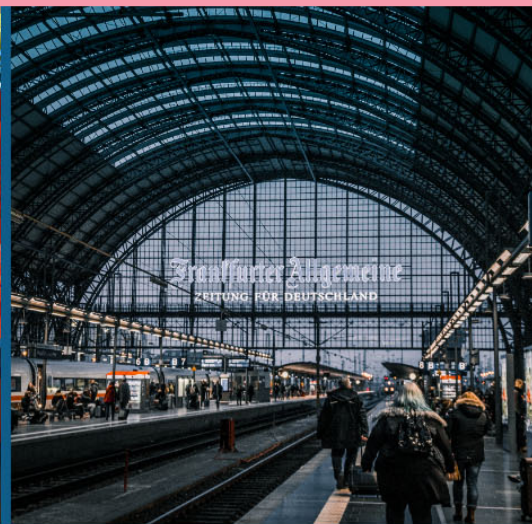




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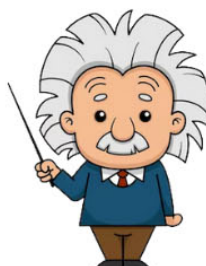
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ODDÍL 4. LÉKAŘSKÉ VĚDY

§4.1 STUDY AND CHARACTERIZATION OF HIGHER CARBOXYLIC ACIDS OF BLUEBERRY SEED OIL (*VACCINIUM MYRTILLUS* L.) (Osyp Yu., Lesya Ukrainka Volyn National University, **Osyp M.**, Higher Education "Luts'k Pedagogical College" of the Volyn Regional Council)

Entry. Blueberry seed oil, derived from *Vaccinium myrtillus* L., is gaining attention due to its rich composition of bioactive compounds, particularly higher carboxylic acids. These acids play a crucial role in determining the oil's nutritional, therapeutic, and industrial value. The study and characterization of these components provide insights into their chemical structure, concentration, and potential applications in food, cosmetics, and pharmaceuticals. Understanding the profile of higher carboxylic acids in blueberry seed oil contributes to optimizing its utilization and exploring its health-promoting properties.

The study of biologically active substances of natural origin is a classical direction in pharmacology and medicine. A large number of medicinal products have been developed based on plant raw materials, which specialists in medical practice often prefer over synthetic drugs.

Bilberry (*Vaccinium myrtillus* L.) is an important medicinal, food, and honey-bearing plant. It is a low-growing shrub (15–40 cm, rarely up to 60 cm tall) with branched, sharply ribbed, bare stems and a long creeping rhizome. The stems are ascending or upright, branched, and greenish-brown. The leaves are arranged spirally, nearly sessile, entire, ovate, elliptical, or ovate-elliptical with finely serrated edges, pointed tips, light green on the upper side, paler underneath, smooth or sparsely covered with short hairs along the veins. The leaves are thin and fall off in winter.



The flowers are regular, borne singly in the leaf axils on short pedicels. The corolla is greenish-white with a pinkish tint, spherical-urn-shaped, with fused petals and 4–5 reflexed lobes. The fruit is a spherical black berry. The plant blooms in May–June, and the fruits ripen in July [1].

Bilberry fruits contain carbohydrates (5.3–7.4%), including glucose, fructose, sucrose, and pectin; organic acids (0.90–1.28%) such as citric, lactic, oxalic, malic, and succinic acids. They are rich in vitamins: ascorbic acid (5–6 mg%), thiamine (0.045 mg%), riboflavin (0.08 mg%), nicotinic acid (2.1 mg%), and carotene (0.75–1.6 mg%). The fruits also contain flavonoids (460–600 mg%) like hyperin, astragalin, quercitrin, isoquercitrin, and rutin; anthocyanins such as delphinidin, malvidin, petunidin, idaein, and myrtillin; and phenolic acids like caffeic, quinic, and chlorogenic acids. Additionally, bilberries contain phenols and their derivatives such as hydroquinone, monotropein, and asperuloside; tannins (up to 12% in absolutely dry matter), essential oil, inositol (a cyclic hexatomic alcohol), anthracene derivatives, and compounds of manganese and iron.

Bilberry leaves are also rich in tannins (up to 20% in absolutely dry matter), and contain flavonoids such as kaempferol, rutin, astragalin, hyperin, quercitrin, isoquercitrin, avicularin, and meratin. They include anthocyanins like cyanidin, delphinidin, and petunidin; triterpenoids such as β -amyrin, oleanolic acid, and ursolic acid; and phenolic acids like caffeic, chlorogenic, and quinic acids. The leaves also contain various phenols and their derivatives: Hydroquinone, arbutin, methyl arbutin, asperuloside, monotropein, essential oil, the alkaloid murtin, vitamin C, carotenoids, and citric acid are also present in bilberry. Due to its complex of biologically active substances, bilberry exhibits various biological effects and is widely used in fresh, dried, and processed forms [2, 3, 4, 5, 6].

