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Morphological and anatomical study and quantitative determination of polysaccharides in fruits, pericarp and seeds of Elettaria cardamomum L.

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Abstract: *E. cardamomum is officially included in the Pharmacopoeias of India, United Kingdom, and United States of America. In our country, it is a spice and is not used for medical purposes. The study of the macro- and microscopic structure of fruits and seeds, as well as chemical components, in particular polysaccharides, will allow developing methods for quality control of raw materials for fruits with E. cardamomum seeds. This approach will expand the use of herbal preparations with cardamom in Ukraine. In addition, it will ensure the waste-free use of cardamom in the medical, pharmaceutical and food industries, since only seeds rich in volatile essential oils are used for industrial purposes, and the pericarp is utilized. The article analyzes the current literature data on the phytochemical composition, use in pharmacy and medicine of E. cardamomum. The main morphometric features of E. cardamomum fruits and seeds, which play a key role in the identification of the species, have been established. The anatomical features of fruits and seeds of E. cardamomum were studied under light and electron microscopes, a number of micromorphological features were revealed, in particular, idioblasts with essential oil, angular layer, waxy scales on the surface of seeds. Quantitative determination of polysaccharides in seeds, fruits and pericarp of E. cardamomum was carried out. The highest content of polysaccharides was found in the pericarp - 4.7%, the average in the fruit - 2.8% and the lowest in the seeds - 2.22%.*

Keywords. Elettaria Cardamomum; Polysaccharides; Capsule; Seeds; Light Microscopy; Scanning Electron Microscopy.

Introduction

Stress factors and emotional instability lead to a significant increase in chronic diseases accompanied by systemic inflammation in the human body. The World Health Organization supports the use of plants with a scientific evidence base for the treatment and prevention of somatic diseases that can lead to complications by activating the inflammation process, accompanied by the release of cytokines that affect the repair of damaged tissues. The process of inflammation occurs as a response to aggression in order to repair tissues [2,3]. The modern therapies include anti-inflammatory drugs, which can often have side effects, such as gastric ulcers, heart failure, stroke or heart attack, liver or kidney failure, etc. The search for natural and safe remedies to combat inflammatory processes of various genesis has become a priority among researchers in recent years. In addition, there is a growing interest in

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foods, spices, and dietary supplements that could prevent the development of inflammation in the body at the prevention stage [4,5].

Elettaria cardamomum L., a member of the ginger family (Zingiberaceae), is a well-known plant whose fruits have long been used in cooking, perfumery, and for the treatment of various diseases [2,3]. A series of studies by scientists from around the world have been largely aimed at identifying the antioxidant properties of cardamom [6,7]. For example, scientists from Pakistan measured the antioxidant potential of methanolic extracts of green cardamom (*E. cardamomum*) based on a significant amount of antioxidants, including phenol, compounds – flavonoids, which can inhibit lipid peroxidation due to their antioxidant activity. They concluded that cardamom consumption not only reduces the risk of developing cancer and various diseases, but also prevents the rancidity of fatty foods [4]. Indian researchers paid attention to the study of the chemical components of cardamom essential oil and their potential pharmacological properties. They found out that daily consumption of cardamom capsules is nothing more than a nutraceutical and functional food that can protect a person from many chronic diseases [4,8]. A group of scientists from Tunisia and Canada studied the antibacterial and anti-inflammatory activity of cardamom extracts in periodontal infections. Cardamom extracts showed strong antibacterial activity against common gram-negative periodontal pathogens, including Aggregatibacter ctinomycetemcomitans, Fusobacterium nucleatum, Porphyromonas gingivalis, and Prevotella intermedia. Scientists believe that this activity is probably due to the presence of a large amount of 1,8-cineole in cardamom fruit [9].

Since the chemical composition of cardamom fruit is rich not only in essential oils, but also contains polysaccharides, we focused our research on the quantitative determination of these substances in E. cardamomum fruits. In addition, the study of the main morphological and anatomical features of fruits and seeds will help to distinguish this species from three others available on the European market, and will also allow to apply these identified features in the development of quality control methods of raw materials for *E. cardamomum* fruits and seeds.

Aim

To conduct a morphological and anatomical study and quantitative determination of polysaccharides in fruits, pericarp and seeds of *E. cardamomum*.

