



Updating approaches to the standardization of *Angelica archangelica* L. rhizomes and roots

D.I. Shishkalov¹, I.N. Zilfikarov^{1,2,3}, T.K. Ryazanova⁴, V.M. Ryzhov⁴,
M.A. Abdurakhmanova³, T.A. Ibragimov⁵, V.V. Artemieva¹

¹ Maykop State Technological University,
191 Pervomayskaya Str., Maykop, Russia, 385000

² All-Russian Scientific Research Institute of Medicinal and Aromatic Plants,
7 Grin Str., Bldg. 1, Moscow, Russia, 117216

³ VIFTECH,
84 SSC PMB, Serpukhov, Obolensk, Moscow Region, Russia, 142279

⁴ Samara State Medical University,
89 Chapaevskaya Str., Samara, Russia, 443099

⁵ Dagestan State University,
43a Gadzhiev Str., Makhachkala, Russia, 367000

E-mail: hitmanapp@mail.ru

Received 04 Dec 2025

After peer review 30 Apr 2026

Accepted 26 June 2026

The aim. To update approaches to the standardization of *Angelica archangelica* rhizomes and roots, taking into account modern requirements, during a comprehensive study.

Materials and Methods. Morphological and anatomical features of *Angelica archangelica* rhizomes and roots were studied using light microscopy. Luminescence of tissues and a working standard sample of the coumarins eleutheroside B1 (7-O-glucoside of isofraxidine) were studied by luminescent microscopy, and vascular structures were studied by polarization microscopy. The profile of volatile compounds of lipophilic fractions of water-alcohol extract and essential oil was determined by gas chromatography with mass spectrometric detection (GC-MS). The total coumarins content was determined by UV spectrophotometry.

Results. Rhizomes and roots of *Angelica archangelica* of Altai origin were studied for the first time. The most significant results were obtained at the excitation wavelengths in the range of 330 to 400 nm — light blue luminescence of phloem cells and secretion of schizogenous cavities was observed, due to substances of a coumarin nature. The expediency of using a histochemical reaction with Sudan III solution, which reveals schizogenous cavities, secretion droplets, and cell walls of vascular elements, has been substantiated. For the diagnosis of specific conducting elements in *Angelica* raw material (powder), the use of polarization microscopy is recommended. Using GC-MS for lipophilic fractions of water-alcohol extract and essential oil, a chromatographic profile was established, including substances of both terpene and coumarin nature: prenylated coumarin osthol (up to 49.72 %) and epilobulol (25.61 %). The total coumarin content in the studied raw material was 1.50 ± 0.08 % calculated as coumarin.

Conclusion. A comprehensive approach to the analysis of *Angelica archangelica* rhizomes and roots, involving the use of both widely used (macroscopic, microscopic, spectrophotometric) and limited-use (luminescent, polarization microscopy, and GC-MS) methods, forms the basis for the development of a draft new pharmacopoeial article “*Angelica archangelica* Rhizomes and Roots.”

Keywords: *Angelica archangelica* L.; *Angelica archangelica* rhizomes and roots; light microscopy; luminescent microscopy; polarization microscopy; gas chromatography-mass spectrometry; water-alcohol extract; lipophilic fraction; essential oil; coumarins; terpenes; osthol; epilobulol; standardization

Abbreviations: BASs — biologically active substances; GC-MS — gas chromatography-mass spectrometry; PhM — pharmacopoeial monograph; MPRM — medicinal plant raw material; SPh RF XV ed. — State Pharmacopoeia of the Russian Federation XV edition; GPhM — general pharmacopoeial monograph; WSS — working standard sample; SS — standard sample; UV-light — ultraviolet light.

For citation: D.I. Shishkalov, I.N. Zilfikarov, T.K. Ryazanova, V.M. Ryzhov, M.A. Abdurakhmanova, T.A. Ibragimov, V.V. Artemieva. Updating approaches to the standardization of *Angelica archangelica* L. rhizomes and roots. *Pharmacy & Pharmacology*. 2026;14(4):338-361. DOI: 10.19163/2307-9266-2026-14-4-338-361

© Д.И. Шишкалов, И.Н. Зилфикаров, Т.К. Рязанова, В.М. Рыжов, М.А. Абдурахманова, Т.А. Ибрагимов, В.В. Артемьева, 2026

Для цитирования: Д.И. Шишкалов, И.Н. Зилфикаров, Т.К. Рязанова, В.М. Рыжов, М.А. Абдурахманова, Т.А. Ибрагимов, В.В. Артемьева. Актуализация подходов к стандартизации дудника лекарственного (*Angelica archangelica* L.) корневищ и корней. *Фармация и фармакология*. 2026;14(4):338-361. DOI: 10.19163/2307-9266-2026-14-4-338-361

Актуализация подходов к стандартизации дудника лекарственного (*Angelica archangelica* L.) корневищ и корней

Д.И. Шишкалов¹, И.Н. Зилфикаров^{1,2,3}, Т.К. Рязанова⁴, В.М. Рыжов⁴,
М.А. Абдурахманова³, Т.А. Ибрагимов⁵, В.В. Артемьева¹

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. Грина, д. 7, стр. 1

³ Закрытое акционерное общество «ВИФИТЕХ»,
Россия, 142279, Московская область, г.о. Серпухов, п. Оболенск, ГНЦ ПМБ, к. 84

⁴ Федеральное государственное бюджетное образовательное учреждение высшего образования «Самарский государственный медицинский университет»
Министерства здравоохранения Российской Федерации,
Россия, 443099, г. Самара, ул. Чапаевская, д. 89

⁵ Федеральное государственное бюджетное образовательное учреждение высшего образования «Дагестанский государственный университет»,
Россия, 367000, Республика Дагестан, г. Махачкала, ул. Гаджиева, 43 а

E-mail: hitmanapp@mail.ru

Получена 04.12.2025

После рецензирования 30.04.2026

Принята к печати 26.06.2026

Цель. В ходе комплексного исследования актуализировать подходы к стандартизации дудника лекарственного корневищ и корней с учётом современных требований.

Материалы и методы. Морфолого-анатомические признаки дудника лекарственного корневищ и корней исследовали с помощью световой микроскопии. Люминесценцию тканей и рабочего стандартного образца кумариновой природы элеутерозида В1 (7-О-глюкозид изофраксидина) изучали люминесцентной микроскопией, сосудистые структуры исследовали поляризационной микроскопией. Профиль летучих соединений липофильных фракций водно-спиртового экстракта и эфирного масла определяли методом газовой хроматографии с масс-спектрометрической детекцией (ГХ-МС). Содержание суммы кумаринов определяли методом УФ-спектрофотометрии.

Результаты. Впервые изучены корневища и корни дудника лекарственного алтайского происхождения. Наиболее значимые результаты получены в диапазоне длин волн возбуждения 330–400 нм — наблюдали светло-голубую люминесценцию клеток флоэмы и секрета схизогенных вместилищ, обусловленную веществами кумариновой природы. Обоснована целесообразность применения гистохимической реакции с раствором судана III, выявляющей схизогенные вместилища, капли секрета и клеточные стенки сосудистых элементов. Для диагностики специфических проводящих элементов в сырье дудника (порошок) рекомендовано применение поляризационной микроскопии. Методом ГХ-МС для липофильных фракций водно-спиртового экстракта и эфирного масла установлен хроматографический профиль, включающий вещества терпеновой и кумариновой природы: пренилированный кумарин остол (до 49,72%) и эпиглобулол (25,61%). Содержание суммы кумаринов в исследуемом сырье составило 1,50±0,08% в пересчёте на кумарин.

Заключение. Комплексный подход к анализу дудника лекарственного корневищ и корней, заключающийся в использовании как широко распространённых (макроскопический, микроскопический, спектрофотометрический), так и ограниченных в применении (люминесцентная, поляризационная микроскопии и ГХ-МС) методов, служит основой для разработки проекта новой фармакопейной статьи «Дудника лекарственного корневища и корни».

Ключевые слова: дудник лекарственный; дудника лекарственного корневища и корни; световая микроскопия; люминесцентная микроскопия; поляризационная микроскопия; газовая хроматография с масс-спектрометрической детекцией; водно-спиртовой экстракт; липофильные фракции; эфирное масло; кумарины; терпены; остол; эпиглобулол; стандартизация

Список сокращений: БАВ — биологически активные вещества; ГХ-МС — газовая хроматография с масс-спектрометрией; ЛП — лекарственный препарат; ФС — фармакопейная статья; ЛРС — лекарственное растительное сырье; ГФ РФ — Государственная фармакопея Российской Федерации XV издания; ОФС — общая фармакопейная статья; РСО — рабочий стандартный образец; СО — стандартный образец; УФ-свет — ультрафиолетовый свет.

INTRODUCTION

Garden Angelica (*Angelica archangelica* L.; *A. archangelica*), widely known in folk medicine as “dyagil”, is a perennial herbaceous plant of the genus *Angelica* L. of the *Apiaceae* Lindl. family. In the Russian Federation, it is found in the European part, the Urals, Western Siberia, and the North Caucasus. It grows in moist places: along rivers, streams, banks of water bodies, at the edges of sedge bogs, in sparse forests, and in the forest-tundra. It is widespread in Europe. The species is listed in the Red Books of the Republic of Karelia, Tatarstan, and the Rostov and Saratov regions¹.

Issues concerning the morphological and anatomical characteristics of *A. archangelica* have been investigated by various groups of scientists: diagnostic features of leaves [1], fruits [2, 3], and herbs [4] have been identified. Within the scope of studying underground organs, the works of E.R. Grigoryan et al. [5] and F.A. Shakirova et al. [6] describe the external and microscopic features of whole, chopped, and powdered rhizomes and roots of *A. archangelica*. However, it should be noted that these sources lack descriptions of the results of luminescent and polarization analyses of histological features, which prevents the formation of a complete diagnostic picture of the raw material. Despite the fact that the work of F.A. Shakirova et al. [6] studied the luminescence of the tissues of *A. archangelica* rhizomes and roots. However, the formulation used, “pronounced fluorescence under ultraviolet light,” is subjective: the research materials do not specify the irradiation specifics (wavelength), the color of the fluorescence, or the tissue types exhibiting this luminescence. Furthermore, the researchers did not use reference samples to interpret the chemical nature of the luminescent agent.

The component composition of the essential oil and coumarins of the underground part of *A. archangelica* is of particular value for medical practice, as these biologically active compounds (BACs) exhibit a wide spectrum of pharmacological activity: antibacterial [7–9], antifungal [10], and anti-inflammatory [11, 12]. Using gas chromatography-mass spectrometry (GC-MS), species-specific components of the essential oil were identified: β -phellandrene, α -pinene, δ -3-carene, limonene [9, 13], and osthol [14–16]; coumarins identified include: osthol [7], angelicin, bergapten, imperatorin, oroselone, and methoxsalen [17–19, 21]. A liquid extract of garden angelica rhizomes and roots is part of the medicinal product (MP) “Iberogast” (Bayer Consumer Care AG, Switzerland), which is used to treat gastrointestinal diseases [20].

¹ Pimenov MG, Ostroumova TA. Umbelliferae of Russia; Moscow: Association of Scientific Publications of the KMC; 2012. 477 p. Russia

Garden angelica rhizomes and roots (*Angelicae archangelicae rhizomata et radices*) is an official medicinal plant raw material (MPRM) included in the pharmacopoeias of the USA, India, and the European Union. Specifically, the American Herbal Pharmacopoeia: Botanical Pharmacognosy—Microscopic Characterization of Botanical Medicines² provides descriptions of microscopic features for two species of angelica—*Angelica archangelica* L. and *Angelica sinensis* (oliv.) Diels. Monograph 01/2013:1857 “*Angelica archangelica* root” of the European Pharmacopoeia, 11th edition³, and “CANDA (Root)” of the Indian Pharmacopoeia, Volume 5⁴, present requirements that standardize the quality indicators of the MPRM of the specified plant. In the Russian Federation, GOST 21569-76 “Rhizomes and roots of garden angelica”⁵ is also in effect, standardizing the quality of raw materials intended for use in the food industry, including those supplied for export. Currently, the State Pharmacopoeia of the Russian Federation, XV edition (SPh RF XV ed.), lacks a pharmacopoeial monograph (PhM) for *A. archangelica* raw material.

The problem of falsification of powdered MPRM requires a comprehensive approach to control, where microscopic analysis remains a key tool. The most effective solution here is a combination of luminescent microscopy, GC-MS profiling, and spectrophotometry. The complementarity of morphological-anatomical and chemical indicators will ensure high identification accuracy and create significant obstacles to the potential falsification of the MPRM discussed in the article.

The data obtained during the study will form the basis for developing a draft of a new PhM “*Angelicae archangelicae* rhizomes and roots”.

THE AIM is to update approaches to the standardization of garden angelica rhizomes and roots through comprehensive research and in accordance with modern requirements.

MATERIALS AND METHODS

Study Object

Samples of *A. archangelica* rhizomes and roots, collected in 2024 in the foothills of the Krasnogorsky

² American Herbal Pharmacopoeia: Botanical Pharmacognosy — Microscopic Characterization of Botanical Medicines. *Angelica archangelica* L. Angelica Root. Radix *Angelicae archangelicae*. CRC Press; 2016. P. 234–236.

³ 01/2013:1857 «*Angelica archangelica* root» European pharmacopoeia 11.0.

⁴ CANDA (Root). The Ayurvedic pharmacopoeia of India Part – I. Volume V. P. 21–23. Available from: <https://www.ayurveda.hu/api/API-Vol-5.pdf>

⁵ GOST 21569-76 Rhizomes and roots of *Angelica officinalis*. Available from: <https://docs.cntd.ru/document/1200022507>. Russian

district of the Altai Territory (garden angelica rhizomes and roots of Altai origin), were used as the study object. Primary processing included cleaning the raw material from soil, washing with cold water, and drying at a temperature of approximately 45°C (artificial drying). The finished samples were purchased from the company IVA (Moscow, Russia) in 2025.

Macroscopic, Microscopic, Microchemical and Histochemical Studies

For the description and evaluation of the external characteristics of the raw material, the provisions presented in the general pharmacopoeial article (GPhM) GPhM.1.5.1.0006 "Roots, rhizomes, bulbs, tubers, cormels" were followed⁶. A Motic DM-39 stereomicroscope (Motic Xiamen, China) with magnifications of $\times 20$ and $\times 40$ and a built-in digital camera was used in the study.

When preparing samples, the requirements of GPhM.1.5.3.0003 "Microscopic and microchemical analysis of medicinal plant raw material and medicinal products of plant origin"⁷ and GPhM.1.5.3.0003.15 "Technique of microscopic and microchemical examination of medicinal plant raw material and medicinal plant preparations"⁸ were adhered to. Transverse sections of rhizomes and roots were prepared manually with a razor blade, as well as on a HM 325 Thermo FS microtome (Thermo Fisher Scientific, USA).

