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QUALIFICATION WORK

on the topic: **«PHYTOCHEMICAL STUDY OF CRANBERRY FRUITS»**

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АНОТАЦІЯ

Журавлина – рослина, яка містить комплекс біологічно-активних речовин з широким спектром фармакологічної активності. Плоди журавлини – перспективна сировина для розробки нових препаратів для лікування багатьох захворювань з огляду на їх ефективність і безпеку. В результаті проведених досліджень у журавлини плодах визначено вміст втрати в масі при висушуванні, загальної золи, золи, нерозчинної в кислоті хлористоводневій та вміст екстрагованих речовин. Проведено визначення якісного складу біологічно активних речовин у журавлини плодах. У журавлини плодах визначено наявність флавоноїдів, дубильних речовин, сапонінів і полісахаридів. Хроматографічними методами дослідження в журавлини плодах виявлені фенольні сполуки. У журавлини плодах визначено кількісний вміст флавоноїдів, гідроксикоричних кислот та полісахаридів. Встановлено, що вміст флавоноїдів у журавлини плодах становив 0,77±1,83 %, гідроксикоричних кислот – 1,09±1,29 % та полісахаридів – 7,13±2,17 %.

Ключові слова: плоди журавлини, флавоноїди, гідроксикорична кислота, полісахариди.

ANNOTATION

Cranberry is a plant with wide range of chemical constituents which exerted many pharmacological effects. There is a great promise for development of novel drugs from Cranberry to treat many human diseases as a result of its effectiveness and safety. The content of loss on drying, total ash, acid-insoluble ash and extractable matter were calculated in Cranberry fruits. Qualitative determination of biologically active compounds in Cranberry fruits was carried out. The presence of flavonoids, tannins, saponins and

polysaccharides were determined in Cranberry fruits. At the base of chromatographic analysis, we can make conclusion that flavonoid glycosides and phenolic compounds are present in Cranberry fruits. Quantitative determination of flavonoids, hydroxycinnamic acid and polysaccharides were carried out for Cranberry fruits. It was determined that content of flavonoids in Cranberry fruits was 0,77±1,83 %. hydroxycinnamic acid – 1,09±1,29 % and polysaccharides – 7,13±2,17%.

Key words: Cranberry fruits, flavonoids, hydroxycinnamic acid, polysaccharides.

CONTENT

INTRODUCTION

Relevance. Cranberry is a particularly rich source of polyphenols, which have been associated in vitro with antibacterial, antiviral, antimutagenic, anticarcinogenic, antitumorigenic, antiangiogenic, anti-inflammatory and antioxidant properties. In vivo, animal models reveal that cranberry extracts can reduce C-reactive protein and proinflammatory interleukins and increase NO synthesis; decrease angiotensin converting enzyme, angiotensin II, and angiotensin II type 1 receptor; suppress Helicobacter pylori infection and improve pancreatic b-cell glucose responsiveness and functional b-cell mass. Some of these actions may underlie the results from clinical studies showing that cranberry products can lower LDL cholesterol and total cholesterol, increase HDL cholesterol (HDL-C) while lowering the oxidative modification of LDL-C, improve endothelial function, lower glycemic responses, elevate plasma antioxidant capacity, modulate ulcerogenic gastric H. pylori colonization, decrease cariogenic Streptococcus mutans and total bacterial counts in saliva, reduce biomarkers of metabolic syndrome.

Research aim. The **aim** of our work was phytochemical study of Cranberry fruits.

Research task. To achieve the aim of the work we have set the following goals:

- to review and systematize literature data about botanical description of Cranberry fruits chemical composition and use;
- identify values for medicinal plant material (moisture content, ash);
- to carry out qualitative analysis of the substances. To identify the main groups of biologically active compounds;
- to carry out the quantitative analyses of active substances of Cranberry fruits.

Object of study. The phytochemical study of Cranberry fruits.

Subject of study. Qualitative analysis and quantitative determination of biological active compound of Cranberry fruits.

Research methods. Chemical reactions and chromatographic methods, in particular paper and thin layer chromatography, were used to study the qualitative composition of Cranberry fruits. Quantitative determination of biological active compound of Cranberry fruits herb were performed by spectrophotometry, gravimetry and determination of indexes of Cranberry fruits.

Practical significance of the obtained results. The qualification work contains the results of phytochemical study of biological active compound of Cranberry fruits. The results of the research can be used in the development of technology and standardization of herbal medicines based on the studied raw materials of Cranberry fruits.

Structure and scope of qualification work. Qualification work consists of an introduction, literature review, 3 experimental parts, conclusions, list of literature.

The work is presented on 46 pages, includes 7 tables, 2 figures, 44 sources of literature.

CHAPTER 1
REVIEW

1.1. Distribution of Cranberry

In North America, the ‘large’ cranberry is referred to as *Vaccinium macrocarpon*, and is cultivated in the northern parts of the country. It is mainly produced in New Jersey, Massachusetts, Oregon, Washington, Wisconsin and, in the Canadian provinces of British Columbia and Quebec. They are also grown for trade purposes in Chile. The ‘large’ cranberry is also present in the commercial farms of Europe such as in Germany, Belarus, Latvia, Lithuania and Russia.

These regions have suitable environmental conditions for cranberries to grow in including sandy soil, plenty of fresh water and a cool resting period for which they can thrive in the growing season. The indigenous people of North America gave the cranberry its English name because the stem, calyx and petals resembled the neck, head and bill of a crane and berry stands for English berry, thus “craneberry”.

The ‘small’ cranberry, also called *Vaccinium oxycoccus,* is harvested in the wild and present in Ireland, the British Isles and Scandinavia, eastern and central Europe, Finland and Germany, the Balkan countries, and Siberia and Japan. It is sold in the markets of the Baltic states, Finland, Poland, Ukraine [7, 42].

1.2. Botanical characteristics of Cranberry

Cranberries grow on trailing vines in beds layered with sand, peat, gravel and clay, which are called bogs or marshes. The ‘large’ cranberry is an evergreen shrub with low growing vines that produce slender wiry stems up to 2 m long and 5–20 cm in height. Leaves are elliptical and grow up to 22 mm long and 9 mm wide [3]. They can also grow light pink flowers. Bees pollinate them. ‘Large’ cranberry fruits can be red, dark red, or dark purple, while those that have not completely matured are a pale pink or white color, which accounts for red and white cranberry juices. The ‘small’

cranberry grows on peat in poorly drained areas with high water with stems that can extend up to 100 cm long. Leaves are ovate and may expand up to 16 mm long and 6 mm wide [6].

1.3. Cultivation of Cranberry

Ripening of ‘large cranberry’ cultivars begins near the end of summer to the beginning of September and continues through October in the U.S. and Canada. The ‘small cranberries’ ripen from late August through September and can be under the snow until spring. The ‘small cranberry’ has more tolerance to cold than the ‘large cranberry’. Only about 5% of cranberries produced in the U.S. are sold fresh and the remaining 95% are processed into products such as juices, sauce, and dried fruits because of their tart flavor. Americans consume an estimated 400 million pounds of cranberries per year, 20% of them during the holidays. The U.S. per capita consumption of cranberries is 2.3 pounds, primarily as juice or juice blends. In 2019, world production of cranberry was 687,534 tons with the U.S. being the primary producer (359,111 tons), followed by Canada (172,440 tons) and Chile (141,338 tons). Cranberry production in the U.S. for 2020 totaled about 7.8 million barrels, mainly by Wisconsin (4.6 million barrels) and Massachusetts (2.1 million barrels) with the least by New Jersey (0.5 million) and Oregon (0.6 million). The U.S. total area harvested was 39,300 acres.

Early European colonists discovered the benefits of cranberry as a medicine and as a food product in North America. Both ‘small’ and ‘large’ cranberries have been used over several decades in North America, and some parts of Asia and Europe, to prevent or cure different diseases. They are mostly known for treating or preventing urinary tract infections and maintaining the digestive system.

Cranberry is abundant in nutritional components and many bioactive compounds that have antioxidant properties. Both American and European cranberry species are rich in many classes of phytochemicals. These include phenolic acids,

anthocyanins, flavones, flavonoids, and organic acids. Cranberry is one of the few fruits that is high in proanthocyanidins, which inhibit adherence of *Escherichia coli* to the urinary tract. The content of phenolic compounds in the cranberries is influenced by aspects such as cultivar, agriculture practices, geographical area, weather conditions, ripeness, harvesting time, and storage settings. The greatest quantity of total phenols is accrued at the beginning of berry ripening. The cultivars grown in colder weather are characterized by higher amounts of phenolics than the same cultivars grown in a mild climate. Consuming cranberries can prevent tooth decay and gum disease, inhibit urinary tract infections, reduce inflammation in the body, maintain a healthy digestion system, and decrease cholesterol levels. This investigation summarizes recent scientific studies as to the health benefits of cranberry due to its phytochemical and antioxidant activity. This review can help promote cranberries as functional foods for consumers interested in maintaining their well-being and reducing health risks the natural way [6,7, 42].

1.4. Chemical composition of Cranberry fruit

Raw, unsweetened American cranberries contain mainly 87% water and 12% carbohydrates, with lesser amounts of protein, fats and fiber. Small cranberries accrue 2.1–4.9% titrable acidity with citric acid contributing 1.8–2.6%. Large cranberries accumulate less titrable acidity (1.9–2.4%) with citric acid found to be 1.88 mg/g and 6.08 mg/g. Citric, malic and quinic acids were the main acids found in the large cranberry. Cranberry nutritional composition may differ depending on the cultivar, climate, growing conditions, maturity/ripeness stage, time of harvest and storage conditions. The concentration of quinic acid was 3.81–13.3 g/kg, of malic acid was 14.1–43.3 g/kg and of citric acid was 10.8–54.3 g/kg [5,8.20-22].