Materials and Methods

Light microscopy was performed according to the standard method described in the State Pharmacopoeia of Ukraine (SPhU) [10]. Scanning electron microscopy was taken at the N.G. Kholodny Institute of Botany using a scanning electron microscope (JSM-6060-LA, Japan).

Temporary preparations for anatomical and histochemical studies were made in aqueous medium and aqueous glycerol solutions of various concentrations under a ULAB microscope (×40, ×100, ×1000) equipped with a Canon EOS 550 digital microphotographic camera. Each fruit and seed fragment were examined ten times to obtain objective data.

The quantitative determination of polysaccharides in *E. cardamomum* fruits and seeds was established by the gravimetric method [11].

Results

Light microscopy

The fruit of *E. cardamomum* is a capsule, elongate-ovoid, ovoid or broadly ovoid, 0.5 to 2 cm long, triangular, greenish to pale brown, smoky greenish with a slight dull yellow tint. The base of the capsule is more rounded than the top, often with the remains of a pedicel (Fig. 1.A3). The top of the fruit is beak-shaped, formed from the remains of a three- three-lobed-style (Fig. 1.A1).

The capsule, erect, three-nested, formed from a syncarpy gynoecium. The consistency of fresh pericarp is fleshy, juicy. It opens with three longitudinal cracks. The surface of the capsule is striped with shallow furrows (Fig. 1.A2). The capsule is multi-seeded, contains from 10 to 20 seeds, which are arranged in two rows, almost sticking together due to the filmy coverings, forming a compact mass.

Cardamom seeds are dark brown, gray-black to black in color, about 3.5-4.4 mm long and 2.6-3.2 mm wide, unevenly angular, transversely

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Fig. 1. Morphometry of fruits (A) and seeds (B) of E. cardamomum: 1 – top of the capsule, 2 – surface of the capsule, 3 – pedicel.