Identification of microscopic features, as well as micro- and histochemical studies, were performed according to GPhM.1.5.1.0006 "Roots, rhizomes, bulbs, tubers, cormels", GPhM.1.5.3.0003 "Microscopic and microchemical analysis of medicinal plant raw material and medicinal products of plant origin", and GPhM.1.5.3.0003.15 "Technique of microscopic and microchemical examination of medicinal plant raw material and medicinal plant preparations" using a Motik DM 1812 light microscope (Motic Xiamen, China)

with magnifications of $\times 40$, $\times 100$, $\times 400$, and a built-in digital camera. Micro-preparations were stained with a Sudan III solution to reveal lipophilic cytological features. Reagent preparation was carried out according to the methods described in GPhM.1.3.0001 "Reagents. Indicators"⁹.

Diagnosis of the conductive elements of the raw material was performed on a PLM-213 polarization microscope (LOMO, Russia) equipped with a ToupCam SCMO503100KPA video eyepiece (ToupTek Photonics, China); tissue luminescence was studied on an Altami LUM-2 luminescence microscope (Altami, Russia) with magnifications of $\times 100$ and $\times 400$, equipped with a Canon EOS 1200D camera (Canon Inc., Japan) and a high-voltage mercury lamp (HBO 100W) as a light source. Luminescence excitation was carried out through a filter system covering the following spectral range: blue excitation filter—420–550 nm, and ultraviolet filter—330–400 nm. As a reference sample, a working standard sample of eleutheroside B1 (7-O-glucoside of isofraxidin), isolated and provided by the scientific and educational center "Pharmacy" of Samara State Medical University, was used. Its luminescence was studied by irradiating air-dried crystals directly on a microscope slide.

For detailed microscopy of powder elements, alkaline sample preparation was used according to GPhM.1.5.3.0003.15 "Technique of microscopic and microchemical examination of medicinal plant raw material and medicinal plant preparations", method 2.

Extraction of Extract for GC-MS Analysis

Approximately 100 g of raw material, ground to a particle size passing through a sieve with openings of 2 mm in diameter, was placed in a 500 mL conical flask and extracted with a 60% ethanol in a volume sufficient to obtain a "mirror" using fractional maceration. The first infusion was carried out for 24 hours, and the subsequent 3 were for 12 hours with periodic shaking. The extracts were combined, allowed to settle, and then filtered through "red ribbon" filter paper (aqueous-alcoholic fraction). When choosing the extractant, its extraction capacity for the comprehensive extraction of a wide spectrum of BACs contained in the underground part of *A. archangelica* was considered: its ability to extract both low-polarity substances (of terpenoid nature) and the phenolic complex (coumarin derivatives).

Subsequently, liquid-liquid extraction with n-hexane and chloroform was performed, and

⁶ GPhM.1.5.1.0006 «Roots, rhizomes, bulbs, tubers, cormels». State Pharmacopoeia of the Russian Federation, XV ed. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-5/1-5-2/korni-kornevishcha-lukovitsy-klubniklubnelukovitsy/?sphrase_id=1215890. Russian

⁷ GPhM.1.5.3.0003 «Microscopic and microchemical analysis of medicinal plant raw material and medicinal products of plant origin». State Pharmacopoeia of the Russian Federation, XV ed. 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/>. Russian

⁸ GPhM.1.5.3.0003.15 «Technique of microscopic and microchemical examination of medicinal plant raw material and medicinal plant preparations». State Pharmacopoeia of the Russian Federation, XV ed. Available from: <https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-13/1/1-5/1-5-3/1-5-3-3/tekhnika-mikroskopicheskogo-i-mikrokhimicheskogo-issledovaniya-lekarstvennogo-rastitelnogo-syrya-i-l/>. Russian

⁹ GPhM.1.3.0001 «Reagents. Indicators». State Pharmacopoeia of the Russian Federation, XV ed. Available from: <https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-3/reaktivy-indikatory>

the corresponding fractions of substance sums were obtained, which were then subjected to chromatography. When selecting organic solvents, literature data confirming the optimal extraction capacity of these solvents for extracting volatile organic compounds [22–24] were used.

For this purpose, 10.0 mL of the aqueous-alcoholic fraction was placed in a 50 mL conical separatory funnel, and 5 mL of “extra pure” grade n-hexane (EKOS-1, Russia) was added and shaken for 2 min, then allowed to stand until complete phase separation. The hexane fraction was filtered through “red ribbon” filter paper into a 25 mL volumetric flask. Extraction with n-hexane was repeated 2 more times with 5 mL portions, each time filtering into the same volumetric flask. The volume of the solution in the volumetric flask was brought to the mark with n-hexane and mixed (hexane fraction). The chloroform fraction was obtained similarly.

Obtaining Essential Oil

Essential oil was obtained by hydrodistillation in accordance with GPhM.1.5.3.0010.15 “Determination of essential oil content in medicinal plant raw material and medicinal plant preparations”¹⁰ (method 2; 50 g of raw material, ground to a particle size passing through a sieve with openings of 2 mm in diameter, distillation time—3 hours).

GC-MS Analysis

The analysis was performed under conditions described previously [26, 27]. The qualitative composition of the extract and essential oil was determined in accordance with GPhM.1.2.1.2.0004 “Gas Chromatography”¹¹ on a “MAESTRO 7820” gas chromatograph (INTERLAB, Russia) with an Agilent 5975 mass spectrometric detector (Agilent Technologies Inc., USA).

Chromatographic conditions: HP-5MS capillary quartz column, 30 m×0.25 mm×0.25 μm (stationary phase: 5% diphenyl, 95% dimethylsiloxane), mobile phase—helium, flow rate—1 mL/min, column thermostat temperature—isothermal at 40°C for 5 min, heating to 80°C at a rate of 3°C/min, heating to 180°C at a rate of 4°C/min, heating to 280°C at a rate of 8°C/min, isothermal at 280°C for 15 min, evaporator

temperature—270°C, ion source temperature—150°C, quadrupole temperature—230°C, transfer line temperature—280°C, injection without splitting. Sample introduction method—autosampler, sample volume—1 μL.

Identification of volatile components was carried out by comparing linear retention indices and full mass spectra with data from the NIST 2.4 mass spectral library and literature data. During identification, only components with a mass spectral match coefficient above 90% were considered, which corresponds to standard scientific practice. The proportion of each component to the sum of all components was calculated by the internal normalization method based on the areas of the corresponding peaks in the chromatogram constructed by total ion current.

Quantitative Determination of Total Coumarins

For the quantitative determination of total coumarins, a spectrophotometric method was used in accordance with the requirements of GPhM.1.2.1.1.0003 “Spectrophotometry in the ultraviolet and visible regions”¹². Within the scope of standardizing MPRM containing a complex of phenolic compounds, spectrophotometric analysis remains relevant as a normalization method [27, 28] due to its justification in the context of high precision, speed, and satisfactory accuracy for establishing standards for the content of total BAC fractions [29].

The analysis was performed on an SF-56 Spectrophotometer (LOMO, Russia), included in the State Register of Measuring Instruments of the Russian Federation (No. 74729-19), using quartz cuvettes with a path length of 10 mm. Coumarin (Sigma-Aldrich, Germany, cat. No. C4261, purity ≥ 98%) was used as a standard sample (SS). Solutions were prepared using “extra pure” grade solvents (EKOS-1, Russia). Raw material moisture content was determined in accordance with the requirements of GPhM.1.5.3.0007.15 “Determination of moisture content in medicinal plant raw material and medicinal plant preparations”¹³ in a 2B-151 drying oven (Medlabtekhnik, USSR).

¹⁰ GPhM.1.5.3.0010.15 «Determination of essential oil content in medicinal plant raw material and medicinal plant preparations». State Pharmacopoeia of the Russian Federation, XV ed. 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-rastitelnykh>. Russian

¹¹ GPhM.1.2.1.2.0004 «Gas Chromatography». State Pharmacopoeia of the Russian Federation, XV ed. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-2/1-2-1/1-2-1-2-khromatograficheskie-metody-analiza-gazovaya-khromatografiya/?sphrase_id=1215888. Russian

¹² GPhM.1.2.1.1.0003 «Spectrophotometry in the ultraviolet and visible regions». State Pharmacopoeia of the Russian Federation, XV ed. Available from: <https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-2/1-2-1/1-2-1-1-metody-spektralnogo-analiza-spektrofotometriya-v-ultrafioletovoy-i-vidimoy-oblastyakh/>. Russian

¹³ GPhM.1.5.3.0007 «Determination of moisture content in medicinal plant raw material and medicinal plant preparations». State Pharmacopoeia of the Russian Federation, XV ed. Available from: <https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-5/1-5-1/opredelenie-vlazhnosti-lekarstvennogo-rastitelnogo-syrya-i-lekarstvennykh-sredstv-rastitelnogo-prois/>. Russian

Standard Solution A of Coumarin SS.

Approximately 0.005 g (exact weight) of coumarin SS was placed in a 50 mL volumetric flask, dissolved in 96% ethanol, the volume of the solution was brought to the mark, and mixed.

Solution B of Coumarin SS. 2.0 mL of standard solution A was placed in a 25 mL volumetric flask, 2.0 mL of 1M sodium hydroxide solution was added, and the volume was brought to the mark with 70% ethanol and mixed.

Test Solution A. Approximately 1.0 g (exact weight) of raw material, ground to particles no larger than 1 mm, was placed in a 250 mL flask with a ground-glass stopper, and 80 mL of 96% alcohol was added. The flask was attached to a reflux condenser and heated in a boiling water bath for 30 min. After cooling to room temperature, the contents of the flask were quantitatively transferred through a "red ribbon" paper filter into a 100 mL volumetric flask. The residue in the flask was washed with the same alcohol, passing the washings through the same filter, and the volume of liquid in the flask was brought up to the mark with 96% alcohol. The contents of the flask were thoroughly mixed. Then, 25.0 mL of the resulting solution was evaporated to a volume of 2–3 mL, quantitatively transferred to a separatory funnel using 50 mL of purified water. The solution was acidified with 1M hydrochloric acid solution to pH 2–3 and extracted with ethyl acetate three times: first with 50 mL, then twice with 30 mL. The combined ethyl acetate extracts were filtered through 2 g of anhydrous sodium sulfate, evaporated to dryness on a rotary evaporator at 70°C, and the dry residue was dissolved in 70% alcohol and filtered into a 25 mL volumetric flask through a "red ribbon" filter.

Test Solution B. 1.0 mL of Test Solution A was placed in a 25 mL volumetric flask, 2.0 mL of 1M sodium hydroxide solution was added, the volume of the solution was brought to the mark with 70% alcohol, and mixed.

Reference Solution. 2.0 mL of 1 M sodium hydroxide solution was placed in a 25 mL volumetric flask, the volume of the solution was brought to the mark with 70% alcohol, and mixed.

After 10 min, the optical density of Test Solution B and the coumarin reference standard solution B was measured relative to the reference solution on a spectrophotometer at a wavelength of 335 nm in a 10 mm cuvette.

The content of coumarins, calculated as coumarin, in percent (X) was determined by the formula:

$$X = \frac{A \times a_0 \times 2 \times 100 \times 25 \times 25 \times P \times 100 \times 100}{A_0 \times 50 \times 25 \times a \times 25 \times 1 \times 100 \times (100 - W)} = \frac{A \times a_0 \times P \times 400}{A_0 \times a \times (100 - W)},$$

where A is the optical density of Test Solution B; A_0 is the optical density of coumarin reference standard solution B; a is the weight of the raw material, g; a_0 is the weight of the coumarin reference standard, g; W is the moisture content of the raw material, %.

The determination was performed in triplicate analytical replicates ($n = 3$). Results are expressed as the arithmetic mean $\pm$ standard deviation (SD).

The proposed method was validated in accordance with the requirements of GPhM.1.1.0012 "Validation of analytical methods"¹⁴ for the following characteristics: specificity, linearity, analytical range, accuracy, precision (repeatability and intermediate precision).

RESULTS

Macroscopic, Microscopic, Microchemical and Histochemical Analyses

Medicinal raw material "*Angelica archangelica* rhizomes and roots" is a collection of fragments of rhizomes in transverse and longitudinal sections with a rough, transversely wrinkled cork on the surface (Fig. 1F, 1G). The fragments vary in diameter from 2.5 cm to 5 cm and in height up to 2 cm. In cross-section, a brown cortical part with cavities in the parenchyma is visible, likely of rexygenic origin (Fig. 1D). The central part of the rhizome fragments (pith parenchyma) is light gray or light yellow. Upon detailed examination, the pith parenchyma has a radiate texture extending from the center (Fig. 1A, 1C). On some fragments, remnants of young cork and elements of the stem part of the plant are visible (Fig. 1I, 1H).

The raw material also contains large whole fragments of roots up to 15 cm in length and 1.5 cm in width. The surface of the roots is dark brown and has a wavy-wrinkled texture with pronounced attachment points of lateral roots (Fig. 1B).

Roots of secondary structure, described previously by other authors [5], have a pronounced wavy margin in cross-section due to rexygenic cavities—radial ruptures of the cortical parenchyma (Fig. 2A). The surface of the roots is covered with a dark brown (sometimes black) cork layer. In the cortical parenchyma, at low magnification, schizogenous cavities containing drops of yellow or light yellow secretion are visible. The cavities stain pink with Sudan III solution (Fig. 2B) and are better detected in cross-sections, including in powder fragments (Fig. 2C, 6C, 6E).

The wavy cambium zone also stains with Sudan III

¹⁴ GPhM.1.1.0012 «Validation of analytical methods». State Pharmacopoeia of the Russian Federation, XV ed. Available from: <https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-1/validatsiya-analiticheskikh-metodov/>. Russian

solution. At high magnification ($\times 400$), narrow-lumen cambial zone cells with brown protoplast contents are visible (Fig. 2D). The xylem part of the central cylinder is devoid of schizogenous cavities. It generally prevails over the secondary cortex. The xylem is represented by radially arranged groups of vessels, between which are located wide medullary rays, composed of uniform oval xylem parenchyma cells up to 10 cells thick (Fig. 2C). In the phloem parenchyma and medullary ray parenchyma, starch is present in the form of small simple grains, almost spherical in shape, up to $6\ \mu\text{m}$ in size.

Vessels in cross-section are of varying sizes with noticeably thickened cell walls, which stain pink with Sudan III solution (Fig. 2E). In longitudinal sections, three types of vascular elements are diagnosed: mainly scalariform, reticulate, and, less commonly, spiral vessels. It should be noted that the best contrast of vascular elements, including in powder, is achieved with polarized microscopy with a λ -light filter (Fig. 2F).

The species specificity of the medicinal raw material is closely related to the chemical composition of the producing plant. It is known that *A. archangelica* rhizomes and roots contain phenolic compounds of the coumarin group, which allows the luminescence characteristics to be interpreted as a pronounced diagnostic feature, additionally used to confirm the authenticity of the raw material.

