75 ± 0.22
17.342 ± 0.016	β-Phellandrene	0.13 ± 0.04	0.00 ± 0.00
17.663 ± 0.022	trans-β-Ocimene	0.09 ± 0.03	0.00 ± 0.00
18.66 ± 0.29	γ-Terpinene	0.24 ± 0.07	0.33 ± 0.10
19.338 ± 0.342	cis-Sabinene hydrate	0.03 ± 0.01	0.24 ± 0.07
20.76 ± 0.121	n.i.	0.02 ± 0.01	0.19 ± 0.06
20.98 ± 0.013	Linalool	0.15 ± 0.04	0.92 ± 0.28
22.003 ± 0.03	Menth-2-en-1-ol	0.06 ± 0.02	0.03 ± 0.01
22.201 ± 0.01	Neo-allo-ocimene	0.09 ± 0.03	0.06 ± 0.02
22.308 ± 0.014	α-Campholenal	0.14 ± 0.04	0.48 ± 0.14
22.846 ± 0.014	Pinocarveol	0.22 ± 0.06	1.00 ± 0.30
23.109 ± 0.015	cis-Verbenol	0.54 ± 0.16	3.72 ± 0.56
23.726 ± 0.186	Pinocarvone	0.09 ± 0.03	0.42 ± 0.13
23.786 ± 0.023	Sabina ketone	0.03 ± 0.01	0.00 ± 0.00
24.244 ± 0.008	Verbenyl, ethyl ether	0.06 ± 0.02	0.00 ± 0.00
24.571 ± 0.014	4-Terpineol	0.03 ± 0.01	0.31 ± 0.09
24.806 ± 0.015	α-Thujenal	0.02 ± 0.00	0.00 ± 0.00
25.015 ± 0.012	p-Cymene-8-ol	0.08 ± 0.02	0.43 ± 0.13
25.251 ± 0.014	Myrtenol	0.11 ± 0.03	0.61 ± 0.18
25.309 ± 0.012	Myrtenal	0.08 ± 0.02	0.56 ± 0.17
25.819 ± 0.014	Verbenone	0.32 ± 0.10	1.44 ± 0.22
26.239 ± 0.013	Carveol	0.09 ± 0.03	0.58 ± 0.18
26.577 ± 0.014	Myrtenyl acetate	0.04 ± 0.01	0.12 ± 0.04
27.289 ± 0.014	Carvone	0.07 ± 0.02	0.29 ± 0.09
28.529 ± 0.011	Borneol, acetate	0.66 ± 0.20	1.02 ± 0.15
30.766 ± 0.014	α-Terpinyl acetate	0.06 ± 0.02	0.22 ± 0.07

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Retention Time, M ± SD, min	Compound	Relative Content, M ± SD, %	
		Hexane	Chloroform
31.15 ± 0.023	Nerol acetate	0.00 ± 0.00	0.00 ± 0.00
31.373 ± 0.024	trans-Myrtanol	0.03 ± 0.01	0.09 ± 0.03
31.715 ± 0.012	β-Gurjunene	2.30 ± 0.34	0.15 ± 0.05
31.82 ± 0.02	Geranyl acetate	0.33 ± 0.10	0.24 ± 0.07
31.997 ± 0.044	n.i.	0.14 ± 0.04	0.00 ± 0.00
32.128 ± 0.019	beta-Elemen	0.09 ± 0.03	0.00 ± 0.00
32.489 ± 0.01	α-Zingiberene	0.10 ± 0.03	0.08 ± 0.02
32.894 ± 0.009	α-Bergamotene	1.26 ± 0.19	0.16 ± 0.05
33.07 ± 0.013	Santalen	0.44 ± 0.13	0.05 ± 0.02
33.17 ± 0.021	Caryophyllene	2.88 ± 0.43	0.28 ± 0.08
33.366 ± 0.013	Elixene	0.06 ± 0.02	0.00 ± 0.00
33.524 ± 0.016	α-Farnesene	1.94 ± 0.29	0.28 ± 0.08
33.897 ± 0.03	Isocaryophyllene	0.21 ± 0.06	0.49 ± 0.15
33.996 ± 0.023	Epi-β-Santalene	0.10 ± 0.03	0.06 ± 0.02
34.182 ± 0.02	(E)-β-Famesene	1.63 ± 0.24	0.10 ± 0.03
34.249 ± 0.021	β-Sesquiphellandrene	2.66 ± 0.40	0.24 ± 0.07
34.395 ± 0.023	cis-β-Farnesene	2.25 ± 0.34	0.31 ± 0.09
34.505 ± 0.013	γ-Muurolene	0.13 ± 0.04	0.03 ± 0.01
34.992 ± 0.043	β-Cubebene	0.83 ± 0.25	0.65 ± 0.20
35.204 ± 0.021	n.i.	0.69 ± 0.21	0.04 ± 0.01
35.467 ± 0.009	β-Selinene	0.18 ± 0.06	0.00 ± 0.00
35.676 ± 0.003	α-Selinene	0.12 ± 0.04	0.11 ± 0.03
35.851 ± 0.011	Chamigren	0.47 ± 0.14	0.06 ± 0.02
35.965 ± 0.014	β-Bisabolene	1.75 ± 0.26	0.54 ± 0.16
36.187 ± 0.038	Sesquicineole	0.07 ± 0.02	0.09 ± 0.03
36.482 ± 0.006	n.i.	0.59 ± 0.18	0.00 ± 0.00
36.993 ± 0.082	Humulene	0.14 ± 0.04	0.14 ± 0.04
38.421 ± 0.018	Caryophyllene oxide	1.54 ± 0.23	2.65 ± 0.40
39.137 ± 0.116	Carotol	25.9 ± 3.9	30.10 ± 4.52
39.384 ± 0.059	Humulene oxide II	0.32 ± 0.09	0.13 ± 0.04
39.708 ± 0.009	Cedrenol	0.28 ± 0.08	0.34 ± 0.10
40.233 ± 0.008	Daucol	1.02 ± 0.15	3.27 ± 0.49
40.568 ± 0.01	β-Eudesmol	0.10 ± 0.03	0.54 ± 0.16
41.083 ± 0.009	n.i.	0.38 ± 0.11	1.02 ± 0.15
41.127 ± 0.001	Elemol	0.30 ± 0.09	0.00 ± 0.00
41.811 ± 0.008	Eudesm-7(11)-en-4-ol	0.20 ± 0.06	0.51 ± 0.15
47.683 ± 0.006	n.i.	1.04 ± 0.16	1.13 ± 0.17
47.863 ± 0.006	Hexadecanoic acid, ethyl ester	0.78 ± 0.23	3.61 ± 0.54
47.923 ± 0.006	n.i.	0.42 ± 0.13	0.74 ± 0.22
48.256 ± 0.006	n.i.	0.78 ± 0.23	1.28 ± 0.19
50.326 ± 0.006	Ethyl Oleate	3.37 ± 0.51	9.47 ± 1.42
50.628 ± 0.033	Stearic acid, ethyl ester	0.38 ± 0.11	0.58 ± 0.17
51.313 ± 0.007	n.i.	2.54 ± 0.38	5.06 ± 0.76
52.336 ± 0.012	Bicyclo[4,4,0]dec-5-ene, 1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)	5.19 ± 0.78	11.84 ± 1.78
	Component Classes (average values):		
	Monoterpenes:	29.0	8.94
	Monoterpenoids:	3.17	12.2
	Sesquiterpenes:	19.6	3.74
	Sesquiterpenoids:	29.7	37.6
	Aromatic compounds:	2.23	2.45
	Fatty acids:	4.53	13.7
	Other:	5.19	11.8