Table 1.1.

Constituens of Cranberry

Name	Amount	Name	Amount
Water	87 g	Vitamin E	1.3 mg
Energy	46 kcal	Vitamin K	5 µg
Carbohydrates	12 g	Vitamin A, as retinol	3 µg
Sugars	4.3 g	Vitamin A, IU	63 IU
Dietary fiber	3.6 g	Calcium	8 mg
Fat	0.1 g	Iron	0.23 mg
Protein	0.5 g	Magnesium	6 mg
Thiamine (B_1)	0.012 mg	Manganese	0.27 mg
Riboflavin (B_2)	0.02 mg	Phosphorus	11 mg
Niacin (B_3)	0.101 mg	Potassium	80 mg
Pantothenic acid (B_5)	0.295 mg	Sodium	2 mg
Vitamin B_6	0.057 mg	Zinc	0.09 mg
Folate (B_9)	1 µg	Copper	0.06 mg
Vitamin C	14 mg	Selenium	0.1 µg

Glucose (3.44 g), fructose (0.67 g), and sucrose (0.16 g) contribute to the simple sugars in 100 g of raw cranberries. Large cranberries constitute 3.4 to 7.1% of monosaccharides, while small cranberries have a lesser amount of sugars, 2.2 to 6.0%. Cranberries contain mostly glucose and fructose with glucose accounting for 58.9 to 65.9% of monosaccharides. The large cranberry contains more sucrose (3.9–5.3%) than in the small cranberry (0.01–0.5%). Fructose was the main sugar identified in these cranberry fruit cultivars with a range of 58.9 to 68.7% of total sugar, followed by

glucose ranging from 29.6 to 39.3% and sucrose ranging from 1.7 to 1.9%. The sweetness of the cranberries is due to these three monosaccharides, fructose, glucose and sucrose [9-16].

Cranberries contain a wide range of water-soluble and fat-soluble vitamins. Antioxidants in cranberries predominantly come from a rich source of Vitamin C, Vitamin E and Vitamin K. The small cranberry comprises 15.3 to 30% of ascorbic acid, the main active form of Vitamin C. A higher amount (47.5%) was detected in the large cranberry. Of the six cultivars of cranberry fruit (*Vaccinium macrocarpon*) grown in a horticultural farm with similar conditions in a region of Poland, 'Pilgrim' had the highest content of Vitamin C (20.74 mg/100 g fresh matter (fm)) and 'Red Star' had the lowest content (10.07 mg/100 g fm). Cranberry also contains a small amount of fat, Omega-3 and Omega-6, which are important for the human diet.

Minerals encompassed 0.19 to 0.28% fresh weight (fw) of the small cranberry. European cranberries (*Vaccinium oxycoccos*) and American cranberries (*Vaccinium macrocarpon*) showed similar average mineral content for the macronutrients, potassium (67.19 and 72.51 mg/100 g, respectively), calcium (12.74 and 10.19 mg/100 g, respectively), and sulfur (8.14 and 7.85 mg/100 g, respectively). Potassium and nitrogen were the major minerals identified in these two varieties of cranberry fruits. American cranberry contained a greater mean amount of iron (0.72 mg/100 g) than the European variety (0.31 mg/100 g). Manganese and boron (2.59 and 0.09 mg/100 g, respectively) were higher in the European cranberry fruit than the American cranberry (0.19 and 0.065 mg/100 g, respectively). This suggests cranberry fruit is a good source of minerals.

Cranberries contain chemically diverse, secondary metabolites, polyphenols that have antimicrobial and antioxidant properties. Chemical composition of cranberries varies due to environmental conditions as well as the ripening process. Cranberry cultivars attain the appropriate shape, weight, texture, color, aroma and flavor during the ripening stage.

Both types of cranberries, the large and small, contain many phenolic compounds, such as phenolic acids, flavonoids (anthocyanins and flavonols), and tannins. The large cranberry has been recognized as an important food and healing agent because of these compounds. Flavonoids were the major compounds identified among over 150 compounds in the large cranberry. Flavonoids are classified into subgroups that include anthocyanins, flavonols, and proanthocyanidins. The large cranberry was found to contain 13 anthocyanins, 16 flavonols, and 26 phenolic acids and benzoates. The small cranberry includes flavonoids, such as anthocyanins, catechins, and flavones. Forty-eight polyphenols (including 19 flavonols, 8 anthocyanins, 7 phenolic acids and 14 flavan-3-ol oligomers) were identified in three cranberry cultivars in a study of different maturity ripening stages in Poland. The lowest amount of polyphenols was observed in the immature and semi-mature ripening stages and increased in the overripe, commercially mature cranberry. In order from highest to the lowest amounts of polyphenol classes that were detected in the cranberry fruits are flavan-3-ols (41.5–52.2%), flavonols (18.6–30.5%), anthocyanins (8.0–24.4%), and phenolic acids (5.0–12.1%). Among the three cranberry species, *Vaccinium macrocarpon* Ait., *Vaccinium oxycoccos* L., and *Vaccinium vitisidaea* (lingonberry grown in North America and Europe), 4624 compounds were identified with about 8000–10,000 phytochemicals found in each type [17,20].

1.4.1. Phenolic acids

Cranberries constitute phenolic acids that include hydroxybenzoic and hydroxycinnamic acids. Cranberry has high amounts of benzoic acid and lower amounts of 2,4-dihydroxybenzoic acid, *p*-hydroxybenzoic, and *o*-hydroxybenzoic acids. The hydroxycinnamic acids in cranberry are *p*-coumaric, sinapic, caffeic, and ferulic acids. The total amount of phenolic compounds in cranberry fruit cultivars depends on the cultivar and berry ripening [14,15].

Table 1.2

Phenolic compounds and triterpenoids of Cranberry fruit

Name	Polyphenol Content and Triterpenoids
Anthocyanins	
Delfinidyn derivatives	31.27–43.87 mg/100 g dm
Delfinidyn-3-O-glucoside	1.1–1.8 mg/100 g dm
Cyanidin derivatives	442–967 mg/100 g dm
Cyanidin-3-O-galactoside	119.9–180.0 mg/100 g dm
Cyanidin-3-O-glucoside	5.5–7.3 mg/100 g dm
Cyanidin-3-O-arabinoside	64.5–95.6 mg/100 g dm
Peonidin-3-O-galactoside	131.3–310.3 mg/100 g dm
Peonidin-3-O-arabinoside	42.9–95.2 mg/100 g dm
Peonidin derivatives	192–666 mg/100 g dm
Malvidin derivatives	29.85–58.85 mg/100 g dm
Malvidin-3-O-arabinoside	1.4–1.9 mg/100 g dm
Total anthocyanins	695–1716 mg/100 g dm
Phenolic acid	
p-Coumaric acid	2–245 μg/g dw
p-Coumaroyl hexose	8.6–13.9 mg/100 g dm
p-Coumaroyl hexose isomer	3.6–50.0 mg/100 g dm
p-Coumaroyl derivatives	210–451 mg/100 g dm
Chlorogenic acid	72.00–129.62 mg/100 g dm
Caffeic acid	5–123 μg/g dw

Name	Polyphenol Content and Triterpenoids
Caffeoyl hexoside	92.7–190.2 mg/100 g dm
Caffeoyl hexoside isomer	10.9–17.5 mg/100 g dm
Caffeoyl and derivatives	39.93–68.28 mg/100 g dm
Ferulic acid	4–39 μg/g dw
Total phenolic acid	327–649 mg/100 g dm
Flavonols	
Myricetin-3-O-galactoside	156.5–348.4 mg/100 g dm
Myricetin-3-O-glucoside	1.8–6.6 mg/100 g dm
Myricetin-3-O-pentoside	6.3–55.6 mg/100 g dm
Myricetin-3-O-glucoronide	19.0–38.5 mg/100 g dm
Myricetin-arabinoside	8–273 μg/g dw
Sinapoyl derivatives	4.36–5.82 mg/100 g dm
Myricetin derivatives	496–926 mg/100 g dm
Quercetin-3-O-galactoside	294.6–375.8 mg/100 g dm
Quercetin-3-O-pentoside	21.2–122.9 mg/100 g dm
Quercetin-3-O-glucoside	4.8–11.5 mg/100 g
Quercetin-p-conmaroyl-hexoside	1.3–13.3 mg/100 g dm
Quercetin-3-O-rhamnoside	6.2–13.3 mg/100 g dm
Quercetin-rutinoside	12.0 mg/100 g fw
Quercetin-acetyl-glucoside	13.58 mg/100 g fw
Quercetin derivatives	107–225 mg/100 g dm

Name	Polyphenol Content and Triterpenoids
Methoxyquercetin hexoside	1.7–25.7 mg/100 g dm
Methoxyquercetin pentoside	3.4–61.0 mg/100 g dm
Methoxyquercetin derivatives	33.31–43.04 mg/100 g dm
Total flavonols	643–1088 mg/100 g dm
Flavan-3-ols and proanthocyanidins	
(+)-Catechin	2.79–7.53 mg/100 g dm
(−)-Epicatechin	27.46–56.84 mg/100 g dm 47.5–60.8 mg/100 g dm
A-type PA-trimer	27.82–76.94 mg/100 g dm
A-type PA-tetramer	41.51–65.61 mg/100 g dm
B-type PA-dimer	12.62–36.75 mg/100 g dm
B-type PA-trimer	0.04–2.93 mg/100 g fw
Polymeric proanthocyanidins	651–1109 mg/100 g dm
Sinapyl hexose	2.0–3.3 mg/100 g dm
Total flavan-3-ols and proanthocyanidins	860–1283 mg/100 g dm
Sum Phenolic compounds	3428–3936.4 mg/100 g dm
Total polyphenols	192.1–3742 mg/100 g fm
Triterpenoids	
Ursolic acid	1044–1714 mg/kg dm

Name	Polyphenol Content and Triterpenoids
Oleanolic acid	894–1137 mg/100 g dm
Betulinic acid	635–824 mg/kg dm;
Sum Triterpenoids	2892–3671 mg/kg dm
Total Sterols (β-sitosterol and stigmasterol)	107.83 mg/g fw

Total phenolic acids ranged from the lowest concentration of 327 mg/100 g dry matter (dm). Cranberries accrued the highest amount of total phenols at the beginning of the ripening process. The average concentration of phenolic acids in the three cranberry cultivars during the ripening process ranged from 236.8 mg/100 g dm to 351.5 mg/100 g dm. The content of phenolic acids [8-15].