During the examination of transverse sections of *A. archangelica* rhizomes and roots, the luminescence characteristics of their tissues were analyzed. Thus, the phloem parenchyma of the cortex, in the excitation range of 330–400 nm, exhibits a light blue luminescence, mainly due to the protoplasts of the cells (Fig. 3A). The oily secretion drops in the schizogenous cavities have a characteristic blue luminescence in this spectral region. It should be noted that the epithelial cells lining the cavities do not luminesce and are visually identified as a dark border against the luminescent elements of the parenchyma and the secretion filling the cavity lumens (Fig. 3A). In the excitation range of 420–550 nm, the phloem parenchyma and resinous secretion luminesce bright yellow (Fig. 3B).

The xylem part of the cross-section also has a characteristic bright blue luminescence in the excitation range of 330–400 nm due to the lignified cell walls of vessels and medullary ray cells. In the excitation range of 420–550 nm, the luminescence is weaker and yellow (Fig. 3C, 3D, 3E). The cork tissue in the visible spectrum is brown due to the polyphenol suberin.

The cork tissue cells are irregularly, almost

rectangularly shaped with remnants of protoplasts at the periphery (Fig. 3F). The light blue luminescence in the excitation range of 330–400 nm (Fig. 3G) and the dirty yellow luminescence in the excitation range of 420–550 nm (Fig. 3H) of the cork tissue cell walls are characteristic due to the presence of polyphenols (suberin), including simple phenolic compounds (Fig. 3D, 3E).

The epithelial cells of the cavities are thin-walled, rectangular in cross-section (Fig. 4A). The protoplasts of the epithelial cells are amorphous, light yellow, and stain pink when treated with Sudan III solution. The parenchyma around the cavity is composed of large, collapsed or wavy cells with noticeably thickened cellulosic cell walls compared to the thin-walled cells of the main parenchyma (Fig. 4B). To interpret the chemical nature of the luminescent agents, we analyzed the luminescence of the coumarin-based reference standard of eleutheroside B1 (Fig. 4F, 4G, 4H). It was found that the reference standard of eleutheroside B1 in the excitation range of 330–400 nm exhibits bright blue luminescence (Fig. 4G), similar to that of secretion drops in schizogenous cavities (Fig. 4C, 4D, 4E).

To test previously known and newly described characteristics of *A. archangelica* rhizomes and roots, model powders with particle sizes passing through sieves with mesh diameters of 0.5 mm and 0.25 mm were analyzed. Under a magnifying glass ($\times 20$, $\times 40$), the rhizome and root particles appear uniformly brown (Fig. 5A, 5B). When the dry powder is irradiated in the excitation range of 330–400 nm, the powder particles luminesce bright blue (Fig. 6A), characteristic of *A. archangelica* tissues described above. In the excitation range of 420–550 nm, the luminescence changes color to yellow (Fig. 6B).

Upon examination of a powder sample prepared according to the requirements of the SPH RF XV ed., fragments of phloem parenchyma with recognizable structures of schizogenous cavities are diagnosed (Fig. 5C). When treated with Sudan III solution, the secretion drops stain orange-pink. Additionally, upon irradiation of the powder in the excitation range of 330–400 nm, the secretion drops luminesce bright blue (Fig. 6C, 6E).

The powder also frequently contains vascular element fragments of varying sizes, mainly reticulate and spiral vessels (Fig. 5D). Under UV light in the excitation range of 330–400 nm, these fragments luminesce blue (Fig. 6D, 6F). Fragments of transverse sections of vascular elements are sometimes encountered. Typically, these are groups of vessels of different sizes (Fig. 5F).

It should be noted that the best contrast of denser powder elements is achieved using a polarizing microscope with a λ -light filter. In this case, vascular elements of various types are easily diagnosed in the general mass of weakly stained fragments by the bright staining of the dense cell walls (Fig. 5G, 5H).

Cork tissue fragments are easily diagnosed by the specific brown staining of the cell walls (Fig. 5E). Phloem parenchyma fragments are barely visible under normal light. In the excitation range of 330–400 nm, some fragments luminesce due to crystalline inclusions, presumably of coumarin origin (Fig. 6G, 6H, 6I).

Determination of Essential Oil Content

The essential oil content in *A. archangelica* rhizomes and roots was 0.09% calculated on a dry basis (with a raw material moisture content of 9.2%).

Gas Chromatography-Mass Spectrometry

GC-MS analysis allowed for the determination of the volatile organic compound profile of the lipophilic fractions of the aqueous-alcoholic extract and the essential oil of *A. archangelica* rhizomes and roots of Altai origin (Table 1).

The main group of biologically active substances in the hexane and chloroform fractions of the aqueous-alcoholic extract is represented by prenylated coumarins (49.94% and 44.42%, respectively), which constituted half of all identified components. The effective extraction of these coumarins with hexane is due to the prenylation process: an isoprene fragment ($-C_5H_9$) is attached to the coumarin nucleus at the C8 or C6 position, which increases lipophilicity and enhances their solubility in nonpolar solvents.

Furanocoumarins are present in the lipophilic fractions of the extract at 7.14% in hexane and 12.67% in chloroform. The hexane fraction also contains twice as much monoterpenoids (5.07% and 2.90%), sesquiterpenes (12.63% and 4.82%), sesquiterpenoids (9.58% and 3.49%), and significantly exceeds the chloroform fraction in monoterpene content (26.03% and 12.04%), which is reflected in the characteristic differences in the polarity of the extractants. The proportion of pyranocoumarins, methoxycoumarins, aromatic compounds, and fatty acids was minor (less than 2%) (see Table 1).

Analysis of the essential oil showed the dominant role of monoterpenes (46.2%), sesquiterpenes (43.5%), and sesquiterpenoids (35.23%). Monoterpenoids also constitute a significant portion—13.53%. Prenylated coumarins (osthol) are present in the essential oil (1.54%) due to their physicochemical properties. The absence of furanocoumarins, pyranocoumarins, and fatty acids is related to their high molecular weight and limited volatility during hydrodistillation (see Table 1).

According to the data presented in Table 1, the dominant component in the lipophilic fractions of the aqueous-alcoholic extract is the prenylated coumarin osthol. Its proportion averaged 49.72% in the hexane and 44.06% in the chloroform fractions of the total peak area of all compounds. Also among the leading components in the hexane and chloroform fractions were substances of terpene nature: α -pinene (7.29%, 4.24%); epiglobulol (5.42%, 2.04%); 3-carene (5.02%, 1.82%); D-limonene (3.82%, 1.46%); β -phellandrene (2.13%, 0.79%); bornyl acetate (1.99%, 0.78%); coumarin-based substances: oroselone (3.76%, 2.60%); angelicin (1.84%, 4.18%); oxypeucedanin (0.47%, 1.65%); isopinipenellin (0.38%, 2.48%), respectively.

In the essential oil, the dominant components were epiglobulol (25.61%), 3-carene (15.22%), bornyl acetate (5.89%), α -pinene (4.26%), D-limonene (3.67%), and osthol (1.54%) (see Table 1).

GC-MS chromatograms of volatile organic compounds of the lipophilic fractions of the aqueous-alcoholic extract and the essential oil of *A. archangelica* rhizomes and roots of Altai origin are presented in Figures 7–9.

Quantitative Determination of Total Coumarins

The content of total coumarins in *A. archangelica* rhizomes and roots of Altai origin, determined by spectrophotometric method, was $1.50 \pm 0.08\%$ calculated as coumarin. The analysis was performed in triplicate analytical replicates ($n = 3$), and the relative standard deviation (RSD) did not exceed 1.0%, indicating high reproducibility of the method.

The linearity range of the method was established in the concentration interval of coumarin from 2.0 to 20.0 $\mu\text{g}/\text{mL}$. The linear relationship is described by the equation: $y = 0.0482x \pm 0.0031$ with a coefficient of determination $r^2 = 0.9994$ and a correlation coefficient $r = 0.9997$ ($n = 10$), which confirms a high degree of linear dependence. The analytical range (application range) of the method was established in the same interval of 2.0–20.0 $\mu\text{g}/\text{mL}$, corresponding to 80–120% of the expected content of total coumarins in the test solution (normal concentration approximately 6.0 $\mu\text{g}/\text{mL}$). The accuracy of the method was confirmed by the “addition method” with an average recovery value of 98.9% ($n = 9$) in the range of 98.0–100.2%. Precision indicators met the requirements: the relative standard deviation for repeatability was 0.8% ($n = 6$), and for intermediate precision—1.5% ($n = 3$ days). Specificity was confirmed by the absence of significant absorption by ballast matrix components at 335 nm ($A \leq 0.02$).

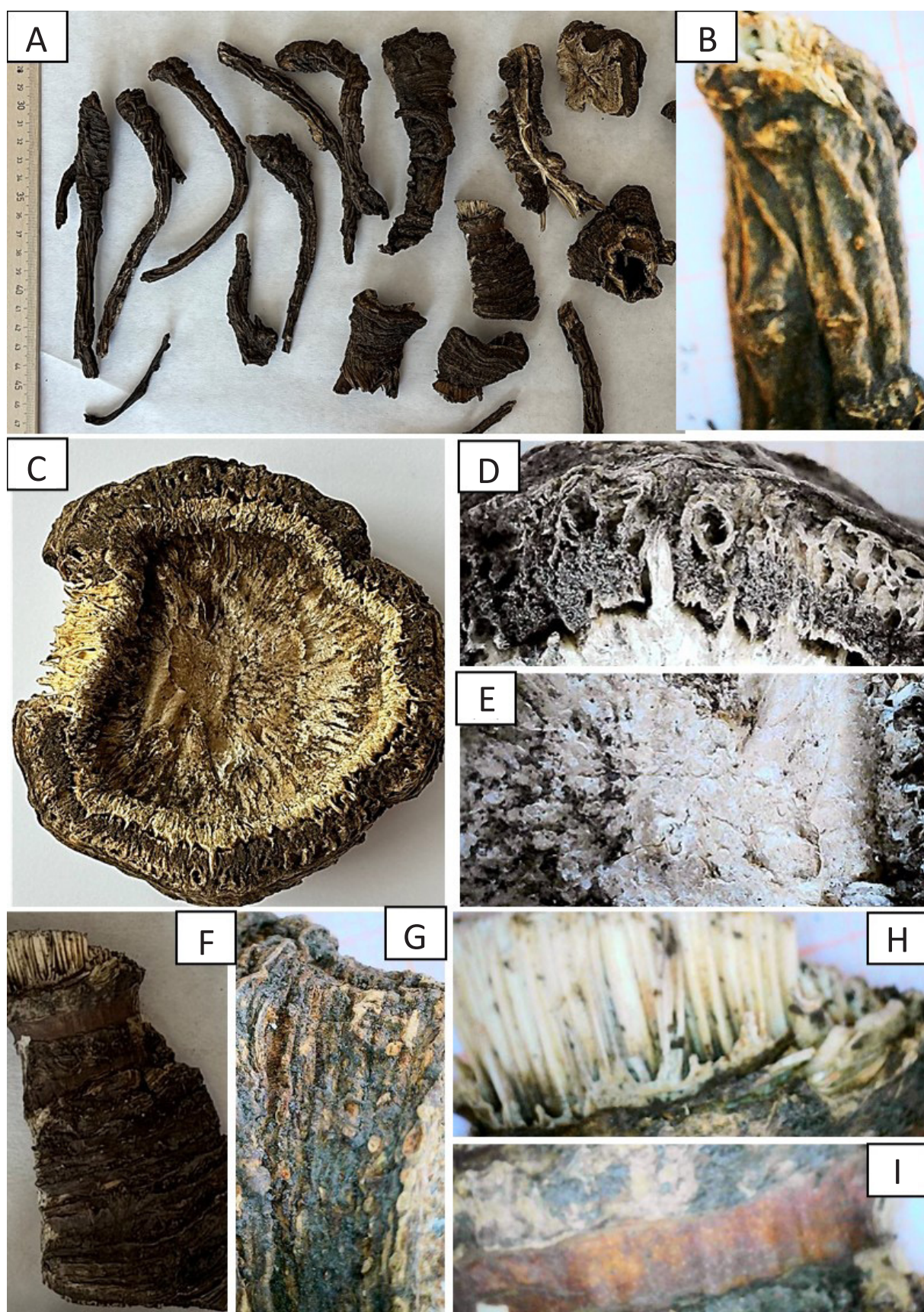


Figure 1 — Appearance and morphological features of *A. archangelica* raw material.

Note: A, collection of raw material fragments; B, wrinkled cork from the root surface; C, rhizome fragments in cross-section; D, cortical part of the rhizome; E, pith of the rhizome; F, rhizome surface fragment; G, rough transversely wrinkled cork of the rhizome; H, stem remnants; I, brown fragments of young cork.

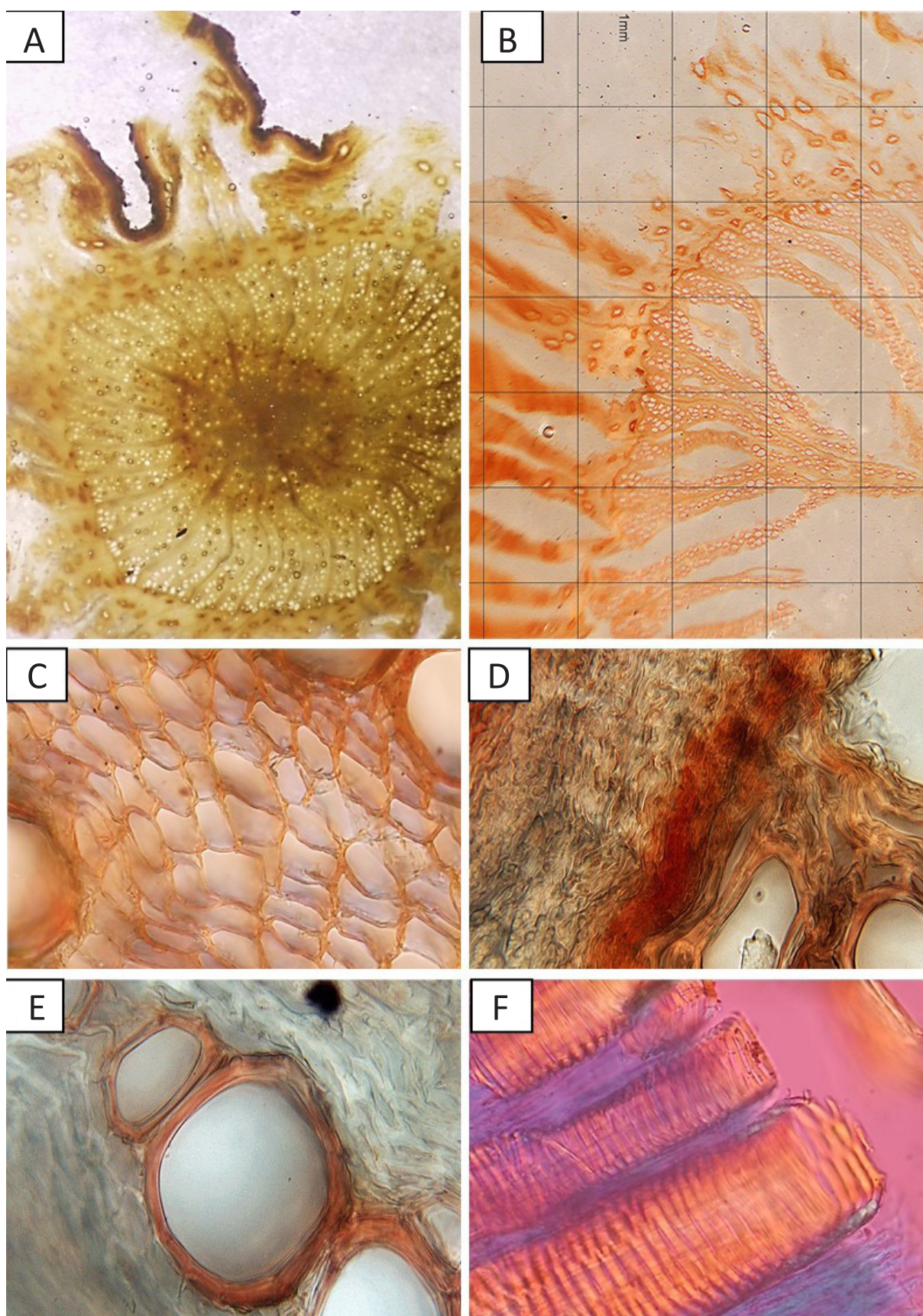


Figure 2 — Conducting tissues of *A. archangelica* rhizomes and roots.