Note: “n.i.” — not identified; SD — standard deviation.

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Table 3 – Results of pharmacognostic testing of Wild Carrot Fruits

Indicator	Method of analysis	Characteristic	
		Whole raw material	Powdered raw material
Loss on drying, %	Gravimetric	5.8	5.8
Essential oil content (30 g + 500 mL H_2O, 4 hours), %	Hydrodistillation	0.17	0.88
Extractives (70 % ethanol), %	Gravimetric	6.6	15.2
Total ash, %	Gravimetric	6.9	7.2
Acid-insoluble ash (in 10% HCl), %	Gravimetric	3.2	–
Sulfated ash, %	Gravimetric	9.8	9.2
Heavy metals, %	Visual colorimetry	Not more than 0.01	Not more than 0.01

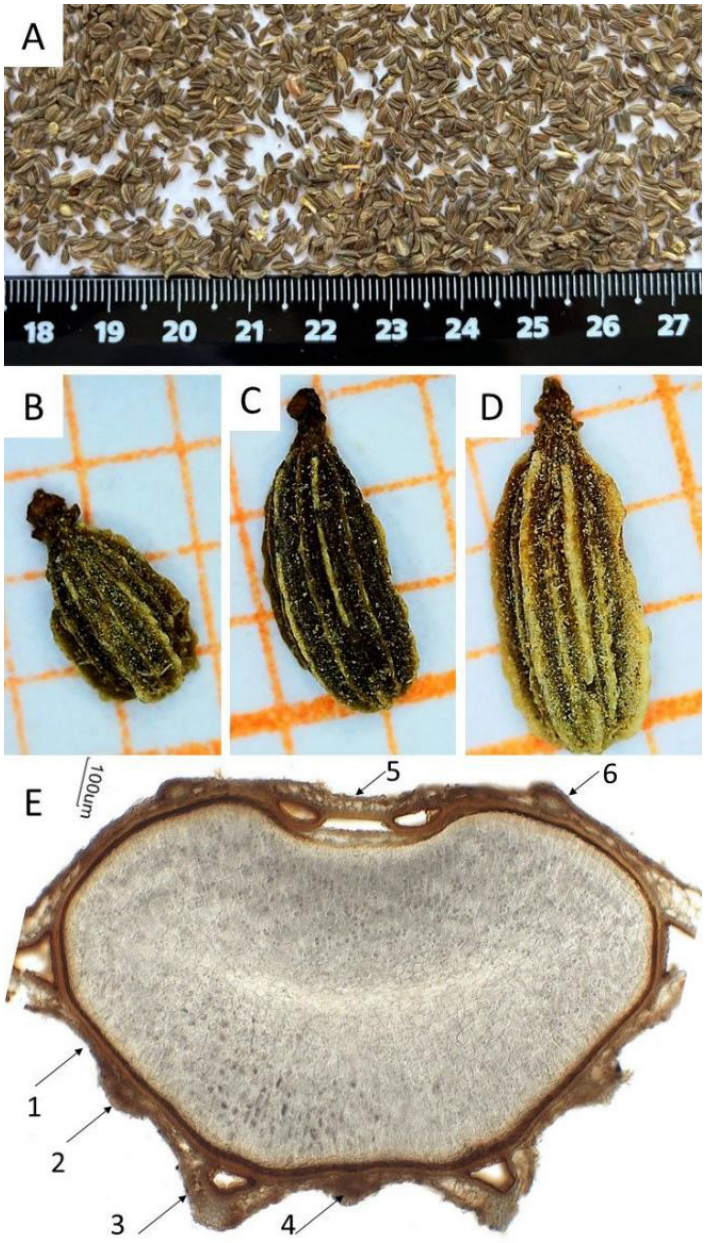

Figure 1 – External view and morphological features of Wild Carrot Fruits.
Note: A — general view of the raw material (industrial sample); B — mericarp up to 2 mm; C — mericarp up to 3 mm; D — mericarp over 3 mm; E — transverse section of mericarp (× 100): 1 — furrowed ridge; 2 — lateral primary ridge; 3 — dorsal secondary ridge; 4 — median primary ridge; 5 — commissural (connecting) ridge; 6 — marginal primary ridge.