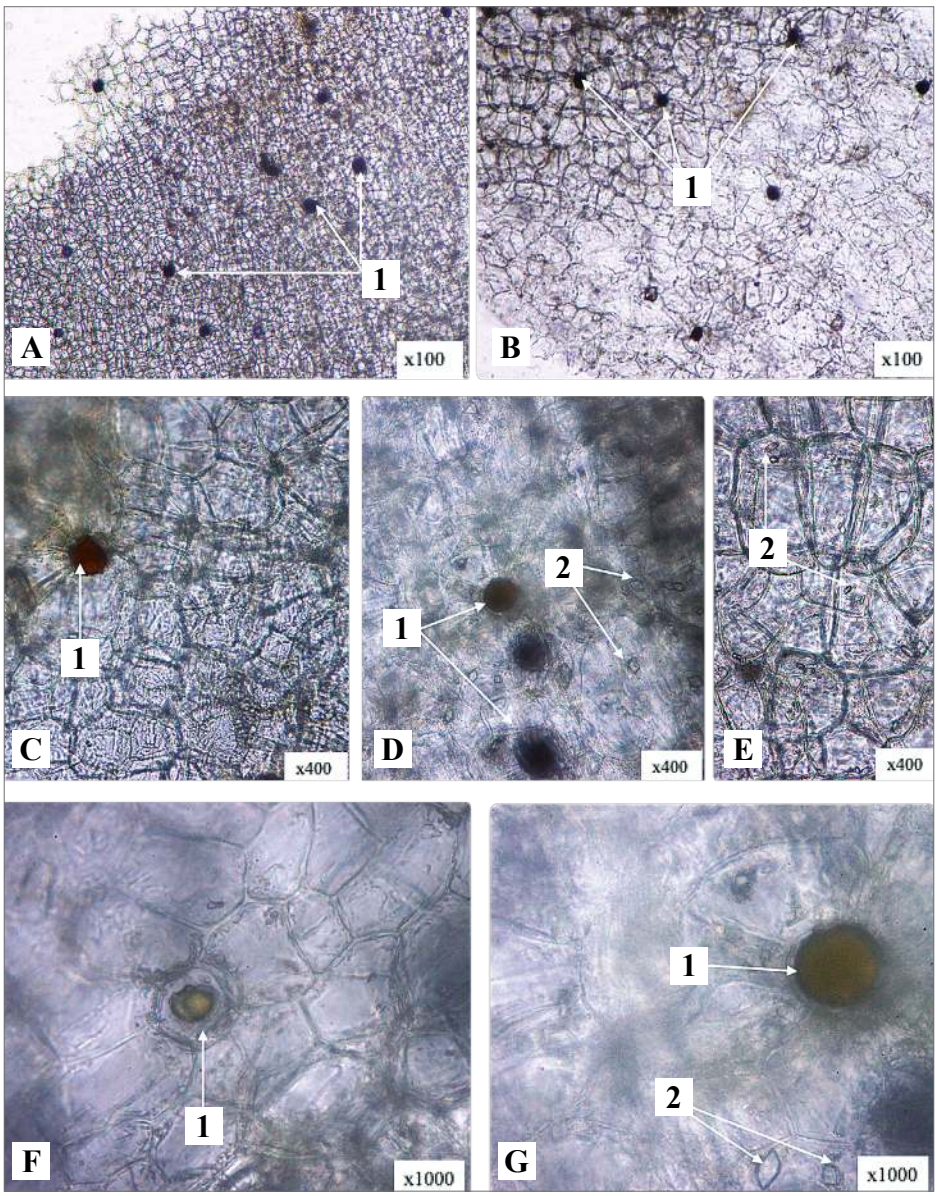


Fig. 2. Light microscopy of the outer (A, C, F) and inner (B, D, E, G) epidermis of the capsule of *E. cardamomum*: A, B – x100, C, D, E - x400, F, G – x1000. 1 – an idioblast cell with brown contents, 2 – single crystals

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wrinkled, without cavities, with a longitudinal channel containing aromatic substances (Fig. 1B). The seeds are enclosed in a colorless membranous shell. It has a characteristic sweetish aroma with notes of eucalyptus and camphor, which quickly disappears when the seeds are ground, and a strongly spicy taste.

The outer and inner epidermises of the cardamom pericarp were examined under a light microscope. The cells of the outer epidermis are multi-angular, unevenly elongated or square in shape with rather thick membranes, among which there are occasionally cells-idioblasts with light brown, lemon or brown contents, which either occupy the entire cell space or are compacted in the middle part of the cell (Fig. 2. A, C, F 1). The idioblast cell is surrounded by 6-8 radially arranged epidermal cells (Fig. 2. F 1). The cells of the inner epidermis are more regular elongated multicornered shape, the cell membranes are thinner, but the layering of rows of cells is thicker. The number of idioblast cells is twice as large as on the outer side and their contents completely fill the cell (Fig. 2. B, D, E, G 1). A significant number of crystalline inclusions in the cells of the inner epidermis of the cardamom capsule was noted (Fig. 2. D, E, G 2).

The cross-section through the pericarp of *E. cardamomum* demonstrates three main components: cuticle (Fig. 3. D 4), main parenchyma (Fig. 3.A-D) and collateral closed vascular bundles (Fig. 3.A, B 3).

The cuticle is characterized by rather large rectangular-elongated cells that extend along the entire surface of the fruit (Fig. 3. D 4). The main parenchyma is multilayered, its cells are trapezoidal, multi- or hexagonal in shape, voluminous, with thick membranes and simple straight pores (Fig. 3. D 5). Vascular bundles are closed collateral with well-defined xylem vessels, passing on longitudinal strips of the fruit surface (Fig. 3. A, B 3).

The pulp of the fruit is dotted with numerous cavities containing a brown substance, which are localized in idioblast cells (Fig. 3. A-C 1). At high magnification, large prismatic crystals are clearly visible, which are found in many