Note: A, root cross-section, general view with a wavy edge (×40); B, fragment of xylem and phloem conducting elements. Stained with Sudan III solution (×40); C, ray parenchyma (×400); D, cambium fragment (×400); E, vascular elements in cross-section. Stained with Sudan III solution (×1000); F, fragment of reticulate vessels in polarized light with λ-filter (×400).

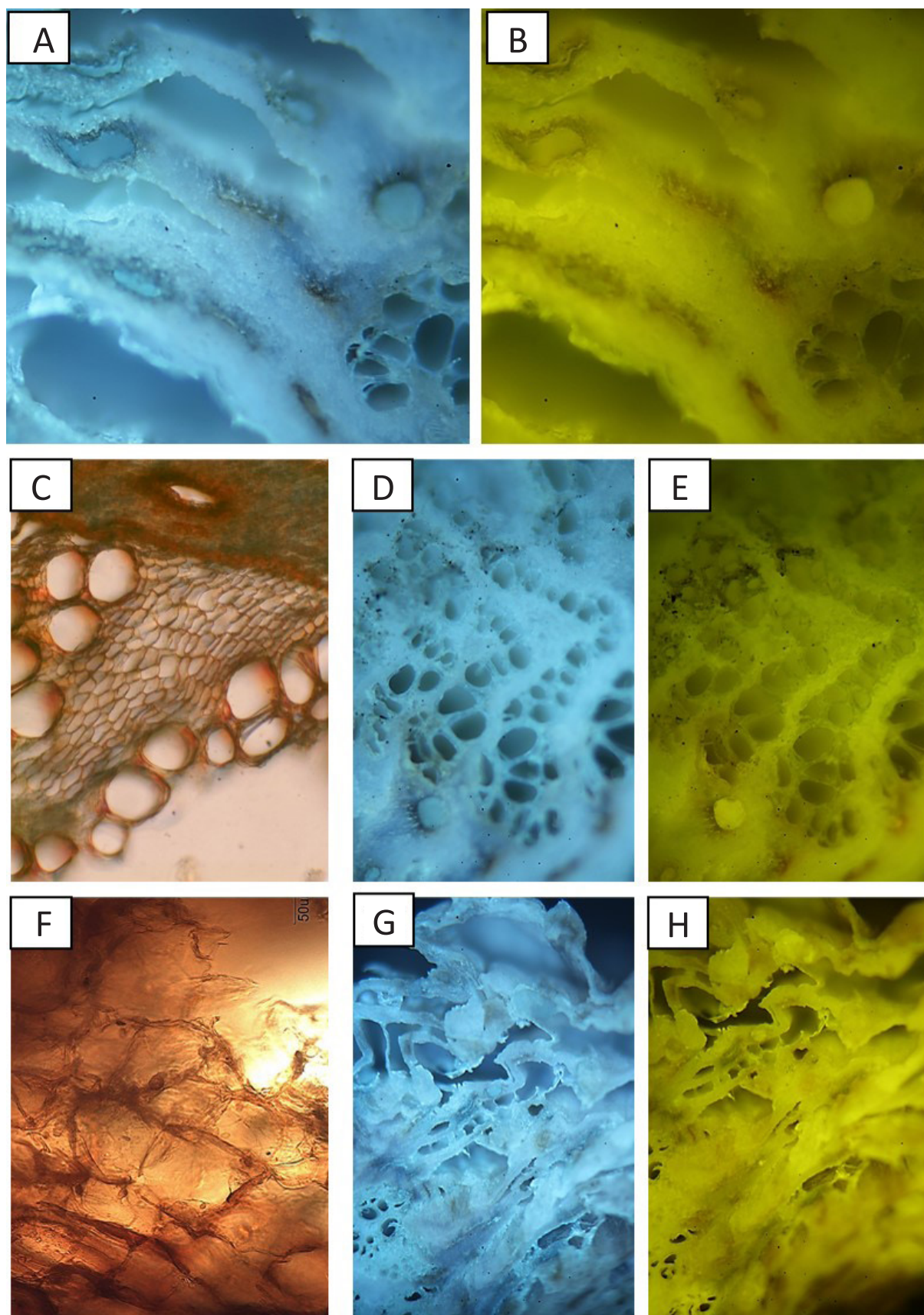


Figure 3 — Luminescence features of *A. archangelica* rhizome and root tissues.

Note: A, phloem in the excitation range of 330–400 nm ($\times 400$); B, phloem in the excitation range of $\lambda = 420\text{--}550$ nm ($\times 400$); C, xylem in visible light ($\times 400$); D, xylem in the excitation range of $\lambda = 330\text{--}400$ nm ($\times 400$); E, xylem in the excitation range of $\lambda = 420\text{--}550$ nm ($\times 400$); F, cork in visible light ($\times 400$); G, cork in the excitation range of $\lambda = 330\text{--}400$ nm ($\times 400$); H, cork in the excitation range of $\lambda = 420\text{--}550$ nm ($\times 400$).

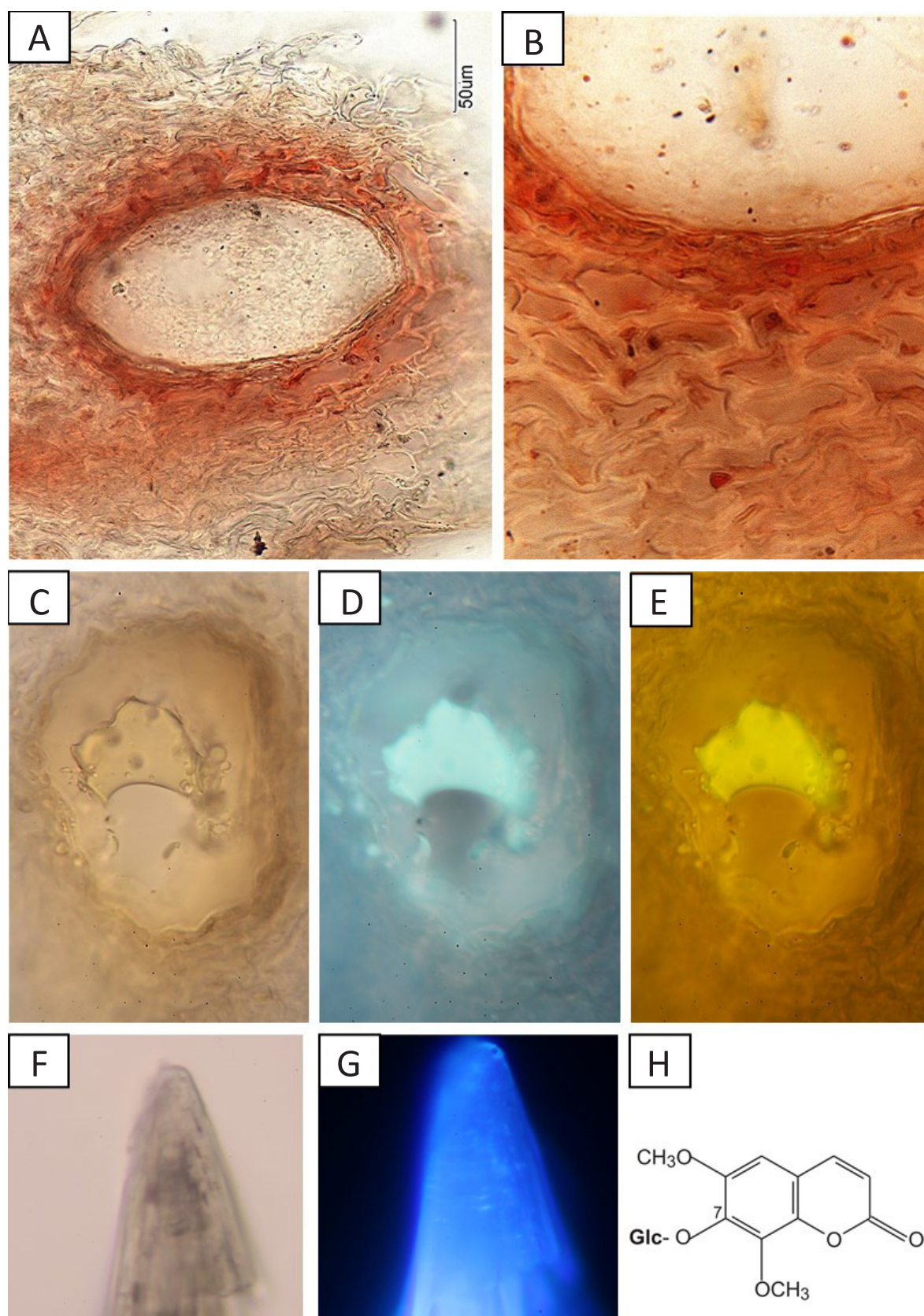


Figure 4 — Histological features of schizogenous cavities of rhizomes and roots of *A. archangelica*.

Note: A, staining of the schizogenous cavity with Sudan III solution ($\times 100$); B, cells of the epithelial layer and fundamental parenchyma around the cavity. Staining with Sudan III solution ($\times 400$); C, oily secretion of the cavity ($\times 100$); D, luminescence of the secretion in the excitation range $\lambda = 360-400$ nm ($\times 100$); E, luminescence of the secretion in the excitation range $\lambda = 420-550$ nm ($\times 100$); F, crystal of a standard sample ($\times 400$); G, luminescence of the standard sample in the excitation range $\lambda = 360-400$ nm ($\times 100$); H, structural formula of the standard sample of eleutheroside B1.

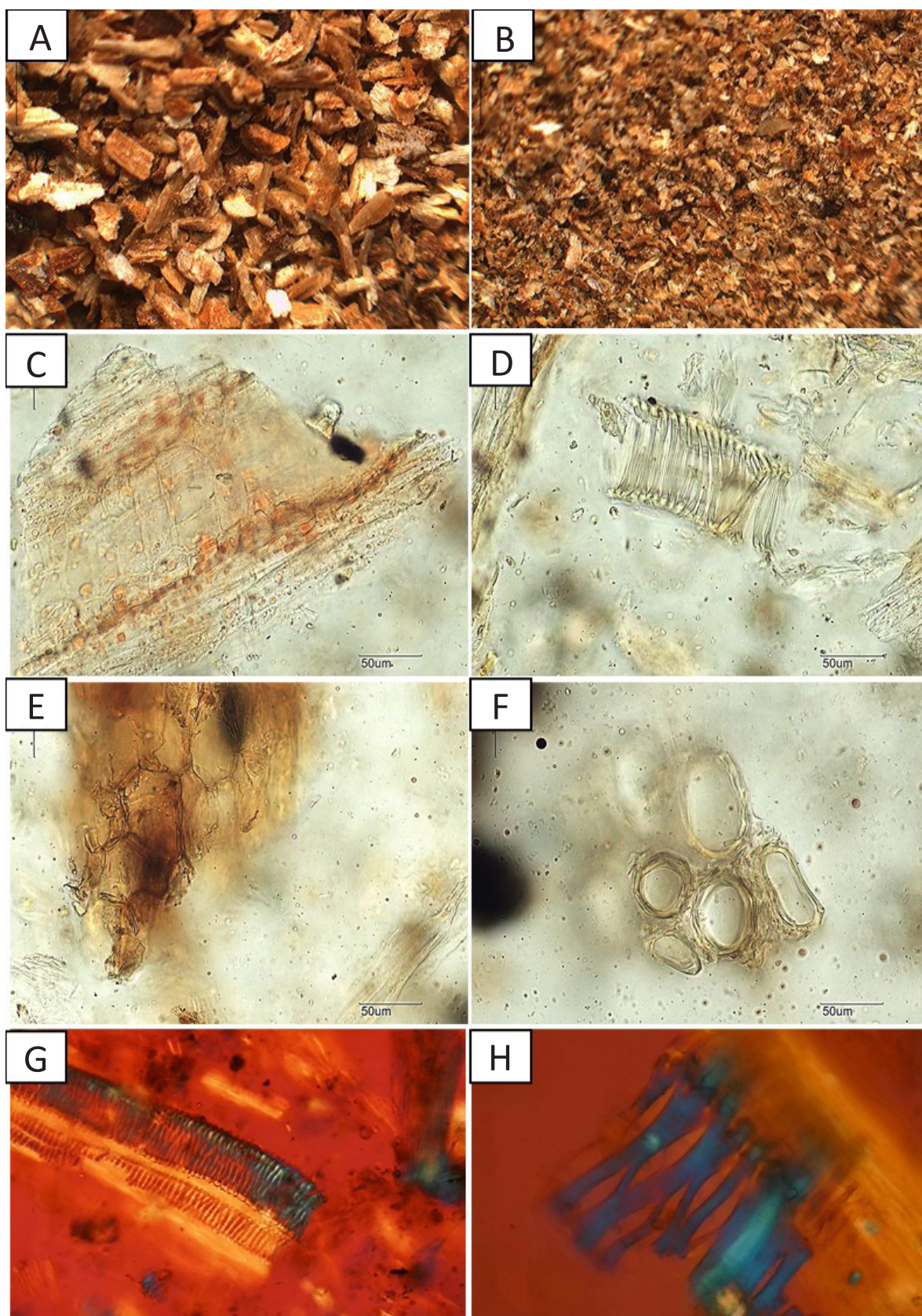


Figure 5 — Main diagnosable elements of the powder of rhizomes and roots of *A. archangelica*.