Bilberry seeds contain fatty oil, which is used as a valuable component in cosmetic products. One of the most important characteristics of fatty oils is their



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fatty acid composition. Particularly significant in plant oils are unsaturated fatty acids such as linoleic and linolenic acids, which are not synthesized in animal organisms but are essential for many biochemical processes.

All oils consist of 99.0–99.5% triglycerides of higher carboxylic acids. In the 1930s, it became known that oils contain substances essential for human life that are not produced in the body but are necessary for normal functioning. These were identified as fatty acids with two or more double bonds in their molecules: linoleic acid (18 carbon atoms and 2 double bonds) and linolenic acid (18 carbon atoms and 3 double bonds), also known as essential or indispensable fatty acids.

Essential fatty acids are the primary building material for cell membranes and the biosynthesis of substances—mediators that regulate metabolic processes (such as prostaglandins and leukotrienes). Membranes, in turn, organize all metabolic and energy processes in the body. A deficiency of essential fatty acids in the body can lead to growth and developmental delays in children.; Disruption of skin permeability, the development of dermatitis, and suppression of reproductive function in adults are consequences of essential fatty acid deficiency in the body [7].

The most important factors determining the nutritional value of oils are the amount and ratio of polyunsaturated fatty acids (PUFAs)— ω -6 (linoleic acid) and ω -3 (linolenic acid)—as well as the balance between monounsaturated fatty acids (MUFAs) and PUFAs.

The human body's requirement for PUFAs is approximately 11 g/day, including 1–3 g/day of ω -3 fatty acids and no more than 10 g/day of ω -6 fatty acids, while the need for MUFAs is about 30 g/day. At the same time, the ratio of MUFAs to PUFAs should be 3:1.

Currently, the ratio of ω -6 to ω -3 in the diet of the average person in Ukraine is approximately 30:1. This is due to the widespread use of sunflower oil in



cooking, which contains a high amount of linoleic acid (50–75%) but almost no linolenic acid [8, 9, 10].

Presentation of the main material. Oil of light-yellow color with a refractive index of 1.4742 was obtained from the seeds of bilberry (*Vaccinium myrtillus* L.) using exhaustive extraction with *n*-hexane. The yield was 18%.

The fatty acid composition of bilberry seed oil was determined using gas-liquid chromatography. The analysis revealed that the oil contains a high proportion of oleic acid (23.7%), linoleic acid (38.1%), and linolenic acid (31.1%). In minor quantities, the oil also contains palmitic acid (5.3%), stearic acid (1.0%), and myristic acid (0.7%).

Bilberry fruits were collected in July 2016 near the village of Krychevychi, Kovel District, Volyn Region. To separate the seeds, fresh berries were mashed, and the resulting mass was diluted with water. In the mixture, the seeds settled at the bottom of the container, while the upper fraction was removed by decantation. This procedure was repeated several times until visually clean seeds were obtained, which were then dried to constant weight.

Oil extraction from crushed bilberry seeds was carried out using exhaustive extraction in a Soxhlet apparatus with *n*-hexane. The obtained extract was filtered, and the solvent was evaporated. Residual *n*-hexane in the oil was removed using a water-jet pump while heating the flask in a water bath.

The fatty acid content in bilberry seed oil was determined using gas-liquid chromatography according to the method described in GOST 30418-96 [11]. The higher carboxylic acids were esterified with sodium methylate, and the resulting methyl esters were separated on a “Kristall 2000M” gas chromatograph (Chromatek) with a DB-FFAP capillary column (USA) and a flame-ionization detector. A set of standard methyl esters of fatty acids (Institute for Scintillation Materials, NAS of Ukraine) was used for instrument calibration.



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Using the exhaustive extraction method with n-hexane, it was determined that the seeds of common bilberry (*Vaccinium myrtillus* L.) contain 18% oil, which is light yellow with a greenish tint. The refractive index is 1.4742.

The results of the study on the fatty acid composition of bilberry seed oil, obtained using high-performance gas-liquid chromatography, are presented in the table:

Carboxylic acid		Mass share, %
Myristic (14:0)	tetradecanoic	0,7 %
Palmitic (16:0)	hexadecanoic	5,3 %
Stearic (18:0)	octadecanoic	1,0 %
Oleic (18:1) ω 9	octadecenoic	23,7 %
Linoleic (18:2) ω 6	octadecadienoic	38,1 %
Linolenic (18:3) ω 3	octadecatrienoic	31,1 %

It has been established that the studied oil contains a significant amount of oleic (23.7%), linoleic (38.1%), and linolenic (31.1%) acids. In much smaller quantities, bilberry oil contains palmitic (5.3%), stearic (1.0%), and myristic (0.7%) acids.