1.4.2. Anthocyanins

Anthocyanins are natural water-soluble pigments that give cranberries their reddish color. Anthocyanins in the small berry were found to be 6 to 10 times higher in the external layer of the berry skin than in the pulp.

The content of total anthocyanins ranged from 695 to 1716 mg/100 g dm for the six cultivars of *Vaccinium macrocarpon* L. Of one hundred and thirty-six wild cranberry fruits, *Vacciniun macrocarpon* Aiton, and two cultivars, observed the total anthocyanins content for all cranberry genotypes to differ significantly. These are cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoise, peonidin-3-galactoside, peonidin-3-glucoside and peonidin-3-arabinoside.

Of the three cranberry fruits (*Vaccinium macrocarpon* L.), consisted of the highest average level of anthocyanins (690.4 mg/100 g dm) when analyzed at the four different maturity stages of ripening and was 50.6% and 6.0%. The major anthocyanins identified in the cranberry cultivars in the ripening study were cyanidin-3-O-

galactoside (32.6–45.0% of total anthocyanins) and peonidin-3-O-galactoside (22.7–32.2%) [17,20].

1.4.3. Flavonoids

Flavonols are abundant in cranberries. Flavonol content ranged from 643 to 1088 mg/100 g dm. These were quercetin 3-O-rhamnoside, myricetin 3-O-arabinoside, quercetin 3-O-galactoside, and myricetin 3-O-galactoside. The flavonol glycosides identified in *Vaccinium vitisidaea* cranberry were myricetin-galactoside, quercetin-rutinoside (rutin), myricetin-arabinoside, myricetin-glucoside, quercetin-galactoside, quercetin-acetyl-glucoside, quercetin-glucoside and quercetin-rhamnoside. Flavonols increased in the cranberry cultivars from the immature to the commercially mature stage by 25%, 9% and 1%. The major flavonols noticed in these cranberry fruit cultivars were quercetin-3-O-galactoside (31.3–38.4% of total flavonols), myricetin-3-O-galactoside (20.4–29.0%) and quercetin-3-O-pentoside (10.1 to 11.8%). Flavonoids are important in plant defense and are strong antioxidants. They also exhibit antibacterial, antiviral, anticarcinogenic, and anti-inflammatory activities. Flavonoids identified in six cultivars of cranberries ranged from the lowest amount of 860 mg/100 g to the highest amount of 1283 mg/100 g dm [9,22].

Jungfer et al. noticed that proanthocyanins A-type trimers varied in the three species of cranberries, the large American cranberry, *Vaccinium macrocarpon* Ait., the small European cranberry, *Vaccinium oxycoccus*, and the lingonberry, *Vaccinium vitis-idaea* L. Only two A-type trimers were detected in *Vaccinium oxycoccus. Vaccinium vitis-idaea* showed the greatest variation with a pattern similar to that of *Vaccinium macrocarpon*. B-type trimers were found in all berries. *Vaccinium macrocarpon* and *Vaccinium oxycoccus* exhibited higher amounts of A-type than B-type trimers and dimers. The amount of procyanidin A2 in the *Vaccinium macrocarpon* varieties ranged from 4.10 to 5.49 mg/100 g of fresh berries. For *Vaccinium vitis-idaea*, the concentration of procyanidin A2 was 2.11 mg/100 g fw for the European berries and 7.98 mg/100 g fw for the Chinese berries. The lowest

concentration of procyanidin A2 was observed in the *Vaccinium oxycoccus* berries at a range of 0.13–0.21 mg/100 g fw. *Vaccinium vitis-idaea* had the highest amount of A-type dimer, followed by *Vaccinium macrocarpon* and the least by *Vaccinium oxycoccus*.

Cranberry is distinct from most foods and other berry fruits in that it is rich in the A-type proanthocyanidins, which inhibit the in vitro adhesion of *Esherichia coli* bacteria to uroepithelial cells to prevent urinary tract infections.

The flavan-3-ols, catechin and epicatechin were observed in all berry samples. (−) Epicatechin is the major constituent of proanthocyanidins, while (+) catechin and (epi) gallocatechins exist in small amounts. The catechin to epicatechin ratios in the cranberry species were found to be different. *Vaccinium vitis-idaea* had the highest total content of 15.48 mg/100 g fw for the European variety and 17.68 mg/100 g fw for the Canadian variety, while the *Vaccinium macrocarpon* varieties had a range of 2.80–5.05 mg/100 g fw and the lowest concentration was in the *Vaccinium oxycoccus* varieties at a range of 0.55–1.94 mg/100 g fw. Borges et al. detected (−)-epicatechin and proanthocyanidin dimers in *Vaccinium oxycoccus* cranberries, but not monomers, which contributes to the antioxidant capacity of the berries. European cranberry contained more epicatechins and less A-type dimers than lingonberry.

All varieties of the *Vaccinium macrocarpon* had higher amounts of epicatechin (2.45–4.46 mg/100 g fw) than catechin (0.33–0.61 mg/100 g fw), while the ratio of epicatechin to catechin in *Vaccinium oxycoccus* (1:1) was lower.

The amount of total phenolic compounds for the five American cranberry cultivars *Vaccinium macrocarpon* ranged from 192.1 mg/100 g fm to 374.2 mg/100 g fm as compared to the European wild-grown cranberry *Vaccinium oxycoccus* at 288.5 mg/100 g. The highest anthocyanin content was found in 77.1 mg/100 g fm and the lowest content 52.1 mg/100 g fm. This was lower for the wild cranberry at 43.4 mg/100 g fm [17,21].

1.4.4. Triterpenoids

Cranberry fruits also contain triterpenoids. Two of the major triterpenoids, ursolic acid and its isomer, oleanic or oleanolic acid are found in the wax of the skin of the cranberry fruit and are mostly responsible for anti-inflammatory, antitumor and anticancer activities. Ursolic acid is present in *Vaccinium oxycoccus* and protects against oxidative damage and lipid oxidation and has strong anti-inflammatory effects.

Wu et al. identified ursolic acid, oleanolic acid, *β*-sitosterol, and stigmasterol in *Vaccinium macrocarpon* cranberry extracts. *β*-sitosterol and stigmasterol showed a total of 107.83 mg/g. The cranberry extract also contained ursolic acid and oleanic acid at concentrations of 372.97 mg/g and 79.16 mg/g, respectively. Oszmiański et al. detected betulinic acid, oleanic acid, and ursolic acid in the fruit. The concentrations of the acids differed between the cultivars and ranged from 37–50% for ursolic acid, followed by 28–35% for oleanolic acid, and 19–28% for betulinic acid.

The ursolic acid content in the cultivars ranged from 22.7–32.2% of total triterpenoids and the amount increased as the fruit ripened [20-22].

1.5. Pharmacological activity

Cranberry fruit is an important source of antioxidants, such as polyphenols (flavonoids, phenolic acids, anthocyanins, tannins), ascorbic acid, and triterpene compounds. They scavenge free radicals, unpaired electrons in their outer orbit and may remove reactive oxygen species that oxidize biological matter. Oxidative stress, extreme amounts of free oxygen radicals in the biological fluids in the human body can cause many diseases. Antioxidant compounds can prevent or reduce oxidative damage to cell structure. Antioxidant activity is influenced by cultivar, genotype, growing season, ripening, processing and storage of cranberry fruit. Their role is critical to preventing the development of chronic diseases such as cardiovascular diseases, aging, diabetes, inflammation, cancer, etc [12,24].

Borowska et al. compared the antioxidant properties of wild cranberry fruit (*Vaccinium* oxycoccus) and five American cranberry cultivars and observed a statistically significant difference between the wild cranberry fruit and the cultivars. The wild cranberry fruit possessed greater antioxidant activity. This study observed the highest scavenging capacity by DPPH radicals to be within the range of 33.87–68.83 μmol/g of fresh mass.

The total content of antioxidants in cranberry was found to be 270 mg/100 g when measured by an amperometric method.

Ascorbic acid is known for its high antioxidant activity since it neutralizes free radicals and other reactive oxygen species which cause tissue damage and diseases. In a study by Brown et al. of the three species of cranberry, *Vaccinium macrocarpon* Ait., *Vaccinium oxycoccos* L., and *Vaccinium vitisidaea*, antioxidant activity showed a negative correlation with the anthocyanin content and a positive correlation with Vitamin C. Borges et al. noticed that Vitamin C has the highest antioxidant capacity (AOC) of 22.6% and (−)-epitecatechin is the major phenolic compound detected at 1121 nmol/g and with peonidin-3-O-galactoside contributing only 14% of the overall AOC. Anthocyanins are the second major group with up to 725 nmol/g (39% of total AOC) of cranberries. A total of 456 nmol/g of flavonols were present (10% of the overall AOC) [11, 19, 39].