Note: A, powder sample with particle size passing through a 0.5 mm sieve (×40); B, powder sample with particle size passing through a 0.25 mm sieve (×40); C, fragment of a cavity with essential oil droplets (×400); D, fragment of a spiral vessel (×400); E, fragment of cork (×400); F, fragment of a group of vessels in cross-section (×400); G, fragment of reticulate vessels in polarized light λ-filter (×100); H, contrast of the cell wall of a fragment of reticulate vessels in polarized light λ-filter (×400).

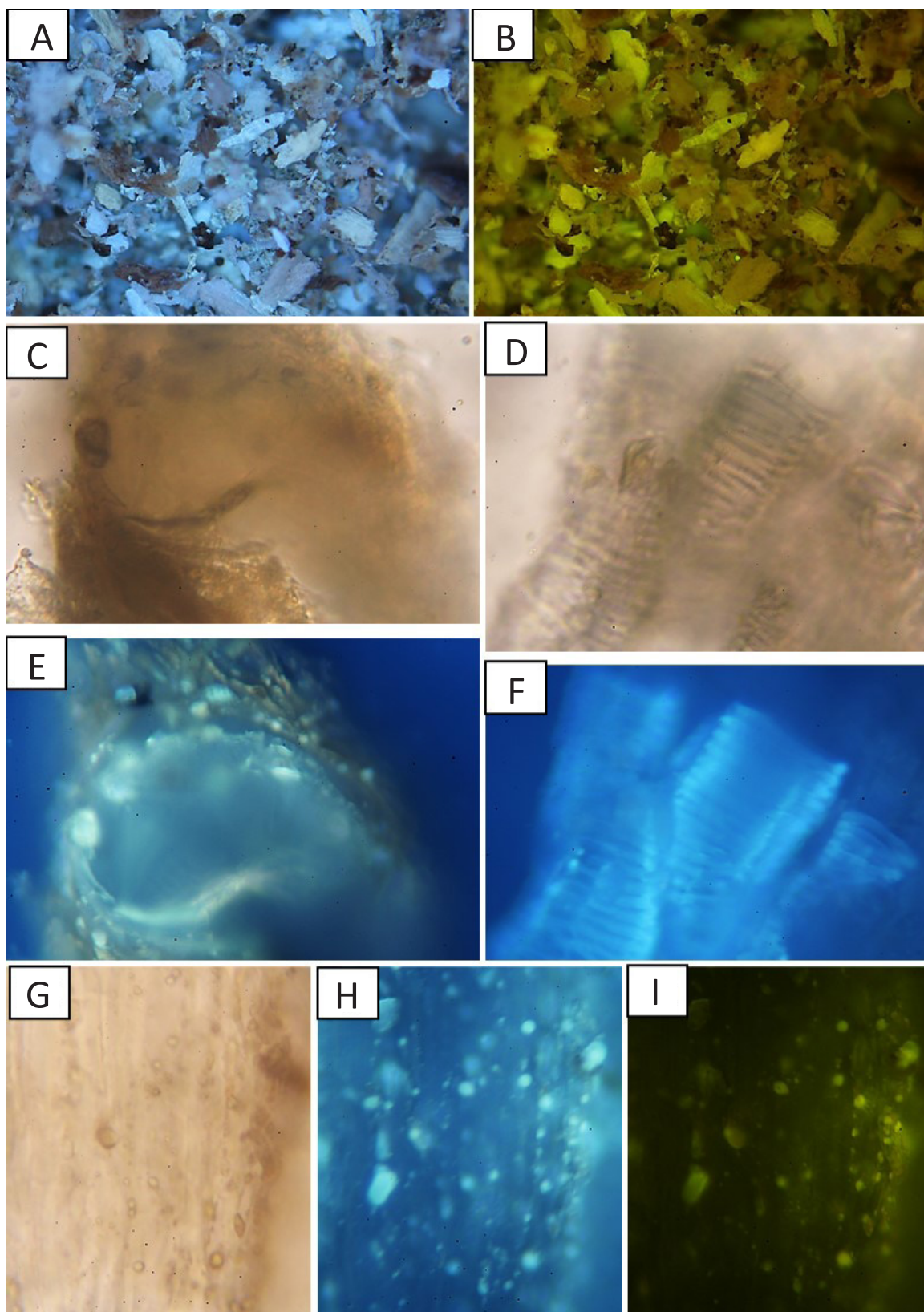


Figure 6 — Luminescence of elements of the powder of rhizomes and roots of *A. archangelica*.

Note: A, dry powder with particle size passing through a 0.25 mm sieve, luminescence in the excitation range $\lambda = 330\text{--}400\text{ nm}$ ($\times 100$); B, dry powder with particle size passing through a 0.25 mm sieve, luminescence in the excitation range $\lambda = 420\text{--}550\text{ nm}$ ($\times 100$); C, fragment of a cavity with oil droplets in daylight ($\times 400$); D, fragment of reticulate vessels in daylight ($\times 400$); E, luminescence of a cavity with oil droplets in the excitation range $\lambda = 330\text{--}400\text{ nm}$ ($\times 400$); F, luminescence of a fragment of reticulate vessels in the excitation range $\lambda = 330\text{--}400\text{ nm}$ ($\times 400$); G, crystalline inclusions in the cells of the fundamental parenchyma ($\times 400$); H, luminescence of crystalline inclusions in the excitation range $\lambda = 330\text{--}400\text{ nm}$ ($\times 400$); I, luminescence of crystalline inclusions in the excitation range $\lambda = 420\text{--}550\text{ nm}$ ($\times 400$).

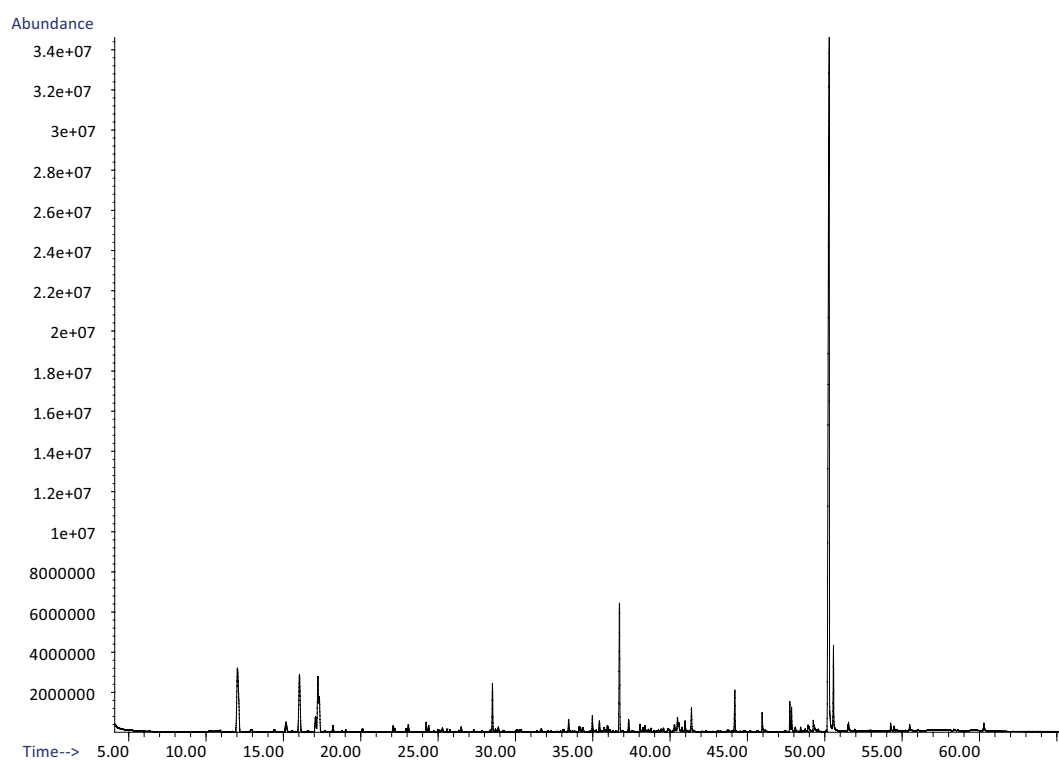


Figure 7 — GC-MS chromatogram of the hexane fraction of the aqueous-alcoholic extract from the rhizomes and roots of *A. archangelica*.

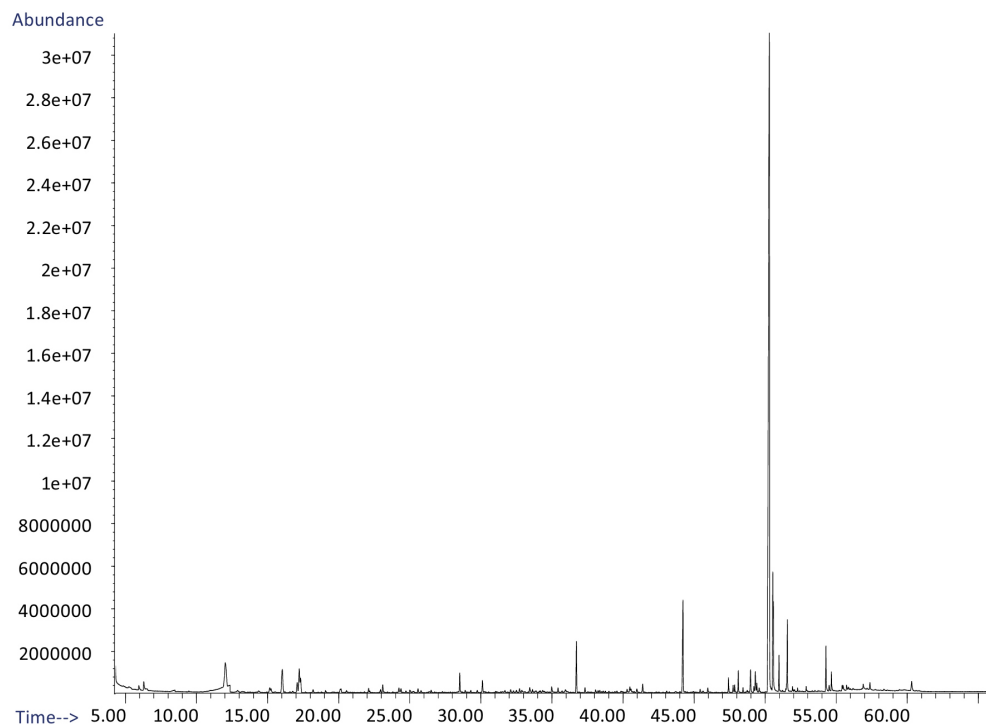


Figure 8 — GC-MS chromatogram of the chloroform fraction of the aqueous-alcoholic extract from the rhizomes and roots of *A. archangelica*.

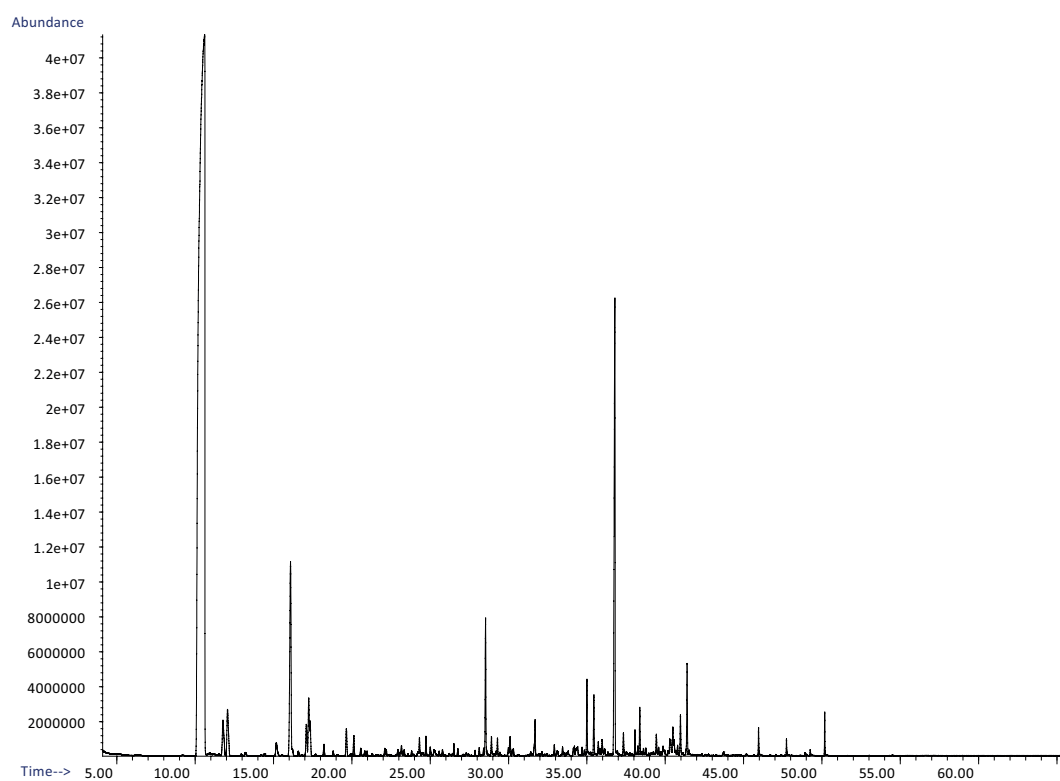


Figure 9 — GC-MS chromatogram of the essential oil from the rhizomes and roots of *A. archangelica*.

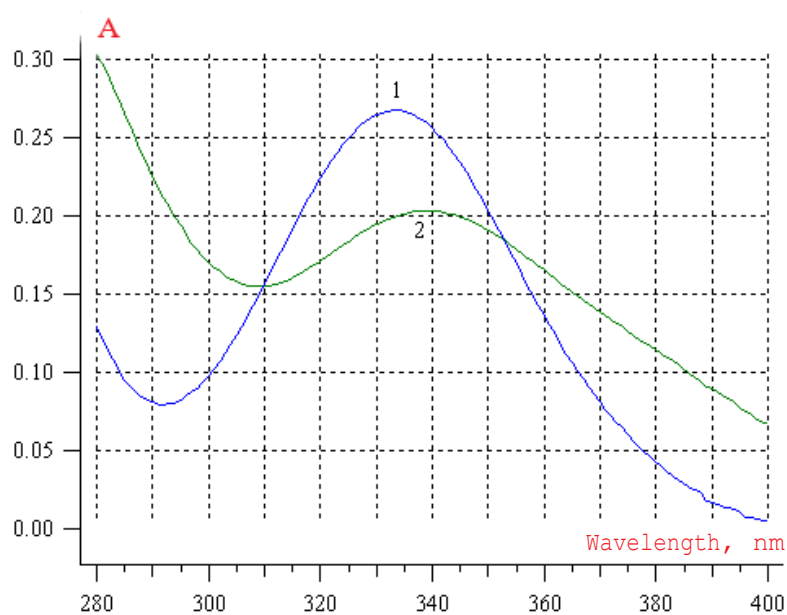


Figure 10 — Electron absorption spectrum of a coumarin standard solution and test solution B from the rhizomes and roots of *A. archangelica*.