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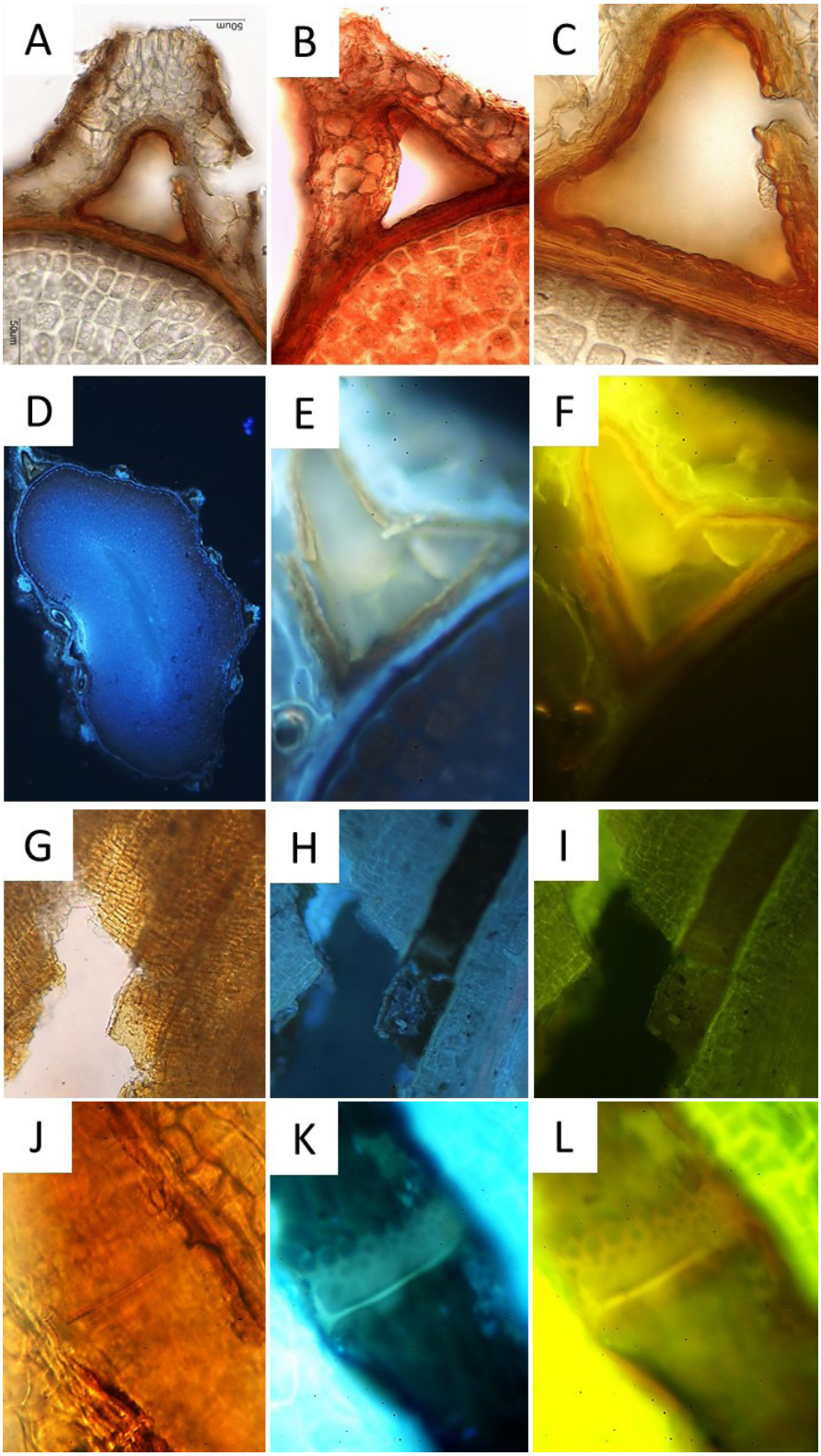

Figure 2 – Histological features of pericarp ridges in cross-section and squashed preparations

Note: A — mesocarp of hollow cells in pericarp ridge (× 400); B — exo- and mesocarp cell walls stained with 1% Sudan III (× 400); C — schizogenous duct in pericarp ridge (× 1000); D — fluorescence of marginal pericarp ridges and embryo tissues under UV light at λ = 330–400 nm (× 40); E — fluorescence of marginal ridge under UV light at λ = 330–400 nm (× 400); F — fluorescence of resinous secretion in marginal ridge under UV light at λ = 420–550 nm (× 400); G — schizogenous ducts in squashed preparation (× 400); H — fluorescence of resin ducts under UV light at λ = 330–400 nm (× 400); I — fluorescence of resin ducts under UV light at λ = 420–550 nm (× 400); J — duct septum under visible light (× 400); K — duct septum fluorescence under UV light at λ = 330–400 nm (× 400); L — duct septum fluorescence under UV light at λ = 420–550 nm (× 400).

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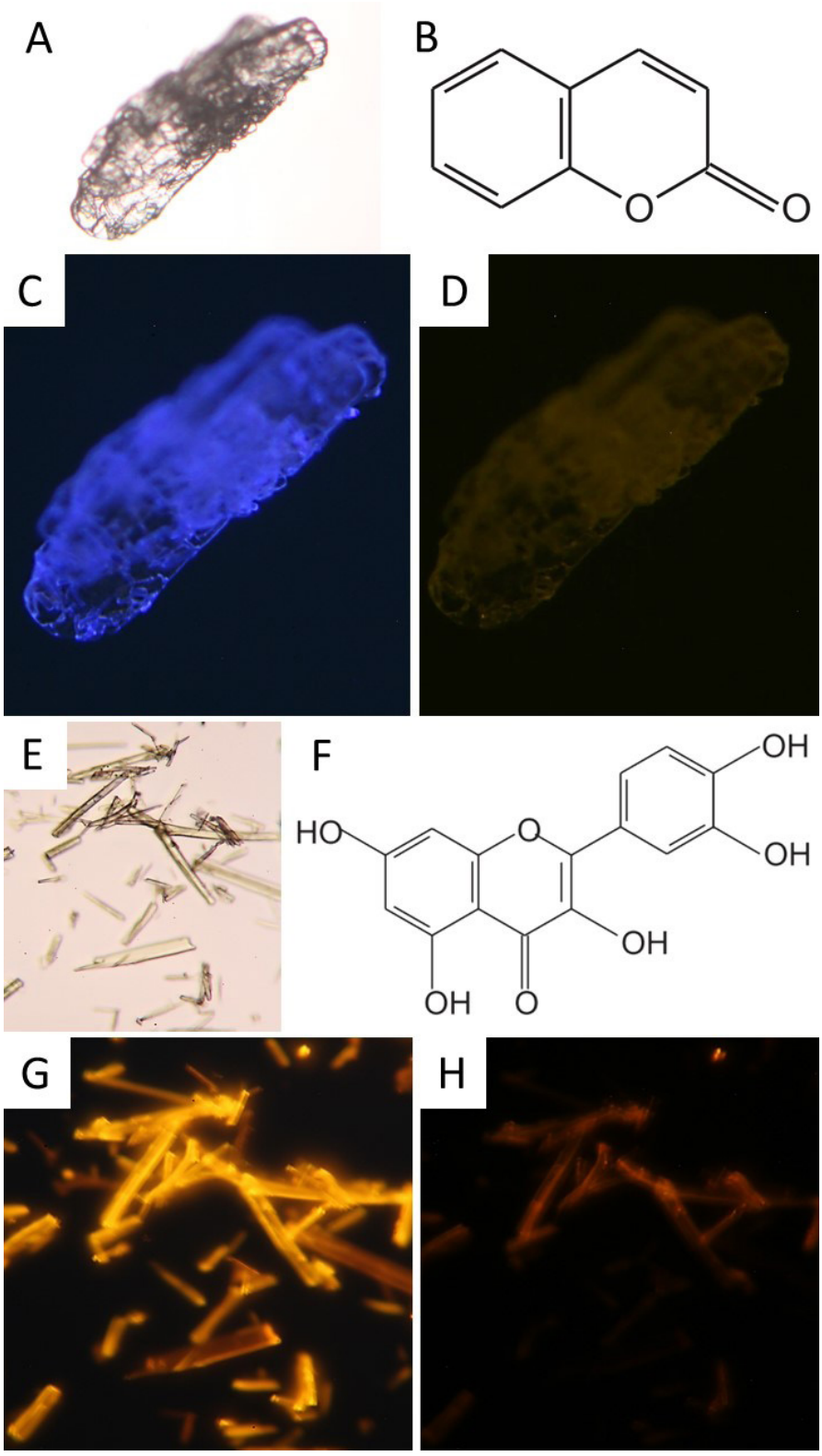