European colonists who arrived in North America immediately recognized cranberries' healing powers, such as poultice for wounds and cure for blood poisoning. Cranberries best-known benefits have been to treat urinary tract infections, which is due to proanthocyanidins (PACs). These tannins prevent *Escherichia coli (E. coli)* bacteria from attaching to cells in the urinary tract and causing infection. Today, more health benefits have been shown due to the phytochemicals, anthocyanins, PACs, and flavonols, found in cranberries. They reduce certain infections, promote a healthy heart, decrease inflammation associated with chronic disease and aging and support digestive health. Cranberries also contain phytochemicals that act as antioxidants,

which reduce oxidative damage to cells that can lead to cancer, heart disease, and other degenerative diseases [41].

In a study of the cranberry effects on human health, since 1984, several articles and reviews have been published. The most common issues on the cranberry effect on human health are investigated in reviews. Cranberries have an anti-bacterial effect and in various forms (juice, concentrated powders, capsules and tablets) have been traditionally used to treat cystitis and urinary tract infections (UTIs). A study noted that the consumption of cranberry juice reduced the number of UTIs by 39% in women. This is mainly due to the PACs content in cranberry, especially proanthocyanidin A. Several studies have confirmed the positive effect of cranberry on urinary tract inflammation, not only for adults but also for children. It was found that PACs contained in cranberries prevent adherence of *E. coli* to uroepithelial cells in the urinary tract [44].

In clinical studies, products containing cranberry were found to prevent recurrent UTIs in young women and middle-aged women. Cranberry juice prevented recurrence of UTIs in children with a 65% reduction shown in the study by Afshar et al. and 43% in the Salo et al. study. On the other hand, cranberry products were found not to significantly reduce recurrence of UTI. Among otherwise healthy college women with an acute UTI, those drinking 8 oz of 27% cranberry juice twice daily did not experience a decrease in the 6-month incidence of a second UTI. Although several studies have shown consuming cranberries had a protective effect against UTI, other studies have not seen positive effects [25-28, 32-35].

Men receiving radiation for prostate cancer usually have a side effect of acute radiation cystitis, inflammation of the bladder. There is no effective treatment for preventing or treating radiation cystitis. In a pilot study, Hamilton et al. determined that the incidence of cystitis was lower in men (65%) when taking cranberry capsules (containing 72 mg PACs) compared with those that took placebo capsules (90%). This study concluded that it may be beneficial for men receiving radiation therapy for prostate cancer to take cranberry capsules instead of antibiotics or ant-inflammatory drugs.

Antibiotics are also used to treat women with chronic cystitis. However, there has been adverse effects and increased risk of resistance with antibiotics. Studies show that cranberry can be used as an alternative. Proanthocyanidins in the cranberries can remove *E. coli* adhesion to the urothelium. The occurrence of acute cystitis decreased when treated by cranberries. A study found that cranberry, D-mannose, a gelling complex, and the two microorganisms *Lactobacillus plantarum* and *Lactobacillus paracasei* significantly improved the uncomfortable symptoms associated with acute cystitis in women. It was suggested that an alternative to antibiotics in the treatment of cystitis and recurrent UTIs are cranberry products. However, there is no conclusive evidence that antibiotics can be replaced completely with cranberry.

Cranberry has a strong anti-oxidant property due to the presence of its rich polyphenol content such as flavonoids, proanthocyanidin dimers and oligomers, which prevents oxidative stress, the precursor to many chronic diseases. Consuming cranberry juice increases the plasma antioxidant capacity while significantly reducing lipid oxidation in women with health problems.

Cranberry helps treat cardiovascular diseases, improving the lipid profile, minimizing the likelihood of atherosclerosis by decreasing low density lipoproteins, reducing blood pressure and increasing high density lipoproteins (good) and preventing metabolic syndrome. Cranberry consumption lowers the risk of type 2 diabetes.

Cranberry is effective against all inflammatory processes, and now it is known that even cardiovascular and oncology diseases lead up to inflammatory responses. Cranberry can be used to prevent stomach ulcers by suppressing the activity of the *Helicobacter pylori* bacterium in the human stomach. It is known that this bacterium can lead to gastritis, ulcer and stomach cancer. Cranberry is active against cancer. Some phytochemicals in cranberry fruit affect cancer-related processes. PACs and flavonoids in cranberries may limit processes involved in tumor invasion and metastasis. *Vaccinium oxycoccos* fruits can suppress the spread of breast cancer cells, which may bring about apoptosis and GI phase arrest. Cranberry extracts inhibit the growth of breast, bladder, prostrate, lung and other tumors [23,24. 38].

Cranberry consumption helps against rheumatoid arthritis with women. Cranberry and its products inhibit the development of tooth decay and periodontal diseases. Other benefits of cranberry include prevention of the following diseases: obesity, infectious diseases, and kidney disease. Cranberry also improves gut microbiota.

Cranberries have been shown to have a high antiviral effect with a positive effect shown between anti-influenza viral activity and total polyphenol content, which indicates high amounts of polyphenols are an important factor in the antiviral effect of berries.

Cranberry also exhibited microbial activity. It slowed the growth of human pathogenic bacteria such as *E. coli*, Salmonella typhimurium, Enteroccocus faecalis, Listeria monocytogenes, Staphylococcus and Bacillus subtilis [44].

Table 1.3

Summarizes all the diseases that cranberry consumption can prevent

Disease Name	**Proposed Mechanism**
Urinary tract inflammation	A-type procyanidins in cranberry demonstrate anti-adhesive activity against *E. coli* to the uroepithelial cells preventing progression of UTIs
Cystitis	A-type procyanidins in cranberry prevent adhesion of *E. coli* to the bladder epithelial cells preventing progression of UTIs
Oxidative stress	Polyphenols in cranberry alleviate intestinal oxidative stress and inflammation while improving mitochondrial dysfunction by quenching reactive oxygen species.

Disease Name	Proposed Mechanism
Cardiovascular	Polyphenols in cranberry may reduce the risk of cardiovascular disease by increasing the LDL resistance to oxidation, hindering platelet accumulation, decreasing blood pressure.
Obesity	Lyophilized cranberries reduced fat accumulation during preadipocyte differentiation by decreasing the number of receptors on the surface of target cells of the mRNA level of adipocyte fatty acid-binding protein (aP2), lipoprotein lipase (LPL), fatty acid synthase (FAS), hormone sensitive lipase (HSL) and perilipin 1 (PLIN1). Therefore, cranberries are effective in preventing the production of adipose tissue.
Type 2 diabetes	Cranberries improved post-prandial glucose concentration due to high fat and inflammation and oxidation in diabetic individuals.
Helicobacter pylori suppression	Non-dialyzable substances from cranberry obstruct the sialic acid-specific adhesion of *H. pylori* to human gastric mucus and to erythrocytes.
Cancers	*Prostate*-Cranberry PACs reduced matrix metalloproteinases (MMP) activity in prostate cancer cells via stimulating and hindering specific MMP regulators, and by disrupting either the phosphorylation status and/or expression of MAP kinase, PI-3 kinase, and NF-κB and AP-1 pathway proteins. *Bladder*-Isorhamnetin and quercetin 3-O-glucoside, the active forms of quercetin may be responsible in prevention of bladder cancer in vivo and diets high in cranberries for the prevention of bladder carcinoma. *Breast*-Cranberry phytochemical may potentially suppress the spread of human breast cancer MCF-7 cells, which is partly due to both the beginning of apoptosis and the G1 phase arrest.

Disease Name	Proposed Mechanism
	Lung-PACs in cranberry can modify gene expression, stimulate apoptosis and induce the cell cycle of human NCI-H460 lung cancer cells.
Rheumatoid arthritis	Quercetin, a flavonoid present in cranberry, is a powerful suppressor of the nuclear factor (NF)-κB-pathway. It also impedes the activities of cyclooxygenase and lipoxygenase, enzymes released after the stimulation of arachidonic acid, which is the initiator of an inflammatory response. Resveratrol, a polyphenol in cranberry, also has been shown to reduce inflammatory genes expression important for cardiovascular disease by regulating the NF-κB and JAK STAT3 pathways in cells.
Tooth decay and periodontitis	Polyphenols in cranberry serve as dental anticaries agents by impeding the production of organic acids and the formation of biofilms by cariogenic bacteria. Additionally, they may reduce inflammation as well as the production and activity of proteolytic enzymes destroying the extracellular matrix in periodontal disease. These polyphenols also interfere with other activities such as formation of biofilm and adhesion of *Porphyromonas gingivalis*, the main disease-causing agent in chronic periodontitis.
Infectious	PAC in cranberries block adhesion to and biofilm formation on target tissues of pathogens
Kidney	Cranberries enriched with PACs can alleviate the complications associated with chronic kidney disease such as oxidative stress, inflammation and gut dysbiosis

Disease Name	Proposed Mechanism
Intestinal microbiota	The rich cranberry content of polyphenols, phenolic acids, isoprenoids and oligosaccharides performing in the gastrointestinal tract may reduce reactive oxygen species, control pathways of inflammation, attach to carbohydrates and proteins on surfaces of bacteria, employ prebiotic effects, and change the transmission of signals between intestinal epithelial cells and the gut microbiota.
Flu virus	High molecular weight substances (NDM) in cranberry inhibited Influenza virus A subtypes (H1N1 and H3N2) and the B type, which was shown by the cytopathic effect on Madine-Darby canine kidney (MDCK) cells and the lack of hemagglutination of red blood cells activity in infected cells.
Microbial	Cranberry phenolic extracts impeded the growth of human pathogenic bacteria: *Escherichia coli*, *Salmonella typhimurium*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus subtilis* in different mechanisms.

CHAPTER 2

DETERMINATION OF INDEXES OF CRANBERRY FRUITS ACCORDING TO THE GENERAL RECOMMENDATION OF PHARMACOPEA

Samples of analysed species, *Cranberry* fresh fruits were bought in December 2021 in Kharkiv Ukraine.