Note: 1, coumarin standard, 2, test solution B.

Table 1 — Component composition of volatile organic compounds in hexane and chloroform fractions of water-alcoholic extract and essential oil from Altai origin *Angelica archangelica* rhizomes and roots

No	Retention time, min $\pm$ SD	Component	Detected in fraction from total area of all components, % $\pm$ SD		
			Hexane	Chloroform	Essential oil
1.	11,73 $\pm$ 0,163	α -Thujene, bicyclic monoterpene	0.1 $\pm$ 0.02	n.d.	3.43 $\pm$ 0.27
2.	12,042 $\pm$ 0,059	α -Pinene, bicyclic monoterpene	7.29 $\pm$ 0.58	4.24 $\pm$ 0.34	4.26 $\pm$ 0.34
3.	12,93 $\pm$ 0,049	Camphene, bicyclic monoterpene	0.29 $\pm$ 0.06	n.d.	0.19 $\pm$ 0.04
4.	13,184 $\pm$ 0,06	2,4(10)-Thuyadiene, bicyclic monoterpene	0.07 $\pm$ 0.01	n.d.	0.34 $\pm$ 0.07
5.	14,421 $\pm$ 0,04	β -Pinene, bicyclic monoterpene	0.25 $\pm$ 0.05	n.d.	0.21 $\pm$ 0.04
6.	15,167 $\pm$ 0,03	β -Myrcene, acyclic monoterpene	0.94 $\pm$ 0	0.3 $\pm$ 0.06	1.3 $\pm$ 0.1
7.	16,056 $\pm$ 0,07	3-Carene, bicyclic monoterpene	5.02 $\pm$ 0.4	1.82 $\pm$ 0.15	15.22 $\pm$ 1.22
8.	16,576 $\pm$ 0,026	α -Terpinene, monocyclic monoterpene	0.11 $\pm$ 0.02	0.05 $\pm$ 0.01	0.32 $\pm$ 0.06
9.	17,089 $\pm$ 0,023	ortho-Cymene, aromatic compound	1.08 $\pm$ 0.09	0.57 $\pm$ 0.06	2.14 $\pm$ 0.17
10.	17,238 $\pm$ 0,034	D-Limonene, monocyclic monoterpene	3.82 $\pm$ 0.31	1.46 $\pm$ 0.12	3.67 $\pm$ 0.29
11.	17,33 $\pm$ 0,03	β -Phellandrene, monocyclic monoterpene	2.13 $\pm$ 0.17	0.79 $\pm$ 0.08	1.92 $\pm$ 0.15
12.	17,673 $\pm$ 0,026	β -trans-Ocimene, acyclic monoterpene	0.09 $\pm$ 0.02	0.04 $\pm$ 0.01	0.12 $\pm$ 0.02
13.	18,211 $\pm$ 0,025	β -cis-Ocimene, acyclic monoterpene	0.39 $\pm$ 0.08	0.16 $\pm$ 0.03	0.63 $\pm$ 0.06
14.	18,799 $\pm$ 0,021	γ -Terpinene, monocyclic monoterpene	0.07 $\pm$ 0.01	0.04 $\pm$ 0.01	0.28 $\pm$ 0.06
15.	19,059 $\pm$ 0,049	Seidenone, monocyclic monoterpene ketone	0.15 $\pm$ 0.03	0.11 $\pm$ 0.02	0.1 $\pm$ 0.02
16.	19,912 $\pm$ 0,03	4-Carene, bicyclic monoterpene	0.04 $\pm$ 0.01	0.03 $\pm$ 0.01	0.15 $\pm$ 0.03
17.	20,12 $\pm$ 0,015	Terpinolene, monocyclic monoterpene	0.21 $\pm$ 0.04	0.17 $\pm$ 0.03	1.06 $\pm$ 0.08
18.	20,559 $\pm$ 0,025	para-Cymene, aromatic compound	0.03 $\pm$ 0.01	0.13 $\pm$ 0.03	0.45 $\pm$ 0.09
19.	22,101 $\pm$ 0,023	trans-para-2-Menthen-1-ol, monocyclic monoterpene alcohol	0.3 $\pm$ 0.06	0.19 $\pm$ 0.04	0.44 $\pm$ 0.09
20.	22,196 $\pm$ 0,019	Neo-allo-ocimene, acyclic monoterpene	0.18 $\pm$ 0.04	0.07 $\pm$ 0.01	0.29 $\pm$ 0.06
21.	22,942 $\pm$ 0,019	cis-2-para-Menthen-1-ol, monocyclic monoterpene alcohol	0.19 $\pm$ 0.04	0.17 $\pm$ 0.03	0.4 $\pm$ 0.08
22.	23,092 $\pm$ 0,025	Verbenol, bicyclic monoterpene alcohol	0.4 $\pm$ 0.08	0.36 $\pm$ 0.07	0.19 $\pm$ 0.04
23.	23,573 $\pm$ 0,017	para-Menthanone, monocyclic monoterpene	0.03 $\pm$ 0.01	0.01 $\pm$ 0.01	0.14 $\pm$ 0.03
24.	24,238 $\pm$ 0,004	Verbenyl ethyl ether, bicyclic monoterpene ether	0.45 $\pm$ 0.09	0.24 $\pm$ 0.05	n.d.
25.	24,365 $\pm$ 0,105	para-Menta-1,5-dien-8-ol, monocyclic monoterpene alcohol	0.30 $\pm$ 0.06	0.2 $\pm$ 0.04	0.93 $\pm$ 0.11
26.	24,493 $\pm$ 0,022	Menthol, monocyclic monoterpene alcohol	0.02 $\pm$ 0.01	0.03 $\pm$ 0.01	0.21 $\pm$ 0.04
27.	24,729 $\pm$ 0,019	para-Cymenol-8, monocyclic monoterpene alcohol	0.06 $\pm$ 0.01	0.1 $\pm$ 0.02	0.94 $\pm$ 0.12
28.	25,017 $\pm$ 0,036	Krypton, monocyclic monoterpene ketone	0.17 $\pm$ 0.03	0.17 $\pm$ 0.03	0.60 $\pm$ 0.07
29.	25,267 $\pm$ 0,048	cis-Piperitol, monocyclic monoterpene alcohol	0.2 $\pm$ 0.04	0.13 $\pm$ 0.03	0.41 $\pm$ 0.08
30.	25,304 $\pm$ 0,011	Myrcenal, bicyclic monoterpene aldehyde	n.d.	n.d.	0.35 $\pm$ 0.07
31.	25,578 $\pm$ 0,013	Sabinol, monocyclic monoterpene alcohol	0.17 $\pm$ 0.03	0.20 $\pm$ 0.04	0.26 $\pm$ 0.05
32.	25,787 $\pm$ 0,019	Piperitol, monocyclic monoterpene alcohol	0.13 $\pm$ 0.03	0.11 $\pm$ 0.02	0.37 $\pm$ 0.07
33.	26,412 $\pm$ 0,021	3-Carene-2-ol, bicyclic monoterpene alcohol	n.d.	n.d.	0.13 $\pm$ 0.03
34.	26,514 $\pm$ 0,018	Thymyl methyl ether, aromatic ether	n.d.	n.d.	0.57 $\pm$ 0.05
35.	27,630 $\pm$ 0,012	Piperitone, monocyclic monoterpene ketone	n.d.	n.d.	0.10 $\pm$ 0.02
36.	28,407 $\pm$ 0,024	Myrthenyl acetate, bicyclic monoterpene ether	0.11 $\pm$ 0.02	0.05 $\pm$ 0.01	0.39 $\pm$ 0.08
37.	28,524 $\pm$ 0,023	Borneol acetate, bicyclic monoterpene ether	1.99 $\pm$ 0.16	0.78 $\pm$ 0.08	5.89 $\pm$ 0.47
38.	28,906 $\pm$ 0,011	Eucarvone, monocyclic monoterpene	0.27 $\pm$ 0.05	0.10 $\pm$ 0.02	1.11 $\pm$ 0.09
39.	30,307 $\pm$ 0,006	Carvyl acetate, monoterpene ester	0.09 $\pm$ 0.02	0.05 $\pm$ 0.01	0.41 $\pm$ 0.08
40.	31,405 $\pm$ 0,026	Cyclosativene, bicyclic sesquiterpene	0.07 $\pm$ 0.01	0.05 $\pm$ 0.01	0.3 $\pm$ 0.06
41.	32,11 $\pm$ 0,022	β -Elemene, monocyclic sesquiterpene	0.07 $\pm$ 0.01	0.16 $\pm$ 0.03	n.d.
42.	33,444 $\pm$ 0,011	α -Gurjunene, tricyclic sesquiterpene	0.58 $\pm$ 0.06	0.29 $\pm$ 0.06	0.47 $\pm$ 0.09
43.	33,695 $\pm$ 0,012	Sativene, bicyclic sesquiterpene	n.d.	n.d.	0.34 $\pm$ 0.07
44.	34,131 $\pm$ 0,023	β -Caryophyllene, bicyclic sesquiterpene	0.26 $\pm$ 0.05	0.08 $\pm$ 0.02	0.43 $\pm$ 0.09

No	Retention time, min $\pm$ SD	Component	Detected in fraction from total area of all components, % $\pm$ SD		
			Hexane	Chloroform	Essential oil
45.	34,204 $\pm$ 0,012	α -Campholenal, monocyclic monoterpenoid aldehyde	0.21 $\pm$ 0.04	0.08 $\pm$ 0.02	0.48 $\pm$ 0.10
46.	35,437 $\pm$ 0,019	Dihydroagarofuran, tricyclic sesquiterpenoid	0.48 $\pm$ 0.10	0.21 $\pm$ 0.04	2.63 $\pm$ 0.21
47.	35,724 $\pm$ 0,019	α -Murolene, bicyclic sesquiterpene	0.26 $\pm$ 0.05	0.12 $\pm$ 0.02	0.84 $\pm$ 0.09
48.	35,820 $\pm$ 0,043	α -Farnesene, acyclic sesquiterpenoid	0.11 $\pm$ 0.02	0.03 $\pm$ 0.01	0.45 $\pm$ 0.09
49.	35,958 $\pm$ 0,041	β -Bisabolene, acyclic sesquiterpene	0.52 $\pm$ 0	0.07 $\pm$ 0.01	1.18 $\pm$ 0.09
50.	36,126 $\pm$ 0,023	Cuparene, monocyclic sesquiterpene	0.15 $\pm$ 0.03	0.09 $\pm$ 0.02	0.59 $\pm$ 0.07
51.	36,745 $\pm$ 0,085	Epiglobulol, tricyclic sesquiterpenoid alcohol	5.42 $\pm$ 0.43	2.04 $\pm$ 0.16	25.61 $\pm$ 2.05
52.	36,877 $\pm$ 0,019	α -Longipinene, tricyclic sesquiterpene	0.07 $\pm$ 0.01	0.03 $\pm$ 0.01	0.22 $\pm$ 0.04
53.	36,944 $\pm$ 0,008	Humulene, monocyclic sesquiterpene	0.08 $\pm$ 0.02	0.03 $\pm$ 0.01	0.38 $\pm$ 0.08
54.	37,329 $\pm$ 0,013	Elemol, monocyclic sesquiterpene	0.55 $\pm$ 0	0.22 $\pm$ 0.04	1.31 $\pm$ 0.1
55.	38,373 $\pm$ 0,013	Caryophyllene oxide, bicyclic sesquiterpenoid	0.32 $\pm$ 0.06	0.12 $\pm$ 0.02	2.21 $\pm$ 0.18
56.	38,504 $\pm$ 0,016	Globulol, tricyclic sesquiterpenoid	0.11 $\pm$ 0.02	0.06 $\pm$ 0.01	0.32 $\pm$ 0.06
57.	39,431 $\pm$ 0,016	Bulnesol, bicyclic sesquiterpenoid alcohol	0.08 $\pm$ 0.02	n.d.	1.19 $\pm$ 0.10
58.	39,576 $\pm$ 0,007	γ -Eudesmol, bicyclic sesquiterpenoid alcohol	0.23 $\pm$ 0.05	0.08 $\pm$ 0.02	0.60 $\pm$ 0.05
59.	39,859 $\pm$ 0,009	α -Eudesmol, bicyclic sesquiterpenoid alcohol	0.22 $\pm$ 0.04	0.09 $\pm$ 0.02	0.73 $\pm$ 0.08
60.	39,960 $\pm$ 0,010	Agarospirol, bicyclic sesquiterpenoid alcohol	0.18 $\pm$ 0.04	0.06 $\pm$ 0.01	0.38 $\pm$ 0.08
61.	40,289 $\pm$ 0,011	α -Guaiene, bicyclic sesquiterpene	0.48 $\pm$ 0.10	0.20 $\pm$ 0.04	0.82 $\pm$ 0.09
62.	40,484 $\pm$ 0,016	Guaiol, bicyclic sesquiterpenoid alcohol	0.75 $\pm$ 0.08	0.19 $\pm$ 0.04	1.57 $\pm$ 0.13
63.	40,566 $\pm$ 0,015	β -Eudesmol, bicyclic sesquiterpenoid alcohol	0.51 $\pm$ 0.06	0.20 $\pm$ 0.04	0.93 $\pm$ 0
64.	41,384 $\pm$ 0,007	α -Bisabolol, monocyclic sesquiterpenoid alcohol	1.13 $\pm$ 0.09	0.40 $\pm$ 0.08	n.d.
65.	44,203 $\pm$ 0,069	Angelicin, furanocoumarin	1.84 $\pm$ 0.15	4.18 $\pm$ 0.33	n.d.
66.	47,858 $\pm$ 0,009	Hexadecanoic acid, ethyl ester, fatty acid ester	0.91 $\pm$ 0.08	0.29 $\pm$ 0.06	n.d.
67.	48,105 $\pm$ 0,023	Citropten, methoxycoumarin	0.25 $\pm$ 0.05	0.73 $\pm$ 0.08	n.d.
68.	48,441 $\pm$ 0,012	Xanthyletin, pyranocoumarin	0.18 $\pm$ 0.04	0.15 $\pm$ 0.03	n.d.
69.	48,948 $\pm$ 0,093	Methoxsalen, furanocoumarin	0.59 $\pm$ 0.06	0.91 $\pm$ 0.10	n.d.
70.	49,306 $\pm$ 0,023	Bergapten, furanocoumarin	0.10 $\pm$ 0.02	0.85 $\pm$ 0.09	n.d.
71.	49,581 $\pm$ 0,009	Phellopterin, prenylated coumarin	0.14 $\pm$ 0.03	0.17 $\pm$ 0.03	n.d.
72.	50,254 $\pm$ 0,160	Osthol, prenylated coumarin	49.72 $\pm$ 3.98	44.06 $\pm$ 3.52	1.54 $\pm$ 0.12
73.	50,570 $\pm$ 0,030	Orocelon, furanocoumarin	3.76 $\pm$ 0.3	2.60 $\pm$ 0.21	n.d.
74.	51,549 $\pm$ 0,046	Isopimpinellin, furanocoumarin	0.38 $\pm$ 0.08	2.48 $\pm$ 0.21	n.d.
75.	52,882 $\pm$ 0,025	Imperatorin, prenylated coumarin	0.08 $\pm$ 0.02	0.19 $\pm$ 0.04	n.d.
76.	54,268 $\pm$ 0,033	Oxypeucedanin, furanocoumarin	0.47 $\pm$ 0.09	1.65 $\pm$ 0.13	n.d.
77.	55,488 $\pm$ 0,011	Decursin, pyranocoumarin	0.48 $\pm$ 0.1	0.49 $\pm$ 0.10	n.d.
78.	56,002 $\pm$ 0,014	Selinidin, pyranocoumarin	0.24 $\pm$ 0.05	0.29 $\pm$ 0.06	n.d.
79.	60,298 $\pm$ 0,04	Dimethyl-2-oxo-2,8,9,10-tetrahydropyrano(2,3-f) chromene, pyranocoumarin	0.69 $\pm$ 0.08	0.91 $\pm$ 0.11	n.d.
By component classes: average values, % (number of components in the corresponding group of compounds in parentheses)					
Prenylated coumarins			49.94 (3)	44.42 (3)	1.54 (1)
Monoterpenes			26.03 (32)	12.04 (28)	46.2 (34)
Sesquiterpenes			12.63 (23)	4.82 (22)	43.5 (22)
Sesquiterpenoids			9.58 (12)	3.49 (11)	35.23 (11)
Monoterpenoids			5.07 (17)	2.90 (17)	13.53 (20)
Furanocoumarins			7.14 (6)	12.67 (6)	n.d.
Aromatic compounds			1.11 (2)	0.7 (2)	2.59 (2)
Pyranocoumarins			1.59 (4)	1.84 (4)	n.d.
Methoxycoumarins			0.25 (1)	0.73 (1)	n.d.
Fatty acids			0.91 (1)	0.29 (1)	n.d.

