Ф А 2.2.1-32-367

MINISTRY OF HEALTH OF UKRAINE

NATIONAL UNIVERSITY OF PHARMACY

Pharmaceutical faculty

Department of Pharmacognosy and Nutriciology

QUALIFICATION WORK

on the topic:

«PHYTOCHEMICAL STUDY OF HOLY THISTLE FLOWERS AND LEAVES (*SILYBUM MARIANUM* (L.) GAERTN)»

Prepared by: higher education graduate of group Фм20(4,10д) англ-03
specialty 226 Pharmacy, industrial pharmacy, educational and professional program Pharmacy
Nouhaila NOUAMANE

Supervisor: assistant professor of department of pharmacognosy and nutriciology, PhD, assistant professor
Andrey POPYK

Reviewer: head of the department of general chemistry, Dr. Pharm. Sc., professor
Sergii KOLISNYK

Kharkiv – 2025

ANNOTATION

The qualification work is devoted to the phytochemical study of flowers and leaves of the *Silybum marianum*.

The qualification work contains the results of an analytical review of literature data on the botanical characteristics, chemical composition and use of milk thistle raw materials. Phytochemical studies of the qualitative composition, quantitative content of biologically active substances and quality indicators for the leaves and flowers of milk thistle.

The qualification work consists of an introduction, a literature review, an experimental part, general conclusions, a list of references and appendices. The work is presented on 43 pages, includes 10 tables and 50 figures. The list of references contains 37 sources.

Key words: *Silybum marianum*, flowers, leaves, chemical composition.

АНОТАЦІЯ

Кваліфікаційна робота присвячена фітохімічному вивченню розторопші плямистої *(Silybum marianum* L. Gaertn).

Кваліфікаційна робота містить результати аналітичного огляду даних літератури щодо ботанічної характеристики, хімічного складу та застосування сировини розторопші плямистої. Фітохімічних досліджень якісного складу, кількісного вмісту біологічно активних речовин і показників якості для квіток та листя розторопші плямистої.

Кваліфікаційна робота складається зі вступу, огляду літератури, експериментальної частини, загальних висновків, списку використаної літератури та додатків. Робота викладена на 43 сторінках, включає 10 таблиць та 50 рисунків. Список використаної літератури містить 37 джерел.

Ключові слова: розторопша плямиста, квітки, листя, хімічний склад.

TABLE OF CONTENTS

LIST OF SYMBOLS

BAS – biologically active substance;

SPU – State Pharmacopoeia of Ukraine;

TLC – thin-layer chromatography;

PC – paper chromatography.

INTRODUCTION

Rationale. Medicinal products of plant origin occupy a leading position among the drugs widely used in evidence-based and traditional medicine. That is why the search for promising plant sources that can be used to obtain modern and effective herbal remedies is a priority task of pharmaceutical science.

One of the promising objects for phytochemical research is cultivated and wild plants that have a sufficient raw material base but have not been sufficiently studied. Milk thistle (*Silybum marianum* (L.) Gaertn) belongs to such plants. The plant is widespread in many countries of the world, including Ukraine.

According to the literature, milk thistle is used worldwide to treat diseases of the liver, gallbladder, skin, mucous membranes, dermatitis, and to remove toxic substances from the body.

However, only the seeds of milk thistle have been thoroughly studied, and the leaves and flowers of the plant remain insufficiently researched.

That is why the phytochemical analysis of milk thistle flowers and leaves is relevant.

Purpose. The aim of the study was to investigate the phytochemical study of leaves and flowers of milk thistle.

Tasks of the research. To achieve this goal, it was necessary to solve the following tasks:

- To analyze the literature on the botanical characteristics, chemical composition, and use of milk thistle in medicine;

- To study the qualitative and quantitative composition of biologically active substances in the flowers and leaves of milk thistle;

- Establish numerical parameters for the raw materials of milk thistle, namely, weight loss during drying and total ash.

The object of the research: phytochemical study of leaves and flowers of milk thistle.

The subject of the research: study of chemical composition and numerical indicators in raw materials of milk thistle.

Methods of the research.

The qualitative composition of the raw materials was studied using chemical reactions and chromatography. The quantitative content of biologically active substances was determined by titrimetric, spectrophotometric and gravimetric methods.