Figure 3 – Cross-section of Wild Carrot Fruit

Note: A — coumarin crystals under visible light (× 400); B — structural formula of coumarin; C — coumarin fluorescence under UV light at λ = 330–400 nm (× 400); D — coumarin fluorescence under UV light at λ = 420–550 nm (× 400); E — quercetin crystals under visible light (× 400); F — structural formula of quercetin; G — quercetin fluorescence under UV light at λ = 330–400 nm (× 400); H — quercetin fluorescence under UV light at λ = 420–550 nm (× 400).

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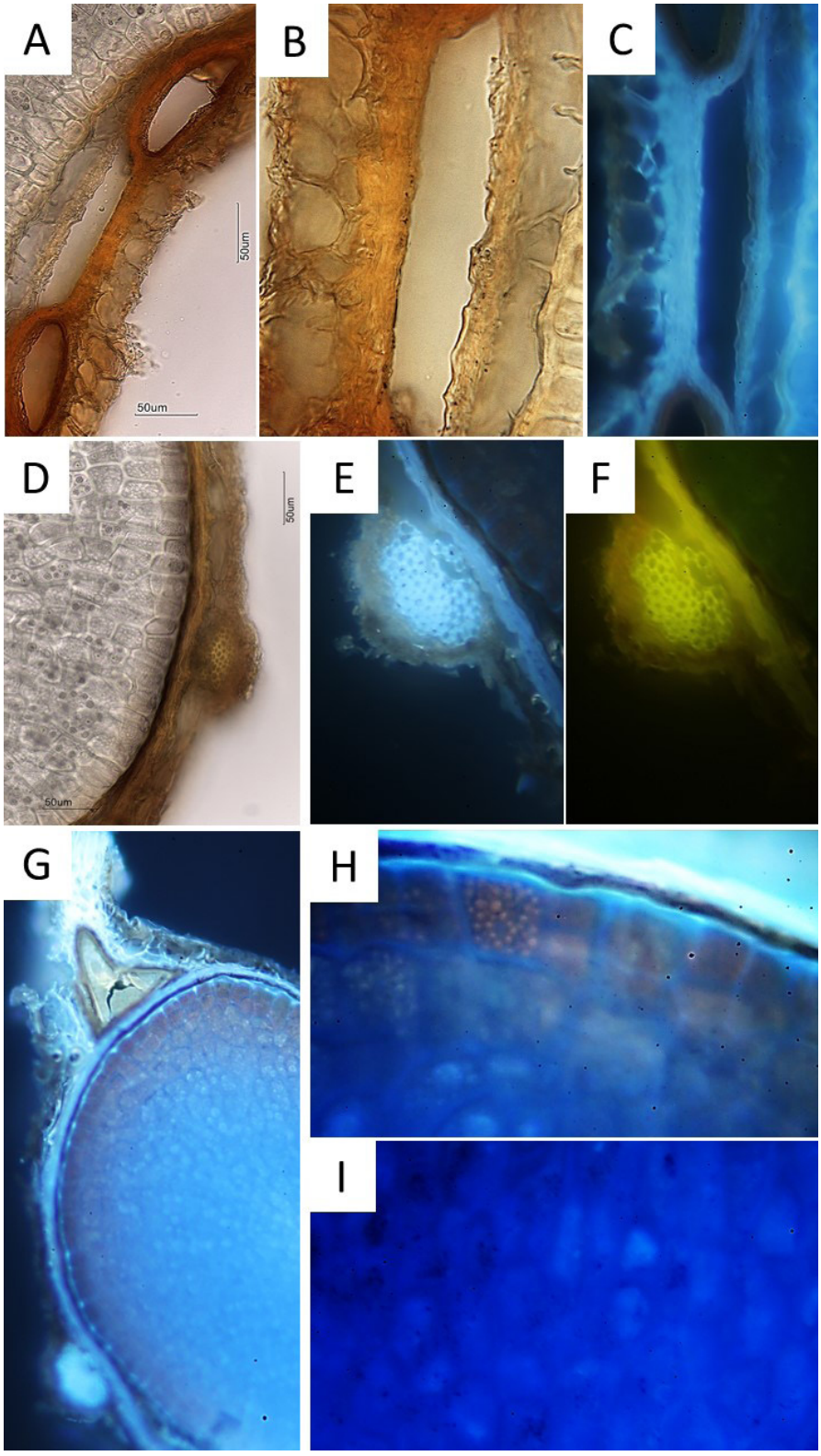

Figure 4 – Secondary pericarp ridges and histology of the dorsal mericarp side (transverse section)

Note: A — schizogenous ducts on the dorsal side of the mericarp (× 400); B — lignified walls treated with 10% sulfuric aniline solution (× 1000); C — fluorescence of connective ridge (carpophore) tissues under UV light at λ = 420–550 nm (× 400); D — bast fibers of the lateral primary ridge (× 400); E — fluorescence of ridge tissue under UV light at λ = 330–400 nm (× 400); F — fluorescence of ridge tissue under UV light at λ = 420–550 nm (× 400); G — fluorescence of embryo protoplasts under UV light at λ = 330–400 nm (× 100); H — red fluorescence of plastids in peripheral endosperm protoplasts under UV light at λ = 330–400 nm (× 400); I — absence of druse fluorescence under UV light at λ = 420–550 nm (× 400).