Note: SD, standard deviation; n.d., not detected.

Table 2 — Validation results of the spectrophotometric method for determining the sum of coumarins in *A. archangelica* rhizomes and roots

Validation parameter	Conditions / range	Acceptance criteria	Result
Specificity	Analysis of "blank" solution	$A \leq 0.05$	$A \leq 0.02$
Linearity	2,0–20,0 µg/mL (n=10)	$r \geq 0.990$	$r = 0.9997$
Analytical range	2.0–20.0 µg/mL (calculated as coumarin)	Corresponds to the linearity range; 80–120% of expected content in raw material	Range established and confirmed
Accuracy	Method of additions	98–102%	98.9%
Repeatability	$n = 6$	$RSD \leq 2.0\%$	$RSD = 0.8\%$
Intermediate precision	$n = 3$ days	$RSD \leq 2.0\%$	$RSD = 1.5\%$

The obtained validation results (Table 2) demonstrate the compliance of the method with the requirements set forth in regulatory documents and are characterized by satisfactory specificity, linearity, accuracy, and precision, which confirms the suitability of the proposed method for the purpose of pharmacopoeial standardization of *A. archangelica* medicinal raw material by total coumarin content. Figure 10 shows the absorption spectrum of the coumarin reference standard solution B and Test Solution B (maximum at 335 nm).

DISCUSSION

For the first time, morphological-anatomical and gas chromatographic studies of *A. archangelica* rhizomes and roots of Altai origin were conducted.

The results of the study of external and microscopic features are consistent with previously published data [5, 6]. For the first time, luminescence characteristics of anatomical structures of *A. archangelica* raw material were discovered in comparison with the reference standard of eleutheroside B1, which preliminarily allows for the interpretation of the localization of BACs. The most diagnostic in this case is the light blue luminescence of phloem cells and secretion in schizogenous cavities in the excitation range of 330–400 nm, attributed to coumarin-based substances. Histological features of the marker characteristic—schizogenous cavities—have also been clarified, which will allow for the identification of the object under study during acceptance control of medicinal raw material, for example, in comparison with *Angelica sylvestris* L. [30, 31]. Methods for diagnosing *Angelica archangelica* rhizome and root powder have been improved, and the expediency of using luminescent and polarized microscopy in the diagnosis of specific schizogenous cavities, vascular elements, and crystalline inclusions has been confirmed. Analysis of microscopic features of medicinal raw material

powder is a mandatory criterion for the acceptance of raw materials and quality control of drugs based on *Angelica archangelica* rhizomes and roots. The obtained data will allow for the development of the "Identification. Microscopic Features" section of the draft of pharmaceutical monograph "*Angelica archangelica* rhizomes and roots".

Previously identified in *A. archangelica* rhizomes and roots: prenylated coumarins—osthol [7], umbelliferone, imperatorin, isoimperatorin, fellopterin; hydroxycoumarins—umbelliferone; furanocoumarins—angelicin, bergapten, psoralen, xanthotoxin, oroselone, methoxsalen, xanthotoxol, archangelicin, pimpinellin, isopinipenellin [17–19, 21]. In raw material harvested in the Republic of Bashkortostan, the leading components were oroselone (71.36%), osthol (5.09%), bergapten (2.74%), methoxsalen (2.65%), angelicin (1.69%) [17]. The present study found that the leading component of the aqueous-alcoholic extract is osthol (49.72%). Apparently, it is the leading component of *A. archangelica* rhizomes and roots of Altai origin. For the first time, cypten, xanthelatin, decursin, and selinidin were identified here.

The content of essential oil in the underground part of *A. archangelica* varies significantly depending on the region of plant growth. For example, in the Republic of Bashkortostan—0.82% [32], near Mount Ozren, Serbia—0.10% [8], in the Ternopil region, Ukraine—0.13% [33], in the high-altitude regions of India in Potiwas (2200 m)—0.28%, Rudraprayag (3300 m)—0.33%, Uttarkashi (3500 m)—0.35% [34]. The essential oil content in the studied sample was 0.09%, which, according to monograph 01/2013:1857 "*Angelica archangelica* root" of the European Pharmacopoeia 11th edition, does not meet the normative value—not less than 2 mL/kg (0.2%). The low essential oil content may be due to the place and time of harvesting, climatic conditions, soil chemical composition, and other factors, as well as the drying temperature (45°C), which does not meet the requirements for essential oil-bearing raw materials

(40°C)^{15,16}, which must be considered when organizing the harvesting of medicinal raw materials.

In the study by A. Forycka et al., it is indicated that the essential oil of *A. archangelica* of Romanian, Finnish, and Serbian origin contains from 62 to 122 components [13]. In the Kemerovo region, Russian Federation, 75 components were found [15], and in Serbia—88 compounds. The largest part of the component composition of the essential oil is made up of monoterpenes and monoterpenoids [35, 36]. In the essential oil of *A. archangelica* growing in the Republic of Bashkortostan, sesquiterpenoids predominate, which are 2.6 times more than monoterpenoids [19]. In the present study, 62 components were identified in the essential oil of Altai *A. archangelica* (possible component volatilization), the basis of which is formed by monoterpenes (46.2%), sesquiterpenes (43.5%), and sesquiterpenoids (35.23%). Monoterpenoids were found in smaller quantities (13.53%).

Essential oil from the northern regions of Europe was characterized by a high level of α -pinene (65.3%), α -phellandrene (5.9–15.4%), and δ -3-carene (15.3%), while in samples from the south, its level was the lowest (0–0.2%); in the east and northeast, α -pinene was dominant (from 15.7% to 20.8%); in the northeast, high concentrations of β -phellandrene (13.5–16.9%), δ -3-carene (15.4–16.9%), limonene (8.0–9.0%), and sabinene (5.0–7.5%) were noted, whereas the concentration of the latter in the east was higher (5.9–14.85%); in the southeast, β -phellandrene (13.8–18.5%), α -pinene (11.4–15.0%), δ -3-carene (10.8–11.9%), α -cymene (6.8–10.6%), and α -phellandrene (5.9–8.6%) were present [13, 14]. The dominant components of the Siberian subspecies of *A. archangelica* are limonene (30.47%) and α -pinene (23.6%) [15]. The volatile organic compound profile of the essential oil of *A. archangelica* of Altai origin is most similar to that of flora from Lithuania and Serbia [14, 16], where the prevailing compounds are: α -pinene (19.4%, 29.7%), δ -3-carene (16.0%, 14.2%), β -phellandrene (15.4%), 15-pentadecanolide (14.9%), limonene (8.8%), and osthol (8.8%).

In the present study, the essential oil consists of 62 components, among which the leading ones are epiglobulol (25.61%), 3-carene (15.22%), bornyl acetate (5.89%), α -pinene (4.26%), and D-limonene (3.67%),

β -phellandrene (1.92%), osthol (1.54%). It is evident that, despite geographical differences, the essential oil of *A. archangelica* of Altai origin contains species-specific components (3-carene, α -pinene, D-limonene, β -phellandrene, osthol) characteristic of the essential oil of plants from the represented habitats, but in different proportions, which allows it to be considered authentic. Osthol is the only coumarin among *A. archangelica* that is present in the essential oil (1.54%), which is confirmed by literature data [14, 37] and can serve as a marker for confirming the authenticity of the essential oil from the rhizomes and roots of *A. archangelica*. Preliminarily, it can be stated that ortho-cymene, seudenone, 4-carene, neo-allo-ocimene, *para*-menthanone, sabinol, 3-carene-2-ol, thymyl methyl ether, eucarvone, α -gurjunene, α -campholenal, α -farnesene, epiglobulol, α -longipinene, globulol, guayol, bulnesol, agarospirol, and guayol have been identified for the first time in the essential oil of *A. archangelica* rhizomes and roots. The conducted analysis allows us to conclude that the profile of BACs of *A. archangelica* of Altai origin represents a distinct chemotype that requires individual quality assessment standards. The obtained data on the leading components of terpene and coumarin nature will allow for the development of sections on “Identification. Determination of major groups of biologically active compounds”.

The proposed method for quantitative determination of the sum of coumarins, calculated as coumarin, using a spectrophotometric method in the rhizomes and roots of *A. archangelica* is based on the specific chemical properties of coumarin and its derivatives, which contain a benzo- α -pyrone ring and intensely absorb light in the ultraviolet region of the spectrum. During the extraction of the sum of coumarins from the MPRM, 96% alcohol extraction was used, followed by liquid-liquid extraction. Ethanol extraction in this case ensures complete extraction of both free coumarins and their glycosidic forms. In the next step, the aqueous-alcoholic solution of the extract was acidified to pH 2–3, and triple extraction with ethyl acetate was performed, which effectively isolates neutral (molecular) and weakly acidic forms of coumarins from the polar matrix and, consequently, allows for the assessment of their quantitative transfer into the ethyl acetate phase. Ethyl acetate was chosen as the extractant due to its optimal polarity, ensuring high selectivity towards the target analytes and minimal co-extraction of ballast substances such as organic acids, sugars, and pigments. The obtained organic extract was evaporated to dryness, and the residue was dissolved in 70% alcohol

¹⁵ Rules for collecting and drying medicinal plants (collection of instructions); Ministry of Health of the USSR, Chief Pharmacy Department, All-Union. information bureau, etc. Moscow: Meditsina Publ.; 1985. 328 p. Russian

¹⁶ Kurkin VA. Pharmacognosy: a textbook for students of pharmaceutical universities (faculties). Kurkin VA; 6th edition, revised and expanded. Samara: OOO “Polygraphic association “Standard”; 2024. 1268 p. Russian

for subsequent spectrophotometric analysis. During spectrophotometric detection, alkaline hydrolysis of the lactone ring of coumarins causes ring opening, forming the corresponding phenolate anion, which is characterized by a bathochromic shift of the absorption maximum in the wavelength range of 330–340 nm. In the present work, the optical density was measured at a wavelength of 335 nm, which ensures high sensitivity and specificity for the determination of the sum of coumarins in an alkaline medium compared to measurement in a neutral medium. This sample preparation scheme ensures high reproducibility of results and minimizes background absorption that may be caused by accompanying components of the plant material.

It should be noted that the leading coumarin in the studied Altai origin raw material is osthol (see Table 1)—a prenylated coumarin derivative with a molecular mass different from coumarin (molecular mass of coumarin is 146.14 g/mol, molecular mass of osthol is 244.3 g/mol) and molar absorptivity. During the analysis, coumarin (1,2-benzopyrone) was used as a reference standard due to the absence of a certified for osthol, as it represents the basic chromophoric structure for the entire class of coumarins and is widely used in the standardization of MPRM containing coumarins. Consequently, a systematic error is introduced into the results of quantitative determination: the true content of osthol may differ from the obtained value due to differences in spectral characteristics and molecular masses of the standard and determined compounds.

The content of the sum of coumarins, determined by the spectrophotometric method in an alkaline medium at a wavelength of 335 nm, was $1.50 \pm 0.08\%$ calculated as coumarin. The obtained value is within the range characteristic for the rhizomes and roots of *A. archangelica*. According to literature data, the total coumarin content varies from 0.1% to 3.2% depending on the region of growth and harvesting conditions [17, 19, 38]. The result of 1.5% correlates with GC-MS data: with a water-alcoholic extract yield of ~20% and an osthol content of ~49% and 44% in the hexane and chloroform fractions, the calculated coumarin content is 1.5–2.0%, which confirms the reliability of the method. Nevertheless, in the absence of a certified RS for osthol, this approach remains acceptable for pharmacopoeial standardization purposes.

Study Limitations

The presented method for quantitative determination of the sum of coumarins by

spectrophotometry has a key limitation is a potential interference from other UV-active compounds, particularly phenolic carboxylic acids and flavonoid aglycones, which, like coumarins, can be extracted by ethyl acetate from the acidified aqueous phase. Since the final measurement is performed without chromatographic separation, the recorded optical density value at 335 nm may include contributions not only from coumarins but also from the indicated co-extracted substances, which can overestimate the quantitative determination results. Despite this limitation, the data obtained in the present study are valuable for the development of a pharmacopoeial monograph and can be supplemented in subsequent studies with an expanded sample size and the use of chromatographic methods for quantitative analysis.

Thus, the proposed method, based on spectrophotometric determination of the sum of coumarins, can be recommended for inclusion in the draft of pharmacopoeial monograph for RHM, provided that “calculated as coumarin” is clearly indicated and that results need to be confirmed by chromatographic methods when determining the content of specific compounds.