2.1. Determination of loss on drying

Place 10 g of the prepared Cranberry fresh fruits in the test procedure for the plant material concerned, accurately weighed, in a previously dried and tared flat weighing bottle. Dry the sample by one of the following techniques:

- in an oven at 100-105°C;
- in a desiccator over phosphorus pentoxide R under atmospheric pressure or reduced pressure and at room temperature [1-4].

Dry until two consecutive weighing's do not differ by more than 5 mg, unless otherwise specified in the test procedure. Calculate the loss of weight in mg per g of air-dried material. Loss on drying of plant was calculated according to the formula:

$$\boldsymbol{X = \frac{(m - m1).100}{m}}$$

m - weight of the plant material before drying, g;

m_1- weight of the plant material after drying, g;

X- moisture of plant material, %.

It was determined that loss on drying for Cranberry fresh fruits was 85,03 %.

2.2. Determination of total ash

Place about 5 g of the ground material, accurately weighed, in a previously ignited and tared crucible (usually of platinum or silica). Spread the material in an even layer and ignite it by gradually increasing the heat to 500-600°C until it is white, indicating the absence of carbon. Cool in a desiccator and weigh. If carbon-free ash cannot be obtained in this manner, cool the crucible and moisten the residue with about 2 ml of water or a saturated solution of ammonium nitrate R. Dry on a water-bath, then on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes, then weigh without delay. Calculate the content of total ash in mg per g of air-dried material [1-4].

It was determined that content of total ash was 2,00%.

2.3. Determination acid-insoluble ash

To the crucible containing the total ash, add 25 ml of hydrochloric acid (~70g/l) TS, cover with a watch-glass and boil gently for 5 minutes. Rinse the watch-glass with 5 ml of hot water and add this liquid to the crucible. Collect the insoluble matter on an ashless filter-paper and wash with hot water until the filtrate is neutral. Transfer the filter-paper containing the insoluble matter to the original crucible, dry on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes, then weigh without delay. Calculate the content of acid-insoluble ash in mg per g of air-dried material [1-4].

It was determined that content of acid-insoluble ash was 0,5 %.

2.4. Determination of extractable matter

Place about 5.0g of coarsely material, accurately weighed, in a glass-stoppered conical flask. Add 100ml of water and weigh to obtain the total weight including the flask. Shake well and allow to stand for 1 hour. Attach a reflux condenser to the flask and boil gently for 1 hour; cool and weigh. Readjust to the original total weight with the solvent specified in the test procedure for the plant material concerned. Shake well and filter rapidly through a dry filter. Transfer 25 ml of the filtrate to a tared flat-bottomed dish and evaporate to dryness on a water-bath. Dry at 105°C for 6 hours, cool in a desiccator for 30 minutes, then weigh without delay [1-4]. Calculate the content of extractable matter in mg per g of air-dried material. The content of extractable matter was calculated according to the formula:

$$X = \frac{m \cdot 200 \cdot 100}{m_1 \cdot (100-W)},$$

m – weight of dry residue, g;

m_1 – weight of the plant material, g;

W – moisture of plant material, %.

The results of determination of the content of extractable matter are presented in Table 2.1.

Table 2.1.

The results of determination of the content of extractable matter of Cranberry fresh fruits

Solvent	Content of extractable matter
Water	29,67%
40 % ethanol	21,34%
70 % ethanol	23,08%
96 % ethanol	20,57%

Water was the best solvent for extraction of extractable matter of Cranberry fresh fruits.

CONCLUSION

The content of loss on drying, total ash, acid-insoluble ash and extractable matter were calculated in mg per g of Cranberry fresh fruits.

CHAPTER 3

QUALITATIVE DETERMINATION OF BIOLOGICALLY ACTIVE COMPOUNDS CRANBERRY FRUITS

For the qualitative determination of some groups of biologically active compounds, water, 50%, 70%, 96% alcohol (ethanol) extracts of the were prepared.

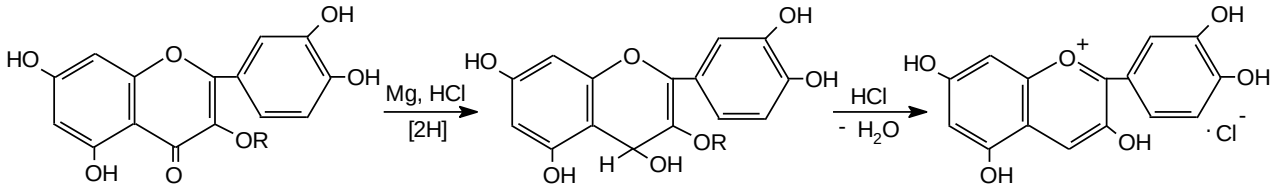
3.1. Determination of Flavonoids

Flavonoids are polyphenolic compounds. They are essentially used to treat capillary and venous disorders: due to its "venoactivity", in other words their ability to decrease capillary permeability and fragility.

For quantitative determination of flavonoids, 5 g of plant material of Cranberry fresh fruits was extracted by 50 ml of 70% alcohol on a hotplate for 20 min with a reflux condenser attached. Extracts were cooled and filtered through a paper filter. The filtrates were used for qualitative determination of flavonoids. As a reference solution 0.1% alcoholic rutin solution was used [1-4].

Briant's modification of cyanidin formation reaction. 1 ml of Cranberry fresh fruits extract, 5 drops of hydrochloric acid and powder of metallic magnesium was added to each test tube. In the test tube with Cranberry fresh fruits coloring appeared (pink color).

1/3 parts on volume of butanol was added to the colored product, produced from reaction of cyanidin formation in test tube, and diluted by water to get 2 layers. After shaking each test tube, colored pigments marked the separation of water phase and the organic phase.



Observation. 0.1% alcoholic rutin solution shows pink color in water layer. In test tube with Cranberry fresh fruits extract, with addition of butanol, colored brown organic later rested on the top, while the water layer, clear in color rested at the bottom. In Cranberry fresh fruits extract test tube appeared a dark red color organic layer on top and light water layer at the bottom.

Reaction with 10% sodium hydroxide solution. 1 ml of obtained Cranberry fresh fruits extract was placed in test tubes, 2 drops of 10 % alcohol solution of sodium hydroxide was added to test tube.

Observation. 0.1% alcoholic rutin solution shows yellow color. In test tube with Cranberry fresh fruits extract yellow color appeared.

Reaction with 2% aluminium chloride. 1 ml of each obtained extract was placed in test tubes, 2 drops of 2 % alcohol solution of aluminium chloride was added to each test tube.

Observation. 0.1% alcoholic rutin solution shows yellow color. In test tube with Cranberry fresh fruits extract, light green- yellow color appeared.

Reaction with iron (III) chloride solution. 1 ml of Cranberry fresh fruits extract, 2 drops of iron (III) chloride was added to each test tube.

Observation. 0.1% alcoholic rutin solution shows green color. In test tube with Cranberry fresh fruits extract green color appeared. A dark green color appeared in test tube with 2 ml of Cranberry fresh fruits extract.

Reaction with vanillin and hydrochloric acid concentrated. 1 ml of Cranberry fresh fruits extract was placed in different test tubes, a few drops of 1 % solution of vanillin and hydrochloric acid concentrated was added to each test tube.

Observation. In test tube with extract of Cranberry fresh fruits light to dark green color appeared.

Conclusion. Flavanoids are present in Cranberry fresh fruits.

3.2. Determination of tannins

Tannins are amophorous, water-soluble, polyphenols (proanthocyanidins, galloyl polyesters) that have tanning properties and astringent taste, can precipitate alkaloids, gelatin, and other proteins from diluted solutions.

Determination of tannins in Cranberry fresh fruits

10 g of plant material was extracted by 50 ml water on a hotplate for 20 min with a reflux condenser attached. Extract was cooled and filtered through a paper filter. The filtrates were used for qualitative determination of tannins.

Reaction with gelatin. 2 ml of obtained extract was placed in test tube, 1 % gelatin solution by drops and 1 drop of 10% solution of HCl (to increase detection sensitivity) was added to the test tube.

Observation. Color of extract in test tube became darker (dark green)

Reaction with alkaloids. 2 ml of obtained extracts were placed in test tubes, 1 % of quinine chloride solution was added to the test tube by drops.

Observation. Derivation of light brown flocculent precipitate was observed for Cranberry fresh fruits extract.

Reaction with iron alum solution. 2 ml of obtained extract was placed in test tubes, 4 drops of iron alum solution was added to the test tube.

Observation. Derivation of dark green color precipitate was observed for Cranberry fresh fruits extract.

Reaction with crystals of sodium nitrite. 2 ml of obtained extract was placed in a test tube. Crystals of sodium nitrite and 2 drops of 10% hydrochloric acid were added to the test tube. This reaction is to distinguish hydrolysable tannin.

Observation. Derivation of red-orange colored appeared in test tube with obtained extract.

Actetic acid and neutral salt of lead acetate. 1 ml of obtained extract placed in test tube, 2 ml of 10% acetic acid and 1 ml of 10% neutral salt of lead acetate was added to the test tube. To filtrate, 5 drops of 1% aluminum solution and 0.1g of crystalline lead acetate was added. Reaction is to determine presence of both tannin groups.

Observation. With 10% acetic acid and neutral salt, extract became clear in color. Precipitate formed with addition of iron aluminum and crystalline lead acetate.

Conclusion. The presence of tannin is evident in Cranberry fresh fruits. Cranberry fresh fruits contain condensed tannin.