Overall, more than 90% of the higher carboxylic acids in the oil are unsaturated. Notably, two-thirds of this amount (69.2%) consists of polyunsaturated fatty acids (PUFAs), specifically linoleic and linolenic acids. These acids are classified as vitamin F because they are not synthesized in the human body but are essential for its normal functioning.

Linoleic acid belongs to the ω -6 class of unsaturated higher carboxylic acids. The human body synthesizes polyunsaturated arachidonic acid (20:4, ω -6) from it, which is present in relatively large amounts in the body. For example, in adrenal phospholipids, its content reaches up to 20%, in the outer membrane of liver



hepatocytes—11%, and in the outer and inner membranes of hepatocyte mitochondria—15.7% and 18.5% of the total fatty acid content, respectively [12].

Arachidonic acid metabolites are endogenous ligands of cannabinoid receptors (CB1 and CB2). The most important of these are arachidonylethanolamide (anandamide) and 2-arachidonylglycerol. These compounds function as neuromodulators and neurotransmitters. Anandamide plays a role in pain mechanisms, depression, appetite, memory, reproductive function, and increases the heart's resistance to the arrhythmic effects of ischemia and reperfusion by activating CB2 receptors [13].

Linolenic acid is classified as one of the most important PUFAs of the ω -3 family for living organisms. In the human body, it is converted into long-chain ω -3 fatty acids: eicosapentaenoic (20:5, ω -3) and docosahexaenoic (22:6, ω -3), which play a crucial role in regulating lipid metabolism, thrombosis, vasodilation, and inflammation [14].

Eicosapentaenoic acid is a precursor of thromboxanes, prostaglandins, and leukotrienes—highly active immune-inflammatory regulators [15]. It prevents rapid blood clotting, reducing the risk of coronary artery thrombosis.

Eicosapentaenoic and docosahexaenoic fatty acids play a central role in maintaining the homeostasis of inflammatory responses [15], preventing inflammatory processes by inhibiting eicosanoid production, promoting the restoration of normal serotonin levels, and releasing substances that facilitate wound healing (protectins and resolvins).

Conclusion

As a result of the conducted research, the fatty acid composition of bilberry seed oil (*Vaccinium myrtillus* L.) was determined, confirming its high biological value and potential for use in the food, pharmaceutical, and cosmetic industries. The oil, extracted through exhaustive *n*-hexane extraction, was light-yellow with a



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refractive index of 1.4742 and a yield of 18%, indicating the efficiency of the extraction method used.

Gas-liquid chromatography analysis revealed that bilberry seed oil contains significant amounts of polyunsaturated fatty acids (PUFAs), particularly linoleic acid (38.1%) and linolenic acid (31.1%), which are essential for the normal functioning of the human body. The high content of these acids highlights the importance of bilberry oil as a source of essential fatty acids that are not synthesized in the human body but are necessary to maintain health. Linoleic and linolenic acids are especially important as building blocks for cell membranes and for the synthesis of biologically active compounds, such as prostaglandins and leukotrienes, which regulate metabolic processes in the body.

In addition to polyunsaturated acids, the oil contains monounsaturated fatty acids, particularly oleic acid (23.7%), which helps reduce cholesterol levels in the blood and positively affects the cardiovascular system. Minor amounts of palmitic acid (5.3%), stearic acid (1.0%), and myristic acid (0.7%) were also detected, which play important roles in metabolic processes.

The high content of ω -3 and ω -6 fatty acids in bilberry seed oil indicates its potential to correct the imbalance of these acids in the modern human diet, which is especially relevant given the excessive consumption of ω -6 fatty acids in the traditional Ukrainian diet. The use of bilberry seed oil can help normalize the ω -3 to ω -6 ratio, which is important for preventing chronic inflammatory processes, cardiovascular diseases, and maintaining overall health.

Given the obtained results, bilberry seed oil can be used not only as a food product but also as a valuable ingredient in cosmetic products due to its moisturizing, antioxidant, and anti-inflammatory properties. The fatty acid composition of the oil promotes the restoration of the skin's lipid barrier, improves its elasticity, and protects against harmful environmental influences.



Thus, the results of this study confirm the promising potential of bilberry seed oil in various industries, including food, pharmaceuticals, and cosmetics. Further research could focus on studying the pharmacological activity of this oil, its effects on the human body, and the potential for its use as a functional ingredient in therapeutic and preventive products.

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