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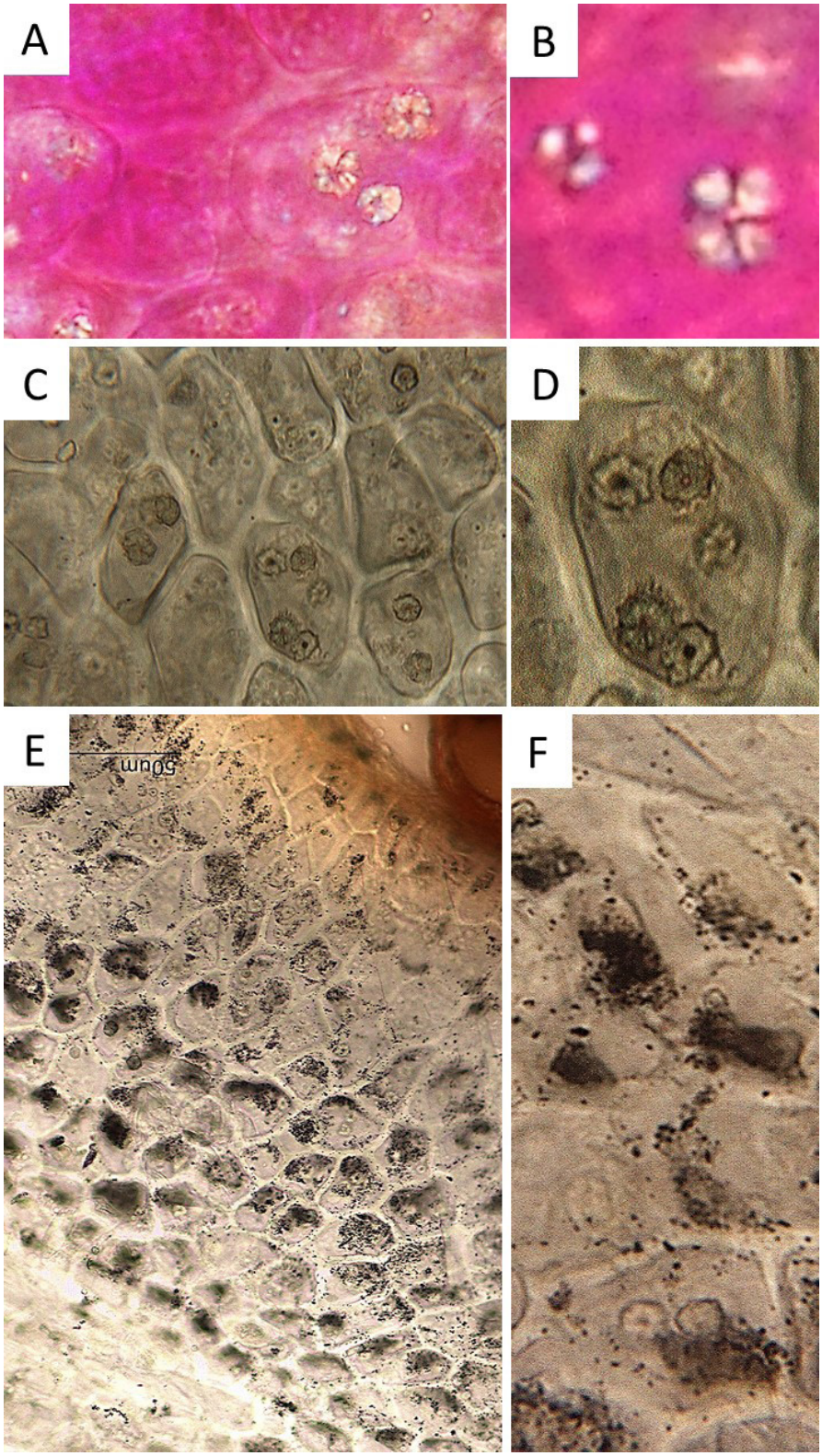

Figure 5 – Crystalline inclusions in embryo tissues

Note: A — small druses in seed endosperm cells under polarization microscopy (× 1000); B — four-rayed druse structure (× 1000); C — view of druses using dark-field condenser (× 1000); D — structure of individual druses (× 1000); E — starch inclusions in embryo parenchyma after Lugol's solution staining (× 400); F — individual parenchyma cells with starch inclusions (× 400).

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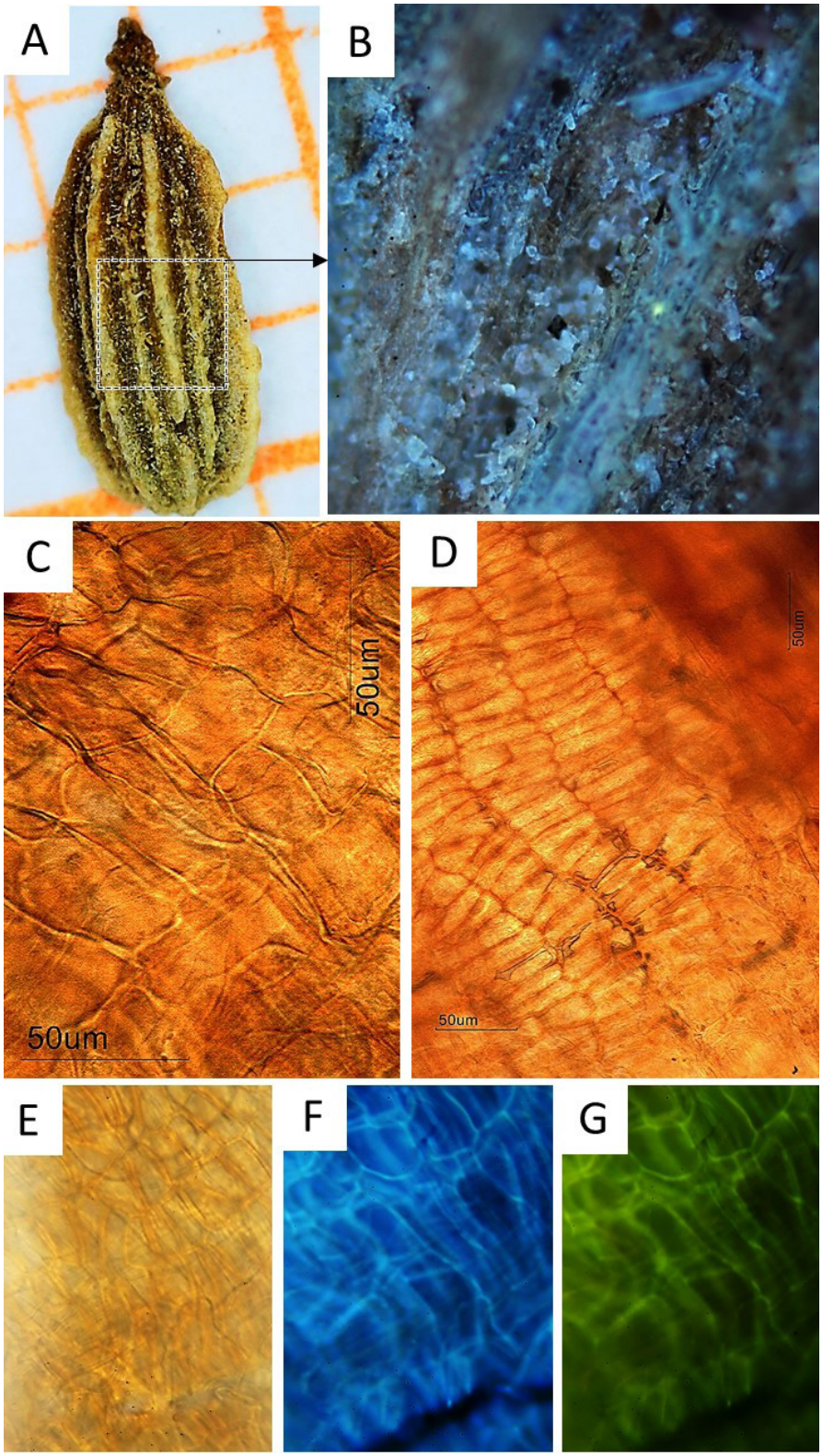