The results were statistically processed.

The practical significance and scientific novelty of the results.

The results of studies of the chemical composition, leaves and flowers of milk thistle contributed to an in-depth study of this plant material. This opens up new prospects for the further use of milk thistle as a valuable medicinal plant, which can be the basis for the development of new medicines.

Approbation of the research results. "Identification of biologically active compounds in raw materials *Silybum marianum* (L.) Gaertn.", one abstract was published at VII International Scientific and Practical Internet-Conference «Current approaches of pharmaceutical science in development and standardization of medicines and dietary supplements that contain components of natural origin», Kharkiv 2025 (Appendix).

The structure and scope of the qualification work – consists of an introduction, a literature review, an experimental part, general conclusions, a list of used literary sources, laid out on 43 pages, including 10 tables, 50 figures, 37 literature sources.

CHAPTER 1
BOTANICAL CHARACTERISTIC, GEOGRAPHICAL DISTRIBUTION, CHEMICAL COMPOSITION, APPLICATION IN MEDICINE AND CULINARY OF *SILYBUM MARIANUM* RAW MATERIALS (LITERATURE REVIEW)

1.1 Botanical characteristic

Milk thistle (*Silybum marianum*) is a diploid (n=34, x=17) plant of the Compositae (*Asteraceae*) family. in culture it is grown as an annual, the full cycle of its development, according to different researchers, takes place in one growing season, the duration of which is from 90 to 130 days, depending on the region.

The root system is tap root, the main mass of roots is in the soil layer 0-50 cm. The stem is straight or branched, 1-1.5 m high, cylindrical, furrowed, bare or slightly spider-webbed, covered with powdery plaque. Its active growth occurs in the phase of mass budding. Lower leaves are gathered in rosettes, obovate-lanceolate or spiny-toothed, upper leaves are pinnately lobed or pinnately dissected, green, shiny, with large white spots. Rosette leaves are petiolate, stem leaves are sessile [4,36,37].

Inflorescence is an elongated, more often globular, single basket 3-6 cm in diameter at the top of the stem or side shoots. The wrapper is shingled, consisting of prickles and green prickly leaflets. The peduncle is flat, fleshy.

Tubular flowers are sessile (Fig. 1.1.), purplish-purple in color, but can be pink and even white. on average, 80 to 100 flowers are formed in an inflorescence, with a lifespan of no more than two days. flowering of one inflorescence lasts an average of 26 days. flower unfolding in the inflorescence is centrifugal. their fragrance is honey-jasmine, quite pleasant [4,22,23].

Fruit (seeds in agronomic practice, Fig. 1.1.) is a seed with a tuft, elliptic or obovate, up to 8 mm long, 2-4 mm wide, 1-3 mm thick, slightly compressed from the sides, glabrous. Surface smooth, sometimes longitudinally wrinkled, shining. Coloration dark brown or black with brown elongate-longitudinal spots, sometimes

grayish. Crests – volatiles, which are formed on the seeds from the bract, contribute to the spread of the fruit by the wind. Sometimes at its strong breeze they spread to a distance of up to 10 m from the mother plant. The weight of 1000 seeds is 25-30 g. On average, 196.8 seeds are set in one inflorescence. It has been found that the weight of one seed from the moment of its setting to maturity increases from 5 to 26 mg [4,10,25,27].

Milk thistle blossoms and ripening of its fruits lasts for a long time – the first flowers appear in July and the last ones in late September. Seeds begin to ripen in July, and the last – in October. Milk thistle is perfectly renewable by seeds, giving a friendly self-seeding, which leads in many places to its feralization and ruderalization.