CONCLUSION

The conducted macroscopic, microscopic, microchemical, and histochemical examination allowed for the summarization of the diagnostic results of *A. archangelica* rhizomes and roots, as well as the supplementation of known structural features with new data on tissue polarization and luminescence. Morphologically, root surface fragments exhibit a wavy-wrinkled texture, while rhizome fragments are covered with a rough, transversely wrinkled cork. The luminescence of *A. archangelica* rhizome and root tissues is due to their complex chemical composition, mainly phenolic compounds. The most diagnostic is the light blue luminescence of phloem cells and the secretion of schizogenous cavities in the excitation wavelength range of 330–400 nm, attributed to coumarin-like substances. The expediency of using polarization microscopy in the diagnosis of specific conducting elements in powdered angelica raw material has been proven. The expediency of using the histochemical reaction with Sudan III solution, which diagnoses schizogenous cavities, secretion droplets, and vascular element cell walls, has been substantiated.

GC-MS analysis of the lipophilic fractions of the water-alcoholic extract and essential oil revealed a total of 79 components, including terpenes and coumarins. It was established that osthol is the leading compound in the coumarin class in the water-alcoholic extract

(up to 49.72%), while in the essential oil, epiglobulol plays a key role (25.61%). This distribution of dominant compounds from different BAC groups confirms the need for separate standardization of polar and non-polar fractions of RHM.

The content of the sum of coumarins determined by the spectrophotometric method was $1.50 \pm 0.08\%$ calculated as coumarin, which corresponds to literature

data and can be proposed as a standard in the draft pharmacopoeial monograph. Despite the indicated limitations related to the use of coumarins as a reference standard, the method demonstrates high precision and is suitable for analyzing the quality of RHM.

Thus, the present study contributes to the standardization of *A. archangelica* RHM and enhances the quality control of herbal preparations based on it.

FUNDING

This study did not have financial support from third-party organizations.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Denis I. Shishkalov — investigation, writing—original draft, writing—review & editing; Ifrat N. Zilfikarov — conceptualization, administration, writing—review & editing, writing—review & editing; Vitaly M. Ryzhov — investigation, visualization, writing—review & editing; Tatyana K. Ryazanova — investigation, formal analysis, writing—review & editing; Timur A. Ibragimov — writing—original draft, writing—review & editing; Madina A. Abdurakhmanova — investigation, formal analysis, writing—review & editing; Vera V. Artemyeva — writing—review & editing. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the study and preparation of the article, read and approved of the final version before the publication).

REFERENCES

1. Marchishin SM, Poteshny IM. Morphologoanatomical analysis of the leaves of the *Angelica sylvestris* L. and *Angelica archangelica* L. Pharmaceutical Journal. 2014;(5):86–92.
2. Liao C. New insights into the phylogeny of *Angelica* and its allies (*Apiaceae*) with emphasis on East Asian species, inferred from nrDNA, cpDNA, and morphological evidence. Systematic Botany. 2013;38(1):266–81. DOI: 10.1600/036364413X662060
3. Forycka A, Morozowska M. Micromorphology and anatomy of fruits of *Angelica archangelica* L. (*Apiaceae*) and their intraspecific differentiation. Herba Polonica. 2020;66(4):1–13. DOI:10.2478/hepo-2020-0018
4. Aktürk C, Özerkan M, Yilmaz A, Kaynak G, Yilmaz Ö. A new distribution area of *Angelica archangelica* L. (*Apiaceae*) around Uludağ (Bursa) and its micromorphological, anatomical, palynological properties. Journal of Biological and Environmental Sciences. 2022;16(47):22–32.
5. Grigoryan ER, Orlovskaya TV. Morphological and anatomical study of rhizomes and roots of *Angelica vulgaris*. Development, research and marketing of new pharmaceutical products; Pyatigorsk State Pharmaceutical Academy. Volume Issue 67. Pyatigorsk: Pyatigorsk State Pharmaceutical Academy; 2012. P. 24–8. EDN: WBONAD. Russian
6. Shakirova FA, Kudashkina NV, Bashirova RM, Fayzullina RR. Anatomical characteristics of leaves and roots of *Angelica officinalis* from the flora of Bashkortostan. Hammermann Readings-2011: scientific and methodological conference, St. Petersburg, February 01–03, 2011. Saint Petersburg State Chemical and Pharmaceutical Academy; 2011. P. 91–2. EDN: VRUZAB. Russian
7. Korpinen RI, Välimaa AL, Liimatainen J, Kunnas S. Essential Oils and Supercritical CO₂ Extracts of Arctic *Angelica* (*Angelica archangelica* L.), Marsh Labrador Tea (*Rhododendron tomentosum*) and Common Tansy (*Tanacetum vulgare*)-Chemical Compositions and Antimicrobial Activities. Molecules. 2021;26(23):7121. DOI: 10.3390/molecules26237121
8. Nemeth S, Paşca B, Teodorescu A, Coita I, Teaha D. Coumarins isolated from the dry roots of *Angelica archangelica* L. and their antibacterial activity. Analele Universităţii din Oradea, Fascicula: Ecotoxicologie, Zootehnie şi Tehnologii de Industrie Alimentară. 2015;XIV:355–62.
9. Aćimović MG, Pavlović SD, Varga AO, Filipović VM, Cvetković MT, Stanković JM, Čabarkapa IS. Chemical Composition and Antibacterial Activity of *Angelica archangelica* Root Essential Oil. Nat Prod Commun. 2017;12(2):205–6.
10. Aćimović M, Rat M, Pezo L, Lončar B, Pezo M, Miljković A, Lazarević J. Biological and chemical diversity of *Angelica archangelica* L. – case study of essential oil and its dioligomer activity. Agronomy. 2022;12:1570. DOI: 10.3390/agronomy12071570
11. Alloush M, Mallion C, Sarker SD, Rahman MM. Coumarins from the roots of *Angelica archangelica* and antibacterial activity against methicillin resistant *Staphylococcus aureus*. Dhaka University Journal of Pharmaceutical Sciences. 2022;20(3):275–81. DOI: 10.3329/dujps.v20i3.59793
12. Fraternale D, Teodori L, Rudov A, Prattichizzo F, Olivieri F, Guidarelli A, Albertini MC. The *In Vitro* Activity

- of *Angelica archangelica* L. Essential Oil on Inflammation. J Med Food. 2018;21(12):1238–43. DOI: 10.1089/jmf.2018.0017
13. Forycka A, Buchwald W. Variability of composition of essential oil and coumarin compounds of *Angelica archangelica* L. Herba Polonica. 2019;65(4):62–75. DOI: 10.2478/hepo-2019-0027
 14. Nivinskienė O, Butkienė R, Mockutė D. The Chemical composition of the essential oil of *Angelica archangelica* L. roots growing wild in Lithuania. Journal of Essential Oil Research. 2005;17(4):373–7. DOI: 10.1080/10412905.2005.9698934
 15. Shchipitsyna OS. Comparative analysis of essential oil from the roots of the Siberian and European subspecies *Angelica archangelica*. Advances in current natural sciences. 2011;(5):126–7. EDN: NQXMVT. Russian
 16. Lunz K, Stappen I. Back to the Roots-An Overview of the Chemical Composition and Bioactivity of Selected Root-Essential Oils. Molecules. 2021;26(11):3155. DOI: 10.3390/molecules26113155
 17. Kudashkina NV, Bashirova RM, Shakirova FA, Galkin EG, Mustafin AG. Biochemical rationale for the use of *Angelica archangelica* L. in the monastery. Traditional medicine. 2015;(2(41)):41–3. EDN: UIWYDR
 18. Härmälä P, Vuorela H, Hiltunen R, Nyiredy S, Sticher O, Törnquist K, Kaltia S. Strategy for the isolation and identification of coumarins with calcium antagonistic properties from the roots of *Angelica archangelica*. Phytochem Anal. 1992;3:42–8. DOI: 10.1002/pca.2800030108
 19. Bashirova RM, Shakirova FA, Kudashkina NV. Coumarins of the roots and leaves of *Angelica archangelica* L. of the Ural region. Bulletin of the Bashkir University. 2013;18(4):1078–80. Russian
 20. Oude Nijhuis RAB, Kuipers T, Oors JM, Herregods TVK, Kessing BF, Schuitenmaker JM, Smout AJPM, Bredenoord AJ. The Effect of STW5 (Iberogast) on Reflux Symptoms in Patients With Concurrent Dyspeptic Symptoms: A Double-blind Randomized Placebo-controlled Crossover Trial. J Neurogastroenterol Motil. 2024;30(1):54–63. DOI: 10.5056/jnm23014
 21. Sigurdsson S, Ogmundsdottir HM, Gudbjarnason S. Antiproliferative effect of *Angelica archangelica* fruits. Z Naturforsch C J Biosci. 200;59(7-8):523–7. DOI: 10.1515/znc-2004-7-813
 22. Nabi MHB, Ahmed M, Mia S, Islam S, Zzaman W. Essential oils: Advances in extraction techniques, chemical composition, and bioactivities. Heliyon. Food Chemistry Advances. 2025;101048. DOI: 10.1016/j.focha.2025.101048
 23. Bhadange YA, Carpenter J, Saharan VK. A comprehensive review on advanced extraction techniques for retrieving bioactive components from natural sources. ACS Omega. 2024;9(29):31274–97. DOI: 10.1021/acsomega.4c02718
 24. Kurkin VA, Sazonova OV, Kurkina AV, Ryazanova TK, Khusainova AI. composition of essential oil of *Tanacetum vulgare* L. from Samara Region. Science and Innovations in Medicine. 2016;(4(4)):58–62. EDN: YKNIEP
 25. Kurkin VA, Vaskova AI, Ryazanova TK Comparative Study of the Constituent Composition of Essential Oil from Herb Species of the Genus *Achillea* L. Pharmaceutical Chemistry Journal. 2025;59(1):56–62. DOI: 10.1007/s11094-025-03364-6. EDN: MKSNJT
 26. Artemyeva VV, Dandash GM, Zilfikarov IN, Shishkalov DI, Ryzhov VM, Ryazanova TK, Bochkareva II, Guseynova ZA, Arutyunov AK. Modern Aspects of Pharmacognostic Analysis of Wild Carrot Fruits (*Daucus carota* L.). Pharmacy & Pharmacology. 2025;13(5):367–84. DOI: 10.19163/2307-9266-2025-13-5-367-384. EDN: ZXJSH
 27. Kurkin VA, Ryazanova TK. Current aspects of quality control and standardization of *Eleutherococcus senticosus* medicines. Drug development & registration. 2022;11(3):152–61. DOI: 10.33380/2305-2066-2022-11-3-152-161. EDN: MRGQXB
 28. Kurkin VA, Kurkina AV, Kosenko AA. The development of methods for determination the total flavonoids in buds of *Populus alba* L. Chemistry of plant raw material. 2024;(2):168–75. DOI: 10.14258/jcprm.20240212904. EDN: AXGYYW
 29. Kompantseva EV, Ayrapetova AY, Saushkina AS. Spectrophotometric Determination of Hydroxycinnamic Acids in Herbal Drugs. Part 1. Direct Spectrophotometry (Review). Bulletin of the Scientific Centre for Expert Evaluation of Medicinal Products. Regulatory Research and Medicine Evaluation. 2024;14(2):181–195. DOI: 10.30895/1991-2919-2024-14-2-181-195. EDN: FAKJMJ
 30. Gorislav AA, Novoselova LV. Anatomical structure of the rhizome of *Angelica sylvestris* L. (Umbelliferae). Innovations in medicine and pharmacy-2018: Proceedings of the remote scientific and practical conference of students and young scientists, Minsk, November 04, 2018; Belarusian State Medical University. Minsk: Belarusian State Medical University; 2018. P. 849–52. EDN: YYULDF. Russian
 31. Abramchuk AV, Karpukhin MYu. Morpho-biological features *Angelica archangelica* L. Bulletin of Biotechnology. 2020;(1(22)):5. EDN: BOTEYC
 32. Bashirova RM, Shakirova FA, Kudashkina NV, Galkin EG, Mustaphin AG. Essential oils roots of garden *Angelica A. Archangelica* Ural Region. Proceedings of the RAS Ufa Scientific Centre. 2014;(1):15–21. EDN: RXOUKZ
 33. Slobodianiuk L. Analysis of the essential oils in leaves and rhizomes with roots of *Angelica archangelica* growing in Ukraine. ScienceRise: Pharmaceutical Science. 2024;(3(49)):63–9.
 34. Chauhan RS, Nautiyal MC, Cecotti R, Mella M, Tava A. Variation in the essential oil composition of *Angelica archangelica* from three different altitudes in Western Himalaya, India. Industrial Crops and Products. 2016;94:401–4. DOI: 10.1016/j.indcrop.2016.08.044
 35. Jovan L, Aćimović M, Durović-Pejčev R, Lončar B, Vukić V, Pezo L, Roljević-Nikolić S, Vrbničanin S, Božić D. Linking weed control techniques to anti-inflammatory potential: Comparative analysis of *Angelica archangelica* L. root essential oil profiles. Industrial Crops and Products. 2024;216:118656. DOI: 10.1016/j.indcrop.2024.118656
 36. Holm Y, Solberg S, Hiltunen R. Variation in *Angelica archangelica* root essential oils. Planta Medica. 2009;75(09):52–6. DOI: 10.1055/s-0029-1234418
 37. Lunz K, Stappen I. Back to the Roots-An Overview of the Chemical Composition and Bioactivity of Selected Root-Essential Oils. Molecules. 2021;26(11):3155. DOI: 10.3390/molecules26113155
 38. Sigurdsson S, Jonsdottir S, Gudbjarnason S. Geographical variation of the furanocoumarin composition of the fruits of Icelandic *Angelica archangelica*. Z Naturforsch C J Biosci. 2012;67(1-2):1–7. DOI: 10.1515/znc-2012-1-201

AUTHORS

Denis I. Shishkalov — graduate student of the Department of Pharmacy, lecturer of the Department of Medical and Pharmaceutical Chemistry, Maykop State Technological University. ORCID ID: 0000-0001-9558-5336. E-mail: hitmanapp@mail.ru

Ifrat N. Zilfikarov — Doctor of Sciences (Pharmacy), Professor of the Russian Academy of Sciences, Head of the Department of Medical and Pharmaceutical Chemistry, Maykop State Technological University; Chief Scientist, All-Russian Scientific Research Institute of Medicinal and Aromatic Plants; Deputy General Director for Science, Head of the Laboratory of VIFITECH. ORCID ID: 0000-0002-8638-9963. E-mail: dagfarm@mail.ru

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

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

Madina A. Abdurakhmanova — senior chromatographic engineer at the laboratory of the VIFITECH. ORCID ID: 0009-0001-7302-237X. E-mail: abdurahmanova.16@mail.ru

Timur A. Ibragimov — Candidate of Sciences (Pharmacy), Assistant Professor of the Department of Analytical and Pharmaceutical Chemistry, Dagestan State University. ORCID ID: 0000-0001-9809-1120. E-mail: aloefarm@mail.ru

Vera V. Artemyeva — senior lecturer at the Department of Medical and Pharmaceutical Chemistry, Maykop State Technological University. ORCID ID: 0000-0001-8467-2899. E-mail: denis7radnet.ru@mail.ru