3.3. Determination of Saponins

10 g each of plant material (Cranberry fresh fruits) was extracted by 50 ml 50% ethanol on a hotplate for 15 min with a reflux condenser attached. Extracts were cooled and filtered through a paper filter. 25 ml of filtrate from Cranberry fresh fruits extract was concentrated to 12.5 ml and used for the froth test, some precipitation tests and determination of chemical nature of saponins. 25 ml of filtrate from Cranberry fresh fruits extact was concentrated to 12.5 ml, to be used for froth test, precipitation test,

and determination of chemical nature of saponins. Alcohol extracts of Cranberry fresh fruits was used for other qualitative tests [46].

Froth test. 2 ml of each obtained extracts were placed in different test tubes, and shaken vigorously during 1 minute.

Observation. Water extract of Cranberry fresh fruits gives a layer of foam in aqueous solution after 15 min standing.

Precipitation tests

1. 2 ml of water extract of plant material was placed in different test tube, 3-4 drops of barium water was added to each test tube.

Observation. Derivation of light precipitate was observed for Cranberry fresh fruits plant material.

2. 2 ml of water extract of plant material was placed in test tubes, 3-4 drops of 10 % of lead acetate solution was added to each test tube.

Observation. Derivation of thick precipitate was observed for Cranberry fresh fruits extract.

Colour Reactions for Cranberry fresh fruits

Lafon's reaction. 2 ml of alcohol extract was placed in test tube, 1 drop of 10 % copper sulfate solution and 1 ml of sulphuric acid concentrated was added and heated carefully.

Observation. Derivation of dark red color was present in extract with Cranberry fresh fruits.

Salkovski's reaction. 2 ml of alcohol extracts was placed in test tube, 1 ml chloroform and 5-6 drops sulphuric acid concentrated was added.

Observation. Derivation of light caramel colored appeared in test tube with Cranberry fresh fruits. With addition of chloroform, layers appeared.

Sanje's reaction. 2 ml of alcohol extract was placed in a test tube. 1 ml of 0.5% alcoholic vanillin solution and 3-4 drops concentrated acid was added to the test tube. The test tube was heated in water bath under temperature condition of 60°C.

Observation. Derivation of yellow color appeared in test tube with Cranberry fresh fruits.

Determination of the chemical nature of saponins in Cranberry fresh fruits

2 graduated test tubes with plug stopper were used for Cranberry fresh fruits. In the first test tube, 5 ml of 0,1 mole/l hydrochloric acid solution was added and 5 ml of 0,1 mole/l sodium hydroxide solution- in the other one. 0,5 ml water extract was added to the both test tubes and shaken vigorously during 1 minute.

Observation: Water extracts gave a layer of foam in aqueous solution after 15 min standing. The heights of foam were equal in both test tubes. It signified presence of triterpenoidal saponins in Cranberry fresh fruits.

Conclusion. Based on froth test, saponins are present in Cranberry fresh fruits extract. The presence of triterpenoidal saponins are evident in Cranberry fresh fruits extract.

3.4. Chromatogram study of Cranberry fresh fruits

Extract obtained for qualitative tests of both plant materials on flavonoids was concentrated and used for chromatographic determination of flavonoids in Cranberry fresh fruits.

Extract was investigated by two-dimensional paper chromatography in solvent systems: n-butanol-acetic acid anhydrous-water (4:1:2), 15% acetic acid.

Chromatogram was viewed in daylight and in ultraviolet light before and after the treating by ammonia solution.

In ultraviolet light spots of brown, yellow, light blue, red, purple were observed, which after treating by reagent acquired a bright yellow, bright blue, light blue, yellow-brown fluorescence, indicating the presence of phenolic compounds. In total 12 spots was determined on chromatogram. Location of spots on the chromatogram of Cranberry fresh fruits is shown in Figure 2.1.

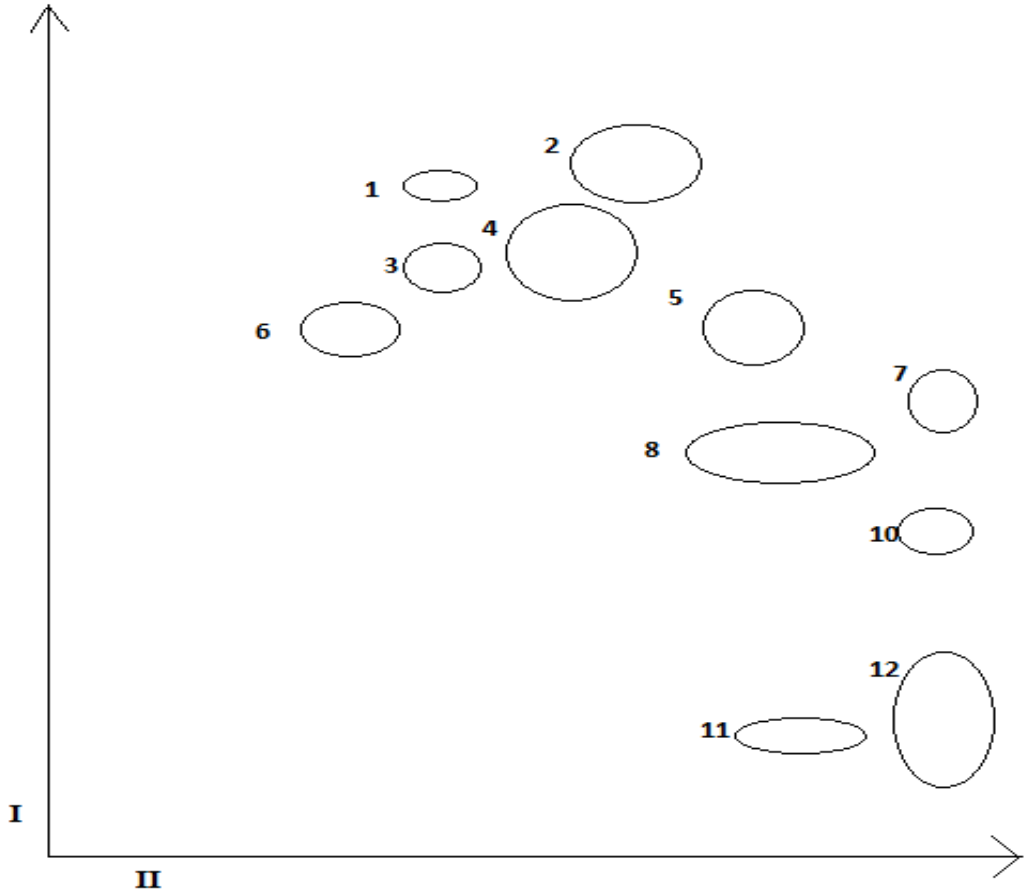


Figure 2.1. Scheme of Cranberry fresh fruits spots location on the two-dimensional paper chromatogram in solvent systems: n-butanol-acetic acid anhydrous-water (4:1:2) (I),15% acetic acid (II).

Conclusion. The presence of phenolic compounds, presumably cinnamic acid, flavonoids was determined in Cranberry fresh fruits.

3.5 Determination of polysaccharides

20.0 g each of accurately weighed crushed plant material (Cranberry fresh fruits) was placed into different glass conical flasks with capacity of 250 ml. 200 ml of water were added to flask. Flask was heated on a hotplate for 1 hour with attached reflux condenser. Water extractions were filtrated and concentrated to the 50 ml volume [4].

10 ml of the concentrated extract was placed into flask. 30 ml of 96% ethanol was added to the extract, mixed and left in a cool place for 12 hours.

Observation. Derivation of abundant light brown flocculent precipitate was observed in Cranberry fresh fruits.

Conclusion. Presence of polysaccharides was determined in Cranberry fresh fruits.

CONCLUSIONS

Qualitative determination of biologically active compounds in Cranberry fresh fruits was carried out.

The presence of flavonoids, tannins, saponins and polysaccharides were determined in Cranberry fresh fruits.

At the base of chromatographic analysis we can make conclusion that flavonoid glycosides and phenolic compounds are present in Cranberry fresh fruits.

CHAPTER 4.
QUANTITATIVE DETERMINATION OF BIOLOGICALLY ACTIVE COMPOUNDS IN CRANBERRY FRUITS

4.1. Quantitative determination of flavonoids

To develop a method for determining flavonoids in Cranberry fresh fruits we recorded spectrum of tinctures from plant materials from 295 to 455 nm.

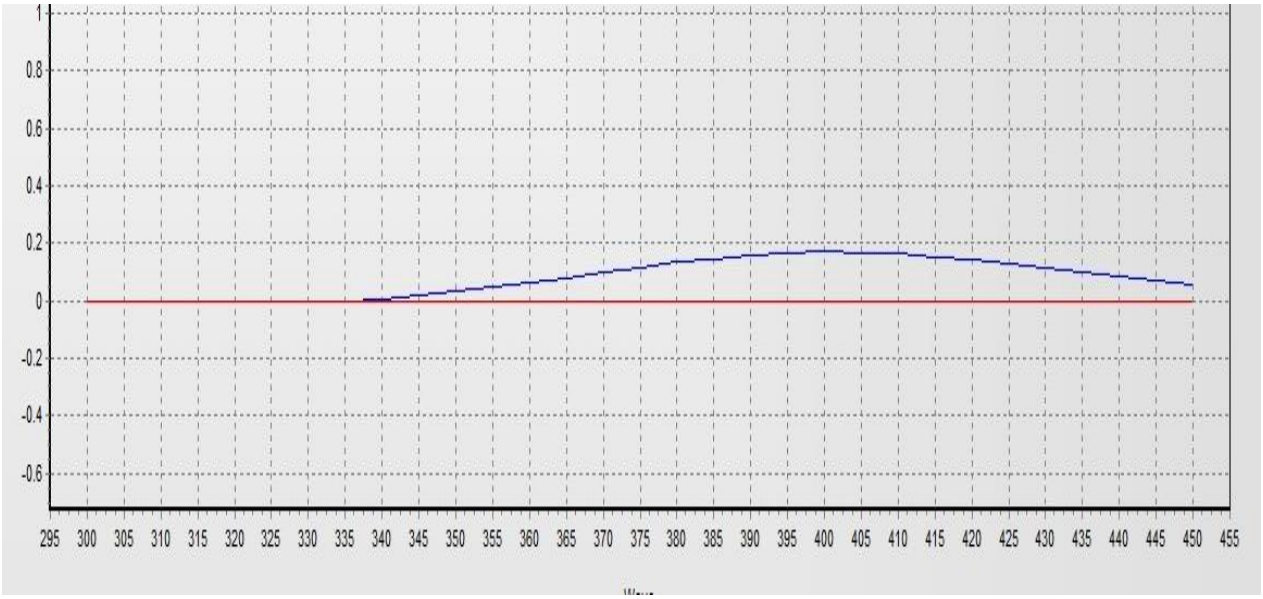


Figure 4.1. Scheme of Cranberry fresh fruits tincture relation absorbance and length of wave