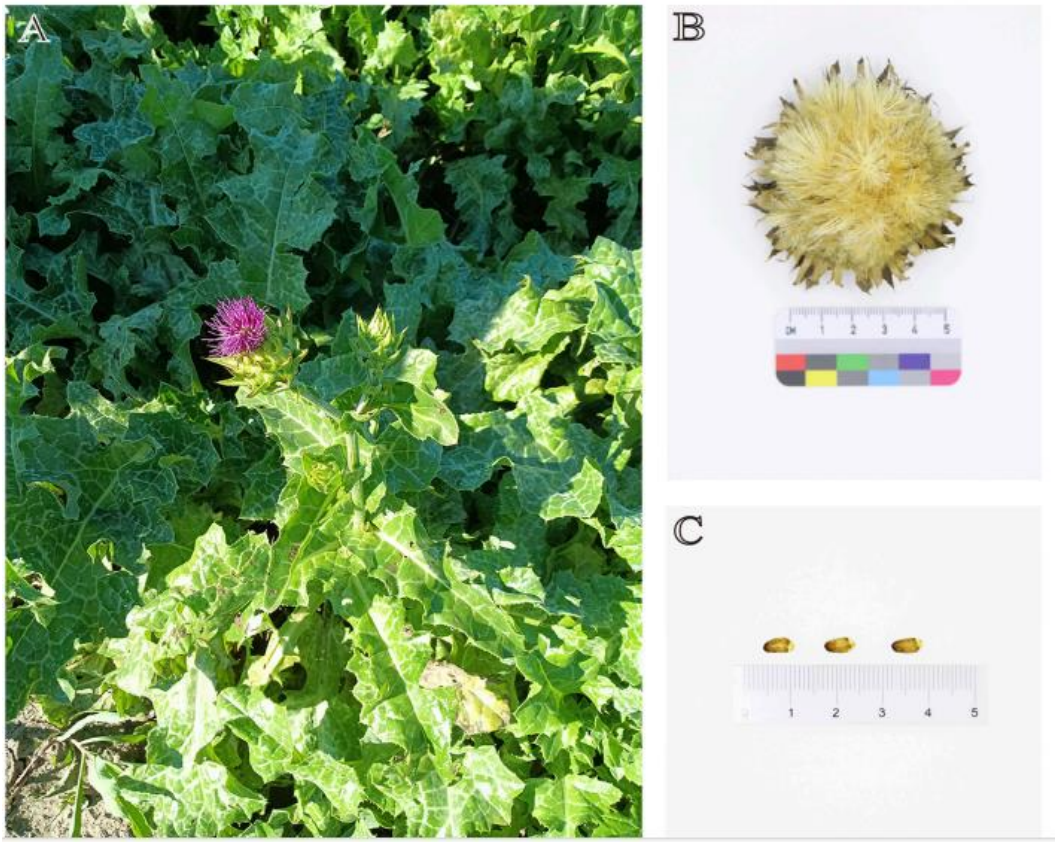

Fig. 1.1. Appearance of plant (A), flower (B), seeds (C)

1.2. Geographical distribution

Milk thistle is native to the Mediterranean [4]. In nature and culture, it is widely distributed in Europe, Asia Minor and Central Asia, North America, North Africa and Australia (Fig.1.2.). This plant is able to adapt to harsh environments such as cold, drought, salinity.

In Ukraine, milk thistle is most widespread in the southern regions – Kherson, Mykolayiv and Odessa, although in almost all other regions of the country its sown areas are registered.

In Europe, milk thistle is traditionally cultivated in Bulgaria, Hungary, Germany, Spain, Poland, Romania [4,20,30]. Its fruits (seeds) are the raw material of export-import operations and are bought by pharmaceutical companies for the manufacture of medicines and products of special food – dietary supplements.

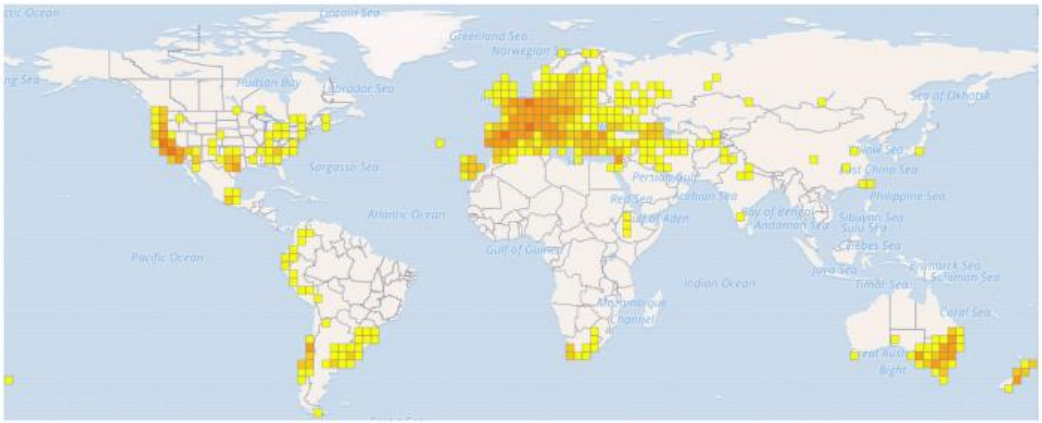

Fig.1.2. Geographical growth of *Silybum marianum*

1.3 Chemical composition

Milk thistle is a unique medicinal plant that contains various groups of BAS [4,10,15,18,22,23,24,25,26,27,28,31,34,35,36,37].