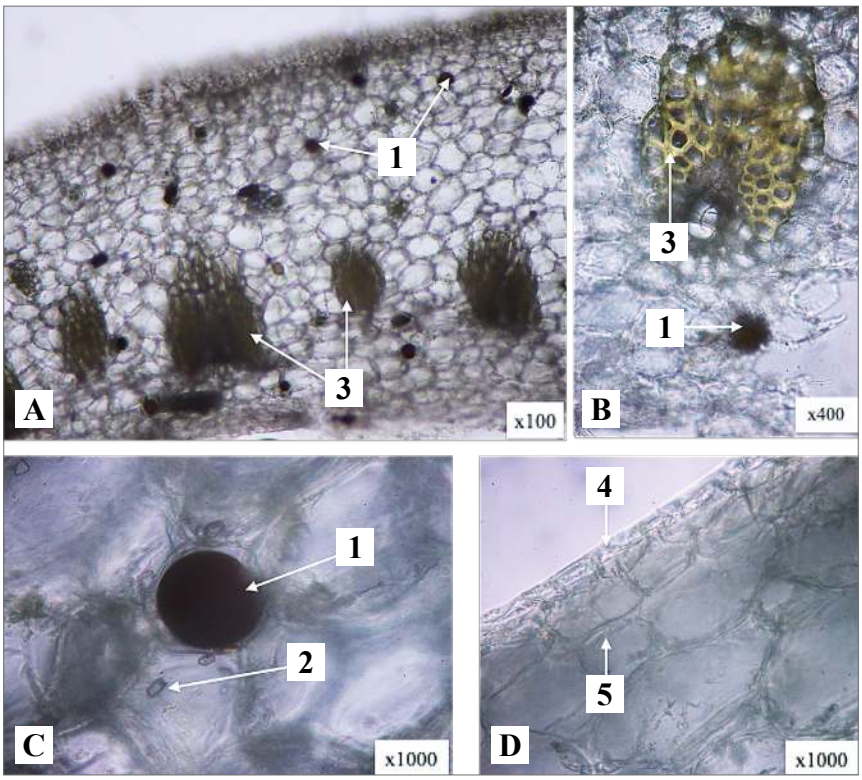


Fig. 3. Light microscopy of a cross-section through the flesh of a pericarp *E. cardamomum*: A – x100, B – x400, C, D – x1000. 1. – idioblast cell with brown contents, 2. – single crystals, 3. – collateral closed vascular bundle, 4. – cuticle, 5. – simple straight pore of parenchyma cell.

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parenchymal cells of the fetal pulp and can also be concentrated around the cavities (Fig. 3. C 2).

To determine the type of the substances that were in the idioblast cells, thin sections of the outer and inner epidermis of the fruit, as well as a cross-section through the capsule, were placed in a Sudan III solution for 5–10 minutes.

We observed pink-orange coloration of cells in the parenchyma that accumulate essential oil (Fig. 4. A-F 1), as well as the accumulation of cutin (Fig. 4. E, F 2) in the outer layer of cells in the cross-section of the capsule.

To study the surface of cardamom seeds, a thin section was made from an *E. cardamomum* seed and examined under a light microscope. The seed epidermis is formed by tightly closed rectangular cells transversely located to the fruit surface (Fig. 5.A).

Magnification of x1000 times allows us to see that the edges of the cells can be not only rectangular, but also beveled at the ends (Fig. 5.B). Through the first layer of integumentary cells, we can see a cluster of ellipsoidal cells (Fig. 5.C 1), which are chaotically arranged in dense rows and densely filled with spherical inclusions of lipid inclusions (Fig. 5.C 2), which, when interacting with Sudan III, are colored orange (Fig. 5.D 3). Also, at a magnification of x1000, single prismatic crystals are present in the cells (Fig. 5.D 4).