Method. Plant material of *Cranberry* fresh fruits was crushed. About 5.0 g of material, (accurately weighed) was placed in a glass conical flask with capacity of 150 ml. 25 ml of 50% alcohol was added to each flask, with a reflux condenser was attached to the flask and boiled on a hotplate for 30 minutes. Hot solution was filtered rapidly in a volumetric flask with capacity of 100 ml through a paper filter. Extraction was repeated twice for each plant material; adding 25 ml of 50% alcohol and boiled for 20 min. Filtrates for each plant material were united. The volume of Cranberry fresh fruits

filtrate in a volumetric flask was leaded to the mark by 50% alcohol and mixed. The volume of Cranberry fresh fruits filtrate in a volumetric flask was leaded to the mark by 50% alcohol and mixed (Cranberry fresh fruits solution A).

2 ml of Cranberry fresh fruits solution A was placed into the volumetric flask with capacity of 10 ml, 1 ml of 2 % alcoholic solution of aluminium chloride was added and leaded to the mark by 70% alcohol and mix. In 40 minutes the absorbance of the obtained solution was measured on a spectrophotometer at a wave-length 415 nm in a cuvette with the thickness of layer 10 mm, solution of comparison consisted of 2 ml Cranberry fresh fruits solution A, 1 drop of acetic acid diluted, leaded to 10 ml in the volumetric flask with capacity of 10 ml.

The absorbance of rutin standard was determined simultaneously.

Content of flavonoids in Cranberry fresh fruits in terms on rutin and absolute-dried material in percents (X) was calculated by a formula:

$$X = \frac{A \cdot m_0 \cdot 100 \cdot 100 \cdot 100}{A_0 \cdot m \cdot 100 \cdot (100 - W)}$$

A is the absorbance of the tested solution;

A_0 is the absorbance of rutin solution;

m_0 is mass of rutin, g;

m - mass of plant material, g.

W - a loss in weight at drying of plant material, %.

It was determined that content of flavonoids in Cranberry fresh fruits was – 0,77±1,83 %.

Table 4.1.

The results of the statistical analysis of the quantitative determination of flavonoids in Cranberry fresh fruits

m	n	X_i	X_{mean}	S^2	S_{mean}	P	t(P, n)	Confidence interval	ε, %
5	4	0,78 0,76 0,77 0,77 0,79	0,77	0,000130	0,00050	0,95	2,78	0,77±0,014175	1,83

4.2. Quantitative determination of hydroxycinnamic acid in calculation on chlorogenic acid

Determination of hydroxycinnamic acid in Cranberry fresh fruits was performed by spectrophotometry.

About 2,0 g (accurate weight) Cranberry fresh fruits was placed in 100 ml flask and added 70 ml of 20% alcohol. The flask was joined to reflux and heated in a water bath for 1 hour. Extraction was carried out twice. Extract was heated for 35 min the second time. Extracts were cooled, filtered through a paper filter, quantitatively transferred to a volumetric flask 100 ml. The volume of filtrate in a volumetric flask was leaded to the mark by 20% alcohol and mixed (Cranberry fresh fruits).

In a volumetric flask of 50 ml were placed 1 ml of Cranberry fresh fruits solution A and the solution was added to the level by 20% ethanol. Absorbance of the solution

was measured on spectrophotometer OPTIZEN at a wavelength of 327 nm. Reference solution was 20% ethanol.

Content of hydroxycinnamic acid (X, %) in calculation on chlorogenic acid was calculated for Cranberry fresh fruits by a formula:

$$X = \frac{A \cdot 100 \cdot 50 \cdot 100}{A_{1\text{см}}^{1\%} \cdot m \cdot 1 \cdot (100 - W)},$$

A – absorbtion of the studied solution;

A_o – absorbtion of the PSS chlorogenic acid solution;

m_o – weight of the PSS of chlorogenic acid, g;

m – weight of the extract, g;

W – the loss in weight at drying, %.

The results of the quantitative determination of hydroxycinnamic acids in the Cranberry fresh fruits are listed in Table 4.2.

Table 4.2

The results of the statistical analysis the quantitative determination of hydroxycinnamic acids

m	ν	X_i	X_{mean}	S^2	S_{mean}	P	t(P, n)	Confidence interval	ε, %
5	4	1,09 1,08 1,09 1,11 1,08	1,09	0,00012	0,005085	0,95	2,78	1,09±0,014	1,29

It was determined that content of hydroxycinnamic acid in Cranberry fresh fruits was - 1,09±1,29 %.

4.3. Quantitative determination of polysaccharides in Cranberry fresh fruits

Carbohydrates are important group of biologically active compounds that make up the plant organisms. They are the main source of energy. It is known, that polysaccharide complexes derived from MPM have diverse pharmacological activity, namely, reparative, analgesic, anti-inflammatory, immunostimulant, expectorant, antibacterial action.

Method.

The plant material (Cranberry fresh fruits) was crushed. About 10.0 g (accurately weighed) of coarsely powdered air-dried material was placed in a glass conical flask with capacity of 250 ml, 200 ml of water was added, a reflux condenser was attached to the flask and flask boiled on a hotplate for 30 minutes; extraction was repeated twice, 200 ml of water was used for the first time, 100 ml – for the second time. Water extracts were united and centrifuged (speed of rotation of 5000 revo) during 10 minutes, decanted in a volumetric flask with capacity of 500 ml through 5 layers of gauze, inlaid in a glass crater with diameter 55 mm and preliminarily washed by water. A filter was washed with water and leaded to the mark by water (solution A).

25 ml of solution was placed in a flask with capacity of 200 ml, 75 ml of 96 % alcohol was added, mixed. Precipitate was filtered through a paper filter. The filter with the precipitate was dried firstly in air and then to constant weight at the temperature 100-105ºC.

$$X = \frac{(m_2 - m_1) \cdot 500 \cdot 100 \cdot 100}{m \cdot 25 \cdot (100 - W)},$$

m_1 is mass of the filter, g;

m_2 is the mass of the filter with the precipitate, g;

m - mass of plant material, g;

W - loss on drying plant material %.

It was determined that content of polysaccharides in Cranberry fresh fruits was – 7,13±2,17%.

Table 4.3

The results of the statistical analysis of the quantitative determination of polysaccharides

m	ν	X_i	X_{mean}	S^2	S_{mean}	P	t(P, n)	Confidence interval	ε, %
5	4	7,21 7,10 6,95 7,13 7,28	7,13	0,00034	0,015530	0,95	2,78	7,13±0,15	2,17

CONCLUSIONS

Quantitative determination of flavonoids, hydroxycinnamic acid and polysaccharides were carried out for Cranberry fresh fruits.

It was determined that content of flavonoids in Cranberry fresh fruits was 0,77±1,83 %. hydroxycinnamic acid – 1,09±1,29 % and polysaccharides – 7,13±2,17%.

GENERAL CONCLUSION

1. We reviewed chemical composition and uses of Cranberry fruits.
2. The content of loss on drying, total ash, acid-insoluble ash and extractable matter were calculated in mg per g of air-dried material.
3. Qualitative determination of biologically active compounds in Cranberry fruits was carried out.
4. The presence of flavonoids, tannins, saponins and polysaccharides were determined in Cranberry fruits.
5. At the base of chromatographic analysis we can make conclusion that flavonoid glycosides and phenolic compounds are present in Cranberry fruits.
6. Quantitative determination of flavonoids, hydroxycinnamic acid and polysaccharides were carried out for Black currant leaves.
7. It was determined that content of flavonoids in Cranberry fruits was 0,77±1,83 %. hydroxycinnamic acid – 1,09±1,29 % and polysaccharides – 7,13±2,17%.

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4. Державна Фармакопея України / Держ. п–во “Науково–експертний фармакопейний центр”. – 1–е вид., 2 допов. – Х. : Держ. п–во “Науково–експертний фармакопейний центр”, 2008. 620 с.
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capacity in women with metabolic syndrome. *Nutr. Res.* 2011;31:190–196. doi: 10.1016/j.nutres.2011.02.003.