Figure 6 – Exocarp surface of Wild Carrot Fruits

Note: A — absence of pubescence on the exocarp (× 20); B — alternating fluorescence of the exo-/mesocarp and schizogenous ducts under UV light at λ = 330–400 nm (× 400); C — exocarp cells (× 400); D — "parquet endocarp" cell arrangement (× 400); E — surface of exocarp cells under visible light (× 400); F — fluorescence of exocarp cells under UV light at λ = 330–400 nm (× 400); G — fluorescence of exocarp cells under UV light at λ = 420–550 nm (× 400).

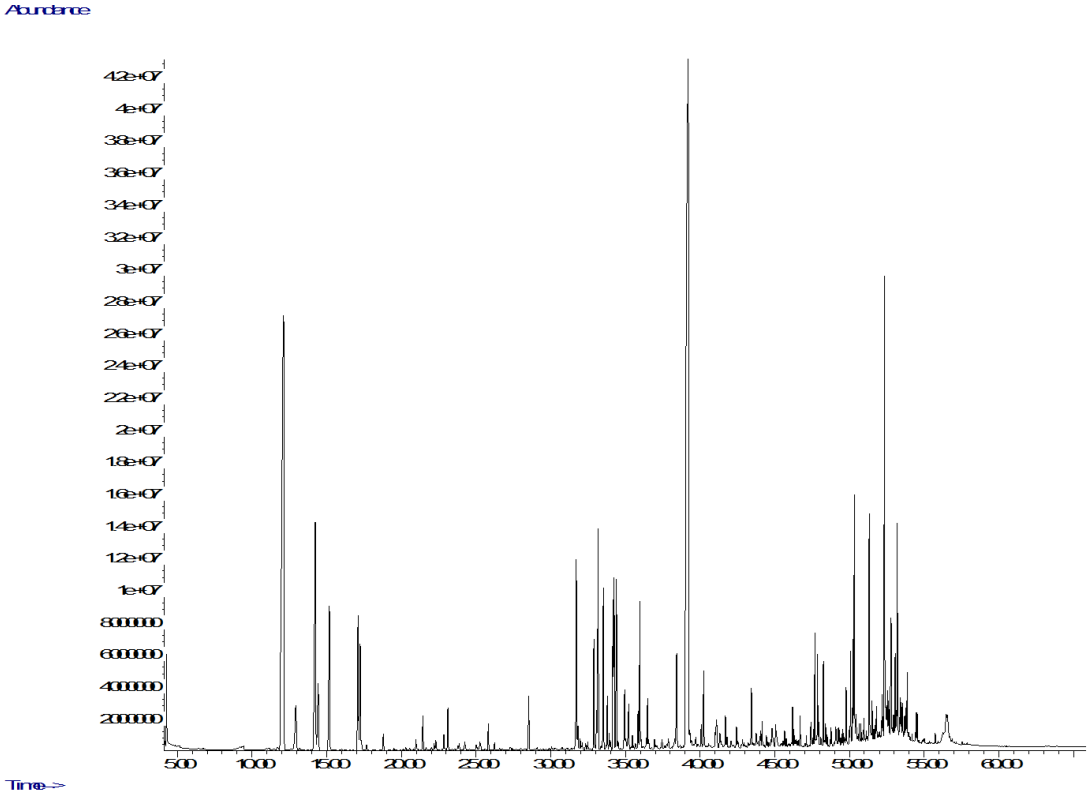

Figure 7 – GC–MS Chromatogram of the hexane fraction of the aqueous-ethanol extract from Wild Carrot Fruits