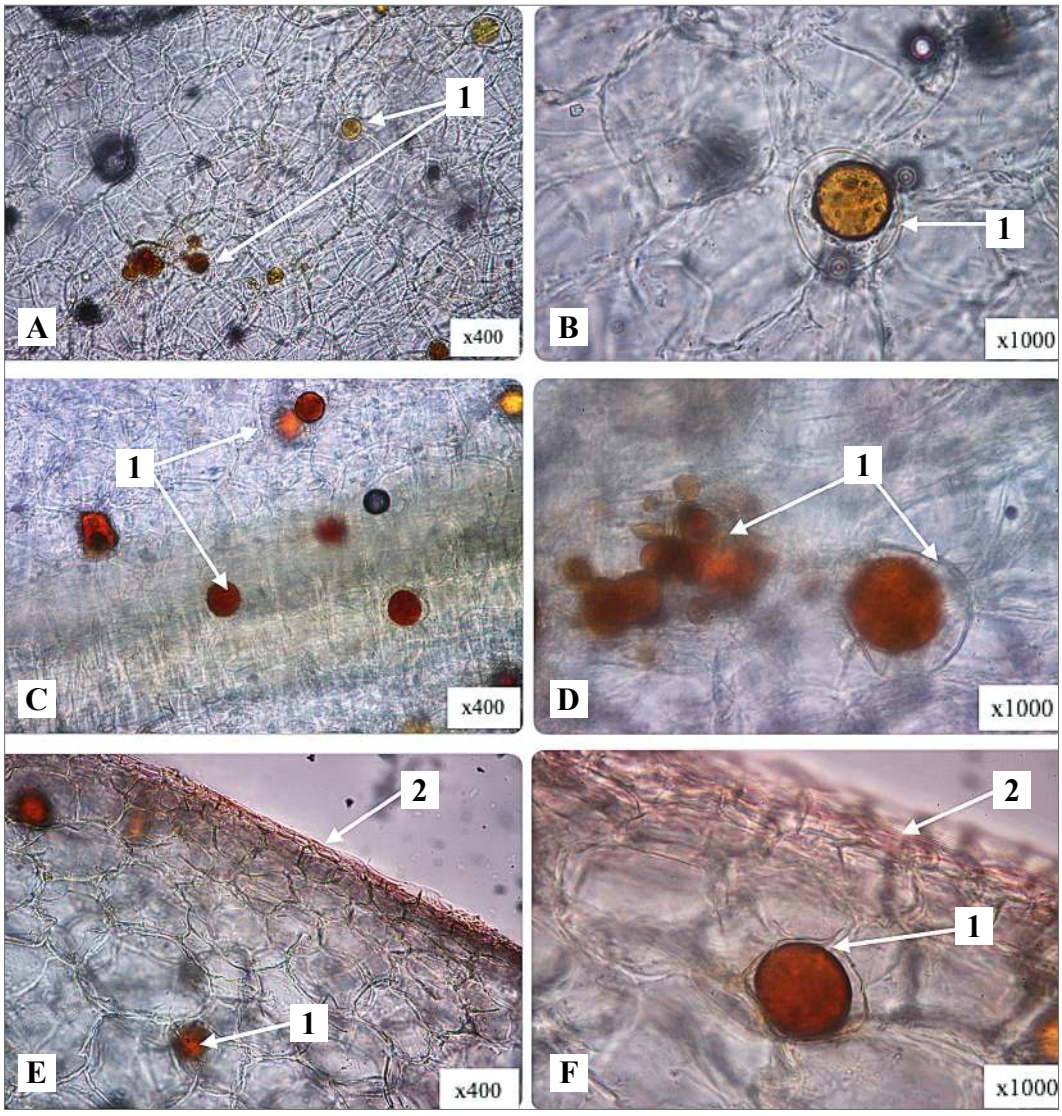


Fig. 4. The result of the reaction with Sudan III for the detection of lipid substances on the outer (A, B) and inner (C, D) surfaces of the fruit and in the cross-section through the capsule (E, F) of *E. cardamomum* as a result of the reaction: A, C, E – x400, B, D, F – x1000; 1. – idioblast cell with essential oil, 2. – cutin layer.

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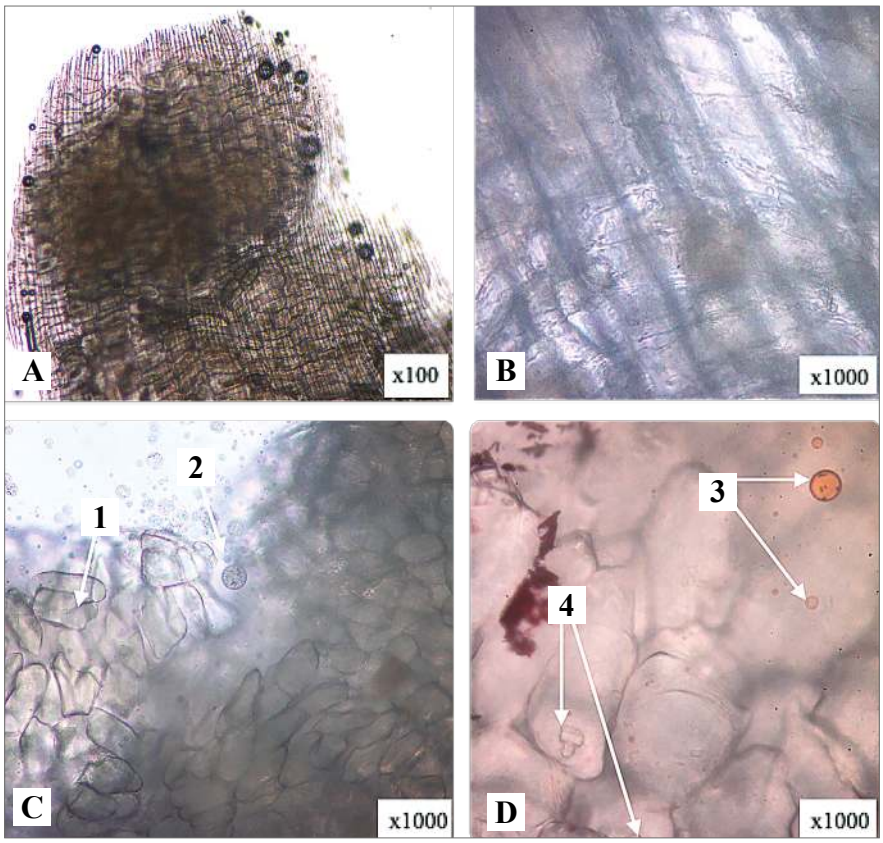