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Ф А 2.2.1–32-366

National University of Pharmacy

Faculty for foreign citizens' education
Department of chemistry of natural compounds and nutriciology

Level of higher education master

Specialty 226 Pharmacy and industrial pharmacy
Educational program Pharmacy

APPROVED
The Head of Department
of chemistry of natural
compounds and nutritiology

_____
Prof. Viktoria KYSLYCHENKO
"28" _September_ 2022

ASSIGNMENT
FOR QUALIFICATION WORK
OF AN APPLICANT FOR HIGHER EDUCATION

Eneje Christian Tochukwu

1. Topic of qualification work: «Phytochemical study of Cranberry fruits», supervisor of qualification work: Viktoriia KUZNIETSOVA, DPharmSc, prof.

approved by order of NUPh from "06" of February 2022 № 35

2. Deadline for submission of qualification work by the applicant for higher education: October 2022.

3. Outgoing data for qualification work: Phytochemical study of Cranberry fruits

4. Contents of the settlement and explanatory note (list of questions that need to be developed):to review and systematize literature data about classification, distribution, biosynthesis and uses of Cranberry;
to carry out qualitative analysis of Cranberry fruits;
to carry out the quantitative biologicaly active compounds of Cranberry fruits

5. List of graphic material (with exact indication of the required drawings):
Tables –7 , pictures –2 _____

6. Consultants of chapters of qualification work

Chapters	Name, SURNAME, position of consultant	Signature, date	
		assignment was issued	assignment was received
1	Viktoriia KUZNIETSOVA, professor of higher education institution of department of chemistry of natural compounds and nutriciology	Viktoriia KUZNIETSOVA 03.10.2022	Eneje Christian TOCHUKWU 03.10.2022
2	Viktoriia KUZNIETSOVA, professor of higher education institution of department of chemistry of natural compounds and nutriciology	Viktoriia KUZNIETSOVA 07.11.2022	Eneje Christian TOCHUKWU 07.11.2022
3	Viktoriia KUZNIETSOVA, professor of higher education institution of department of chemistry of natural compounds and nutriciology	Viktoriia KUZNIETSOVA 05.12.2022	Eneje Christian TOCHUKWU 05.12.2022
4	Viktoriia KUZNIETSOVA, professor of higher education institution of department of chemistry of natural compounds and nutriciology	Viktoriia KUZNIETSOVA 23.01.2023	Eneje Christian TOCHUKWU 23.01.2023

7. Date of issue of the assignment: “28” _September_ 2022

CALENDAR PLAN

№ з/п	Name of stages of qualification work	Deadline for the stages of qualification work	Notes
1	Literature review	October 2022	**done**
2	Determination of indexes of Cranberry fruits	November 2022	**done**
3	Quolitative determination of biologicaly active compound of Cranberry fruits	December 2022	**done**
4	Quantitative determination of of biologicaly active compound of Cranberry fruits	January 2023	**done**

An applicant of higher education _____ Eneje Christian TOCHUKWU

Supervisor of qualification work _____ Viktoriia KUZNIETSOVA

ВИТЯГ З НАКАЗУ № 35
По Національному фармацевтичному університету
від 06 лютого 2023 року

нижченаведеним студентам 5-го курсу 2022-2023 навчального року, навчання за освітнім ступенем «магістр», галузь знань 22 охорона здоров'я, спеціальності 226 – фармація, промислова фармація, освітня програма – фармація, денна форма здобуття освіти (термін навчання 4 роки 10 місяців та 3 роки 10 місяців), які навчаються за контрактом, затвердити теми кваліфікаційних робіт:

Прізвище студента	Тема кваліфікаційної роботи		Посада, прізвище та ініціали керівника	Рецензент кваліфікаційної роботи
• **по кафедрі хімії природних сполук**				
Енедже Крістіан Точукву	Фітохімічне вивчення плодів журавлини	Phytochemical study of Cranberry fruits	проф. Кузнєцова В.Ю.	доц. Кобзар Н.П.

Підстава: подання деканата, згода ректора

Ректор

Вірно. Секретар

Ф А2.8-03-110

ВИСНОВОК
Комісії з академічної доброчесності про проведену експертизу щодо академічного плагіату у кваліфікаційній роботі здобувача вищої освіти
№108273 від «14» листопада 2022 р.

Проаналізувавши випускну кваліфікаційну роботу за магістерським рівнем здобувача вищої освіти денної форми навчання Енедже Крістіани Точукву, 5 курсу, _____ групи, спеціальності 226 «Фармація» на тему: «Фітохімічне вивчення плодів журавлини/ Phytochemical study of Cranberry fruits», Комісія з академічної доброчесності дійшла висновку, що робота, представлена до Екзаменаційної комісії для захисту, виконана самостійно і не містить елементів академічного плагіату (компіляції).

Голова комісії,
професор **Інна ВЛАДИМИРОВА**

17%
31%

Ф А 2.2.1-32-353

REVIEW

of scientific supervisor for the qualification work of the level of higher education master of the specialty 226 Pharmacy, industrial pharmacy

Eneje Christian TOCHUKWU

on the topic: « Phytochemical study of Cranberry fruits»

Relevance of the topic. Cranberry is abundant in nutritional components and many bioactive compounds that have antioxidant properties. Both American and European cranberry species are rich in many classes of phytochemicals. These include phenolic acids, anthocyanins, flavones, flavonoids, and organic acids. Cranberry is one of the few fruits that is high in proanthocyanidins, which inhibit adherence of *Escherichia coli* to the urinary tract.

Practical value of conclusions, recommendations and their validity. The results of research of phytochemical study of Cranberry fruits can be used for the development of new phytomedicine.

Assessment of work. In the course of the work student has shown great responsibility, seriousness and commitment, the ability to work independently and to the scientific interpretation of the results. Eneje Christian Tochukwu proved to be a hard-working, persistent and inquisitive explorer.

General conclusion and recommendations on admission to defend. This qualification work has scientific and practical importance and can be recommended for official defense in the Examination Commission of the National University of Pharmacy.

Scientific supervisor _____ Viktoriia KUZNIETSOVA

«7th » of April 2023

Ф А 2.2.1-32-356

REVIEW

for qualification work of the level of higher education master, specialty

226 Pharmacy, industrial pharmacy

Eneje Christian TOCHUKWU

on the topic: «**Phytochemical study of of Cranberry fruits** »

Relevance of the topic. Cranberry is a plant with wide range of chemical constituents which exerted many pharmacological effects. There is a great promise for development of novel drugs from Cranberry to treat many human diseases as a result of its effectiveness and safety.

Theoretical level of work. The author reviewed the literature on the chemical composition, distribution and use of Cranberry fruits.

Author's suggestions on the research topic. The experimental part started from identification of biological active compounds of Cranberry fruits using chemical tests and paper chromatography. Then the author gives the results of the quantitative determination of the biological active compounds according to the requirements of the State Pharmacopoeia of Ukraine.

Practical value of conclusions, recommendations and their validity. Wissal ben addi in the qualification work dedicated to the qualitative and quantitative analysis of the Cranberry fruits according to general recommendation of Pharmacopoeia. Experimental part was performed on Pharmacopoeia methods of qualitative and quantitative determination of biological active compounds and indexes.

General conclusion and assessment of the work. The qualification work meets the criteria of the qualification work and can be recommended for the official defense in the Examination Commission of the National University of Pharmacy.

Reviewer _____ ass. prof. Natalia KOBZAR

«12th » of April 2023

Витяг
з протоколу засідання кафедри хімії природних сполук і нутриціології
Національного фармацевтичного університету
№ 4 від 18 квітня 2023 року

ПРИСУТНІ: Бурда Н.Є., Журавель І.О., Кисличенко В.С., Комісаренко А.М., Король В.В., Новосел О.М., Попик А.І., Попова Н.В., Процька В.В., Скребцова К.С., Тартинська Г.С., Хворост О.П.

Порядок денний:

1. Щодо допуску здобувачів вищої освіти до захисту кваліфікаційних робіт у Екзаменаційній комісії.

СЛУХАЛИ: про представлення до захисту в Екзаменаційній комісії кваліфікаційної роботи на «Фітохімічне вивчення плодів журавлини» здобувача вищої освіти випускного курсу Фм18(4,10д)анг-09 групи Енедже Крістіан ТОЧУКВУ.
Науковий керівник: професор Вікторія КУЗНЄЦОВА
Рецензент: доцент Наталія КОБЗАР

УХВАЛИЛИ: рекомендувати до захисту в Екзаменаційній комісії кваліфікаційну роботу здобувача вищої освіти Фм18(4,10д)англ-09 групи Енедже Крістіан ТОЧУКВУ на тему «Фітохімічне вивчення плодів журавлини».

Завідувачка кафедри хімії природних
сполук і нутриціології Вікторія КИСЛИЧЕНКО

Секретар кафедри ХПСіН Надія БУРДА

Ф А2.2.1-32-042

НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

ПОДАННЯ
ГОЛОВІ ЕКЗАМЕНАЦІЙНОЇ КОМІСІЇ
ЩОДО ЗАХИСТУ КВАЛІФІКАЦІЙНОЇ РОБОТИ

Направляється здобувач вищої освіти Енедже Крістіан ТОЧУКВУ до захисту кваліфікаційної роботи
за галуззю знань 22 Охорона здоров'я
спеціальністю 226 Фармація, промислова фармація
освітньою програмою Фармація
на тему: «Фітохімічне вивчення плодів журавлини».

Кваліфікаційна робота і рецензія додаються.

Декан факультету _____ / Світлана КАЛАЙЧЕВА /

Висновок керівника кваліфікаційної роботи

Здобувач вищої освіти Енедже Крістіан ТОЧУКВУ може бути допущений до захисту кваліфікаційної роботи в Екзаменаційній комісії.

Керівник кваліфікаційної роботи

_____ Вікторія КУЗНЄЦОВА

«7» квітня 2023

Висновок кафедри про кваліфікаційну роботу

Кваліфікаційну роботу розглянуто. Здобувач вищої освіти Енедже Крістіан ТОЧУКВУ допускається до захисту даної кваліфікаційної роботи в Екзаменаційній комісії.

Завідувач(ка) кафедри
Хімії природних сполук і нутриціології

_____ Вікторія КИСЛИЧЕНКО

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Qualification work was defended

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« ___ » _____ 2023

With the grade _____

Head of the Examination commission,

DPharmSc, Professor

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