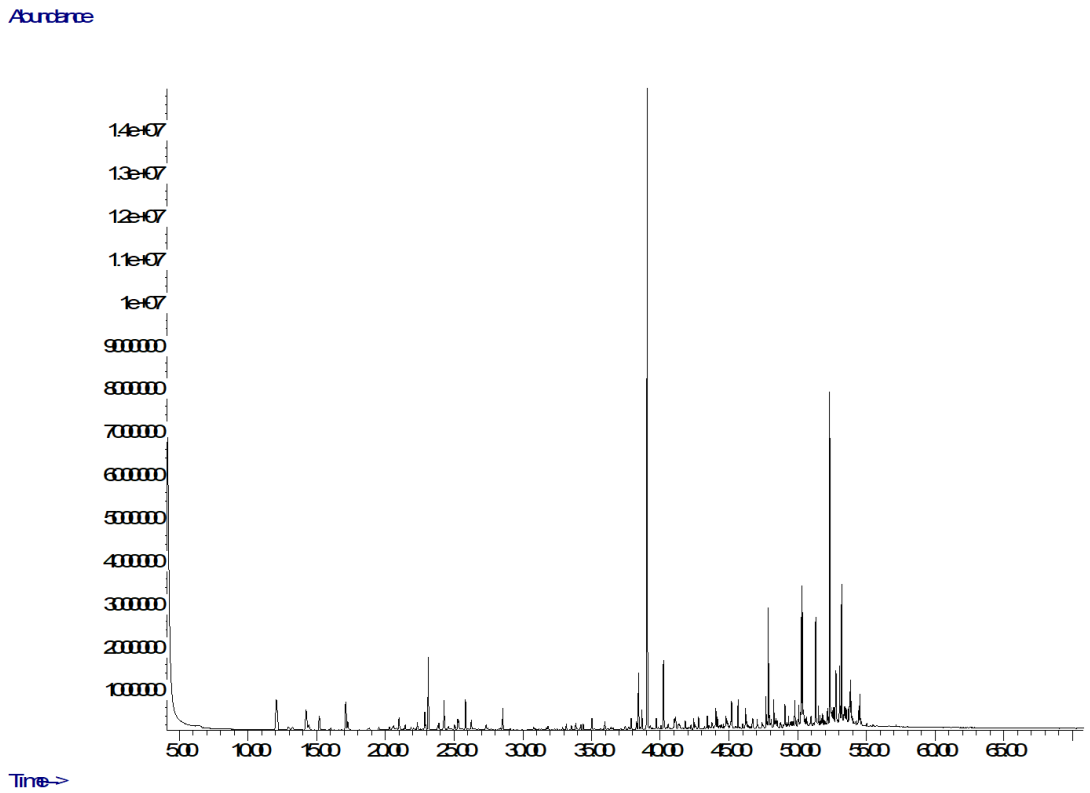

Figure 8 – GC–MS Chromatogram of the chloroform fraction of the aqueous-ethanol extract from Wild Carrot Fruits

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Under UV excitation at 330–400 nm, the druses do not fluoresce. However, the amorphous body of the protoplast emits bluish-blue fluorescence. Toward the periphery of the endosperm, round structures are observed within the protoplasts, showing red fluorescence likely associated with carotenoids and chlorophylls (Fig. 4G–I).

Druses are poorly visible under classical light microscopy (Fig. 5C–D), so polarization microscopy with a dark-field condenser and polarizing filter was used. Under these conditions, numerous druses become clearly visible in nearly every cell of the seed tissue. They are small, round, and up to 10 µm in diameter. Notably, the druses exhibit a unique star-shaped morphology with four symmetrical rays. A distinct central division into four segments is also observed (Fig. 5A–B). This specific druse structure is a valuable diagnostic marker and may be used for taxonomic identification and authentication of “Wild Carrot Fruits” raw material.

Upon treating transverse mericarp sections with ethanol-based Lugol’s solution, small round inclusions stained from dark blue to nearly black are revealed, characteristic of starch (Fig. 5E–F). However, due to their small size and non-specific structure, these inclusions are not suitable as diagnostic features.

When examined under a × 5 or × 10 hand lenses, the surface of the wild carrot fruit appears glabrous (non-pubescent). Observation of the dry fruit surface under UV excitation at 330–400 nm reveals alternating blue fluorescence of the exo- and mesocarp with brown fluorescence of the schizogenous ducts (Fig. 6A–B).

At higher magnification (× 400), the exocarp cells covering the fruit appear thin-walled, parenchymatous, and angular — almost rectangular in shape — with no intercellular spaces. The protoplasts are not detectable.

Upon focusing deeper into the tissue or examining squashed preparations, the endocarp structure becomes visible. The endocarp is composed of elongated, thick-walled cells tightly packed and aligned side-by-side. This distinctive pattern is commonly referred to as a “parquet endocarp” and serves as an important diagnostic feature for species identification. Under visible light, the exocarp surface cells show weak yellow to pale orange coloration. Their fluorescence under UV light is consistent with the previously described cross-sectional fluorescence (Fig. 6C–G).

Chromatographic–Mass Spectrometric Analysis

The GC–MS analysis of wild carrot fruit extracts allowed the identification of the volatile organic compound (VOC) profile. The component composition of the hexane and chloroform fractions of the aqueous-ethanol extract is presented in Table 2. Chromatograms are shown in Figures 7 and 8.

According to the data in Table 2, the predominant component in both fractions was carotol, with an average relative content of 25.9% in the hexane extract and 30.1% in the chloroform extract. Significant differences were noted in the component composition of the two extracts. The hexane extract was rich in monoterpenes (such as α-pinene, sabinene, β-myrcene) and sesquiterpenes (caryophyllene, β-sesquiphellandrene, β-gurjunene). In contrast, the chloroform extract was dominated by oxygenated compounds of essential oils (alcohols, ketones, and esters of monoterpenes and sesquiterpenes).

Numerical Quality Indicators

Determination of numerical quality indicators was performed for wild carrot fruit herbal raw material (MPRM) in two forms: whole and powdered. The loss on drying was preliminarily calculated and amounted to 5.8%. The test results presented in Table 3 allow us to establish criteria for the quality of raw materials.

The given numerical indicators made it possible to establish differences in the content of essential oil and extractive substances in whole and crushed raw materials, which will serve as a justification for choosing approaches to standardization and developing a draft regulatory document “Wild Carrot Fruits”.

DISCUSSION

A morphological and anatomical study of wild carrot fruits confirmed the literature data on the structure of lop-sided plants [9]. The main diagnostic features are the shape and size of mericarps, the number of longitudinal ribs, their color and prominence. We have described for the first time crystalline inclusions in the cells of the embryo parenchyma, represented by druses of a special stellate shape, having four distinct symmetrical rays. In the center of the druse, there is a visually formed division into four parts. The specific structure of the druze is diagnostic in nature and can be used in the process of taxation and authentication of raw materials of “Wild carrot fruits”. Studies of the luminescence of

wild carrot fruit tissues were carried out for the first time. The specificity of the luminescence of secretory canals, seed coat, embryo parenchyma has been proven, and the connection of luminescence with the chemical composition of protoplasts, as well as the release of secretory tissues, has been predicted. The data obtained allow us to develop the sections "Identification. External signs" and "Identification. Microscopic signs" in the draft FS "Wild Carrot Fruits".

According to the results of our research, the predominant components are carotol, α-pinene, daukol, sabinene, β-sesquifellandren. The results of the gas chromatographic analysis generally correspond to the known scientific literature data on the component composition of the essential oils of the studied fruits. The components present in *D. carota* L., include quercetin, pyrrolidine, daucine, daucosterol, tiglinic acid and essential oils, the main components of which are carotene, daucol, copaenol, gerenol, citric acid, pinenic acid and cineolic acid, while for the first three compounds the corresponding percentages are 30.55%, 12.60% and 0.62% [21].