Fig. 5. Light microscopy of the surface of *E. cardamomum* seeds: A – x100, B–D – x1000, 1. – cells filled with reserve substances. 2. – lipid inclusions, 3 – the result of reaction with Sudan III, 4 – single crystals.

Scanning electron microscopy

The study of the fruit surface under a scanning electron microscope revealed a deeply wrinkled surface with numerous plates of epicuticular wax, represented by scarious and unevenly edged, uniformly oriented plates (Fig. 6 A, B). The cuticle is striated, with flattened projections and wavy contours. The surface relief is uneven, with periclinal walls raised relative to anticlinal walls.

The electron scanning microscopy of cardamom seeds showed an unevenly wavy surface structure with transverse striations of the layers and a large amount of epicuticular wax on the seed surface. The base of the seed is widened, and the apex is blunt, narrowed, thus creating an obovate shape (Fig. 6 C, D).

Determination of polysaccharides quantitative content

For the quantitative determination of polysaccharide content, we selected raw materials: fruits, pericarp and seeds, which were ground in a ceramic mortar (Fig. 7).

Extracts were prepared from the raw materials according to the standard method (State Pharmacopoeia of Ukraine, add. 5, 2021). The quantitative determination of polysaccharides in fruits, pericarp and seeds of *E. cardamomum* was carried out in accordance with SPhU 2.5 Monograph "*Plantaginis majoris folia*^{N}". A threefold increase in ethanol was added to 25 ml of the extracts obtained. The extracts with the addition of ethanol were left to cool for an hour and then centrifuged for half an hour. The resulting precipitate was filtered with filter paper and dried to a constant weight (Fig. 8).

The content of polysaccharides in terms of dry matter in the studied samples was: in pericarp – 4.7 ± 0.11%, in fruit – 2.8 ± 0.09%, in seeds – 2.22 ± 0.06%.

The highest quantitative content of polysaccharides was found in cardamom pericarp – 4.7%, and the lowest in seeds – 2.22%. The fruits showed an average result, with the same amount of raw materials taken - 5 g, which can be

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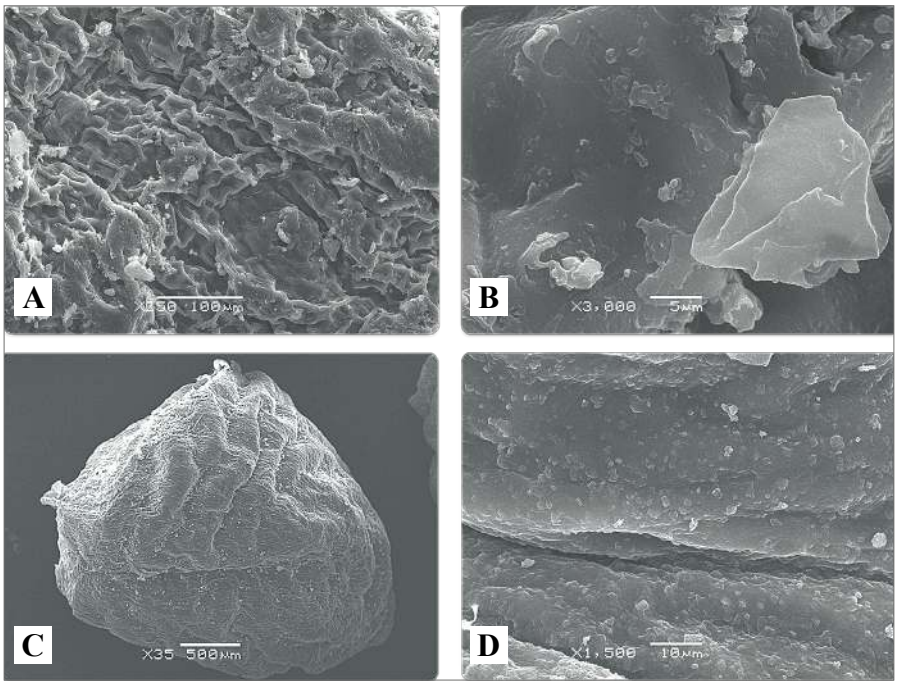


Fig. 6. Electron scanning microscopy of the surface of the capsule (A, B) and seed (C, D) of *E. cardamomum.*



Fig. 7. The crushed raw material of *E. cardamomum*



Fig. 8. The filtered precipitate is ready for complete drying.

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explained by the different weight ratio of seeds and pericarp in cardamom fruits.

Table 1 Quantitative content of polysaccharides in different types of medicinal plant materials

Medicinal plant materials	% polysaccharides	Note
Plantaginis majoris folia	Not less than 12 - 14,7	[11]
Parthenocissus quinquefolia folia	4,9	[12]
Ocimi basilici folia	4,44	[13]
Elaeagni angustifolia fructus	3,48	[14]
Teucrium chamaedrys herba	3,20	[15]
Teucrium montanum herba	2,81	[15]
Thymus serpyllum herba	0,28	[16]

Having analyzed the literature on alternative sources of polysaccharides, it should be noted (Table 1) that cardamom fruits, although they cannot compete with the main pharmacopoeial plant materials containing polysaccharides, may well serve as an alternative source.

Conclusions

A detailed morphological and anatomical analysis of the most important diagnostic features of cardamom fruits, pericarp and seeds using light and electron scanning microscopy was carried out: idioblasts with essential oil, cutin layer, wax flakes on the surface of seeds, which will allow to separate the fruits of this species from possible impurities.

Finally, it can be noted that the quantitative determination of the content of the sum of polysaccharides in fruits, pericarp and seeds of *E. cardamomum* according to the methodology from the monograph DFU 2.5 "*Plantaginis majoris folia*^{N}" [11] found that all parts of the fruit contain polysaccharides. Although the content is not as high as in the leaves of greater plantain, which range from 12% to 14.7%, but still the pericarp can be used as an additional source of polysaccharides with 4.7%, Such use will ensure the waste-free use of cardamom in the medical, pharmaceutical and food industries, since seeds rich in volatile essential oils are used for industrial purposes.

Financing

This study did not receive external funding.

Conflict of interest

There is no conflict of interest in this article. No rewards received.

Consent to publication

All authors of the article are acquainted with the final version of the manuscript and have no objections to its publication. The article does not use personal data and information about patients.

ORCID ID and author contribution

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A – Conception and design of the work, B – Data collection and analysis, C – Responsibility for the statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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Морфолого-анатомічне дослідження та кількісне визначення полісахаридів у плодах, оплоднях та насінні Elettaria cardamomum L.

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Анотація. *E. cardamomum офіційно включений до Фармакопей Індії, Великобританії та Сполучених Штатів Америки [1]. Для нашої країни він є спецією і в медичних цілях не застосовується. Вивчення макро- і мікроскопічної структури плодів та насіння, а також хімічних компонентів, зокрема полісахаридів, дозволить розробити методи контролю якості сировини на плоди з насінням E. cardamomum. Такий підхід розширить використання рослинних*

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препаратів з використанням кардамону в Україні. Крім того, забезпечить безвідходне використання кардамону в медичній, фармацевтичній та харчовій галузях, оскільки для промислових цілей використовують тільки насіння багате на леткі ефірні олії, а оплодні утилізують. У статті проаналізовано сучасні літературні дані щодо фітохімічного складу, застосування у фармації та медицині E. cardamotmit. Встановлено основні морфометричні ознаки плодів і насіння E. cardamotmit, які відіграють ключову роль в ідентифікації виду. Досліджено анатомічні особливості плодів та насіння E. cardamotmit під світловим та електронним мікроскопами, виявлено низку мікроморфологічних ознак, зокрема, ідіобласти з ефірною олією, кутиновий шар, воскові лусочки на поверхні насіння. Проведено кількісне визначення полісахаридів в насінні, плодах та оплоднях E. cardamotmit. Найвищий вміст полісахаридів виявлено у оплоднях - 4,7%, середній у плодах - 2,8% та найнижчий у насінні - 2,22%.

Keywords. Elettaria cardamomum, полісахариди, коробочка, насіння, світлова мікроскопія, електронна скануюча мікроскопія.

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