R. Chizzola in the fruits of samples grown in Vienna, found that the predominant components are α-pinene (from 23.5% to 30.4%), sabine (from 21.5% to 46.6%), geranyl acetate (from 3.9% to 28.1%), β-pinene (3–13.1%), α-thuyene (1–8,8%), γ-terpinene (0.3–4.1%), myrcene (3.4–3.9%), carotene (1.2%) [22]. The main components of wild carrot essential oil samples from Morocco were α-pinene (22.2–23.5%), β-azarone (15.1–16.7%), sabinene (1.2–12.4%), β-bisabolene (4.0%) [23, 24];

The fruits of Daucus carota ssp. carota growing in Uzbekistan were dominated by carotene (69.8%), daucene (9%), trans-α-bergamotene (4.7%), trans-β-farnesene (3.7%), in Portugal — geranyl acetate (28.7–65%), α-pinene (13–27.1%), 11aH-himachal-4-en-1-β-ol (0.5–9.4%), Limonene (1.2–9%), β-pinene (2.3–4.5%). The fruits of other subspecies of *Daucus carota* L. contain such components as geranyl acetate (ssp. major, gummifer, maximus), α-pinene (ssp. major, halophilus, gummifer, maximus), α-azarone (ssp. maximus), and carotol (ssp. gummifer) [25].

The variety of main components and their relative content may be due to the peculiarities of obtaining essential oils, as well as the time and place of harvesting, the influence of climatic conditions, soil chemistry, and other factors. Differences in the component composition can lead to changes in the pharmacological activity of raw materials harvested at different times and in different geographical regions. Nevertheless, it is possible to identify the components that were found in most samples: α-pinene, sabinene, carotol, which can act as markers of MPRM, which allows the development of sections "Identification. Determination of the main groups of biologically active substances" and "Tests. Quantitative definition" in the PhA draft.

The established characteristics of numerical commodity indicators (weight loss during drying, essential oil content, extractive substances content, total ash, ash insoluble in 10% hydrochloric acid, sulfate ash, heavy metal content) indicate the quality of the MPRM and allow the development of the "Tests" section.

Study Limitations

To develop a draft of the PhM "Wild Carrot Fruit" and clarify the quality of raw materials, it is necessary to determine numerical quality indicators and evaluate the component composition of biologically active substances in samples harvested in various geographical regions and climatic zones.

CONCLUSION

Morphological and anatomical analysis performed using modern approaches to microscopic analysis has established the characteristics of carrot fruits: the oval shape of the fruit, narrowed to the tip, brown in color with seven veins; angular schizogenic receptacles with non-cellular partitions; endosperm parenchyma of isodiametric, oval cells; parquet-type endocarp of elongated, thick-walled, tightly closed cells stellate druses in the tissues of the embryo with four symmetrical rays. Luminescence in the excitation range of 330–400 nm mesocarp – bright blue; Epithelial cells are light yellow; tarry secretions are blue.

Chromatography-mass spectrometric study of hexane and chloroform fractions extracted from wild carrot fruits determined the profile of volatile compounds. It was found that the predominant component in both fractions is sesquiterpene alcohol carotol (25.9% and 30.1%, respectively), which is a marker compound in the composition of the studied MPRM.

The identified microscopic features, the main biologically active substance and numerical quality indicators are of practical importance for inclusion in the draft pharmacopoeial monograph "Wild carrot fruits".

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

Vera V. Artemyeva — research conducting, draft writing and editing; Gada M. Dandash — research conducting, preparing raw materials, draft writing and editing; Ifrat N. Zilfikarov — developing the concept, scientific guidance, draft writing and editing; Denis I. Shishkalov — research conducting, correction of photographic materials, draft writing and editing Vitaly M. Ryzhov — conducting research on anatomy and morphology, preparing and correcting photographic materials, draft writing and editing; Tatyana K. Ryazanova — conducting chemical composition research, processing primary data, draft writing and editing; Inna I. Bochkareva — research conducting, draft writing and editing; Ziyarat A. Guseinova — research conducting, draft writing and editing; Artur K. Arutyunov — research conducting, draft writing and editing. All authors confirm that their authorship meets the international ICMJE criteria (all authors have made significant contributions to the development of the concept, research and preparation of the article, read and approved the final version before publication).

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AUTHORS

Vera V. Artemyeva — Senior Lecturer of the Department of Pharmacy of the Maykop State Technological University. ORCID ID: 0000-0001-8467-2899. E-mail: denis7radnet.ru@mail.ru

Gada M. Dandash — PhD student of the Department of Pharmacy, Lecturer of the Department of Morphology, Maykop State Technological University. ORCID ID: 0009-0003-5045-5652. E-mail: gdandache@gmail.com

Ifrat N. Zilfikarov — Doctor of Sciences (Pharmacy), Professor of the Russian Academy of Sciences, Leading Researcher of the Maykop State Technological University; Chief Researcher of the Department of Natural Compound Chemistry of the All-Russian Scientific Research Institute of Medicinal and Aromatic Plants. ORCID ID: 0000-0002-8638-9963. E-mail: dagfarm@mail.ru

Denis I. Shishkalov — PhD student, Assistant of the Department of Pharmacy of the Maykop State Technological University. ORCID ID: 0000-0001-9558-5336. E-mail: hitmanapp@mail.ru

Vitaly M. Ryzhov — Candidate of Sciences (Pharmacy), Assistant Professor of the Department of Pharmacognosy with Botany and Basics of Phytotherapy, Samara State Medical University ORCID ID: 0000-0002-8399-9328. E-mail: lavr_rvm@mail.ru

Tatyana K. Ryazanova — Doctor of Sciences (Pharmacy), Director of the Scientific and Educational Center "Pharmacy," Assistant Professor of the Department of Pharmacy Management and Economics, Samara State Medical University. ORCID ID: 0000-0002-4581-8610. E-mail: t.k.ryazanova@samsmu.ru

Inna I. Bochkareva — Candidate of Sciences (Pharmacy), Assistant Professor of the Department of Pharmacy of the Maykop State Technological University. ORCID ID: 0000-0002-7898-4404. E-mail: bochkarevainna@gmail.com

Ziyarat A. Guseinova — Candidate of Sciences (Biology), Senior Researcher of the Laboratory of Flora and Plant Resources, Mountain Botanical Garden of the Dagestan Federal Research Center. ORCID ID: 0000-0003-0355-4132. E-mail: guseinovaz@mail.ru

Artur K. Arutyunov — Candidate of Sciences (Medicine), Assistant Professor of the Department of Pharmacy of the Maykop State Technological University. ORCID ID: 0000-0003-3883-6631. E-mail: ak.arut17@mail.ru