The chemical composition of the plant includes flavonolignans [4,9,10,11,14,15]: Silybin A, (Fig.1.3), Silybin B (Fig.1.4), Isosilybin A (Fig.1.5),

Isosilybin B (Fig.1.6), Silicristin A (Fig.1.7), Silicristin B (Fig.1.8), Isosilybin C (Fig.1.9), Isosilybin D (Fig.1.10), Silydianin (Fig.1.11), Silymonin (Fig.1.12), 2,3-cis-silybin B (Fig.1.13), (-) Isosilandrin A (Fig.1.14), (-) Isosilandrin B (Fig.1.15), Silandrin A (Fig.1.16), Silandrin (Fig.1.17). Milk thistle contains flavonoids [9,35]: (-)-Epicatechin (Fig.1.18), Catechin (Fig.1.19), Phlorizin (Fig.1.20), Procyanidin B_2 (Fig.1.21), Procyanidin C_1 (Fig.1.22), Kaempferol (Fig.1.23), the plant also contains fatty acids: Palmitic acid (Fig.1.25), Stearic acid (Fig.1.26), Linoleic acid (Fig.1.27).

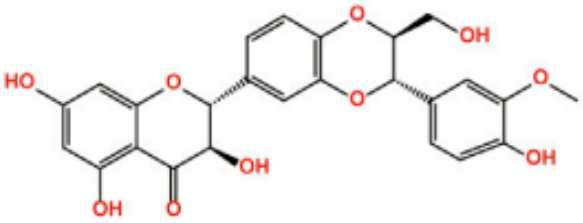

Fig.1.3. Silybin A

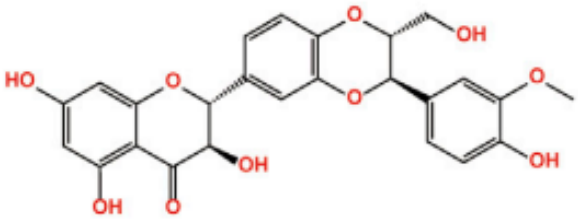

Fig.1.4. Silybin B

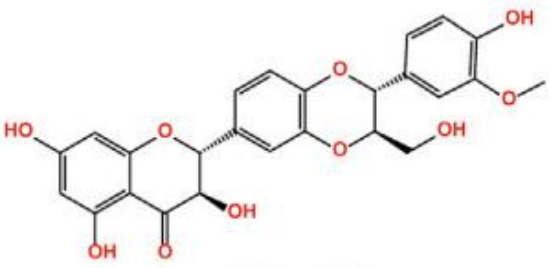

Fig.1.5. Isosilybin A

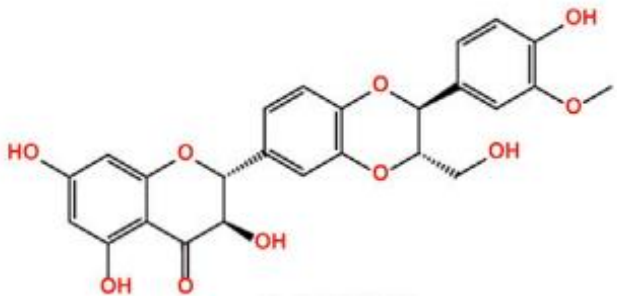

Fig.1.6. Isosilybin B

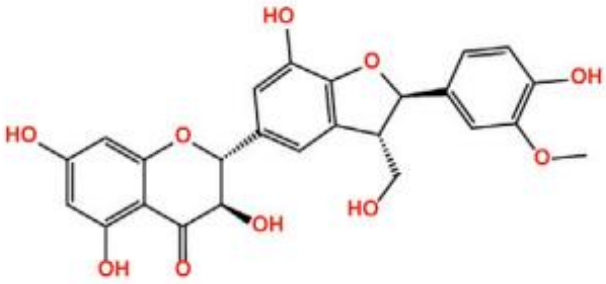

Fig.1.7. Silicristin A

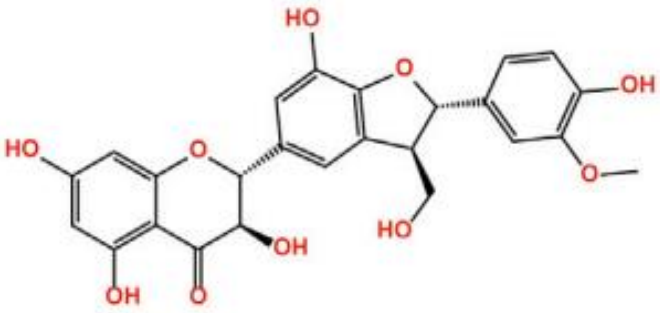

Fig.1.8. Silicristin B

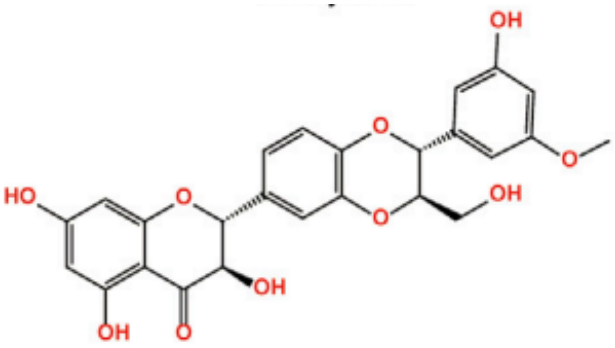

Fig.1.9. Isosilybin C

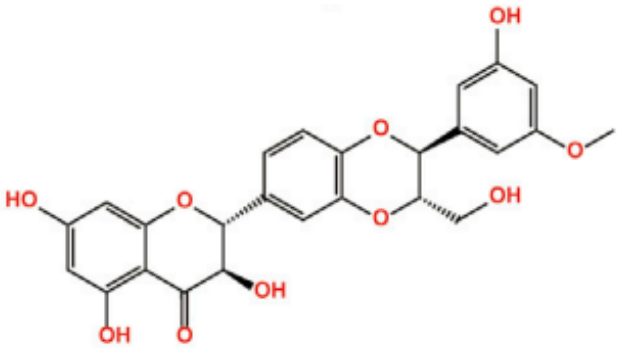

Fig.1.10. Isosilybin D

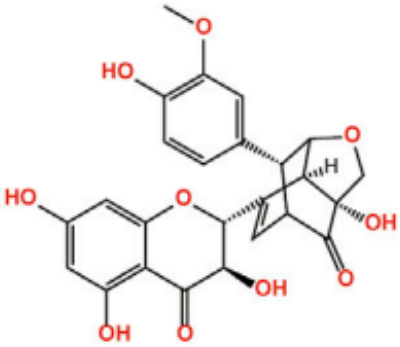

Fig.1.11. Silydianin

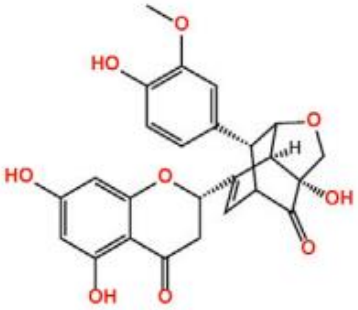

Fig.1.12. Silymonin

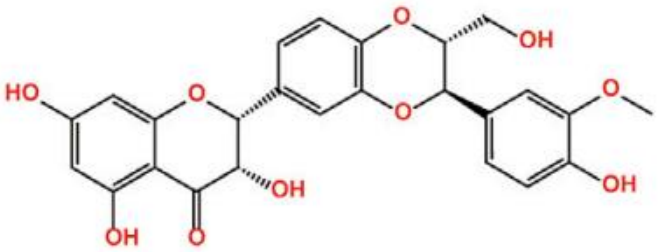

Fig.1.13. 2,3-cis-silybin B

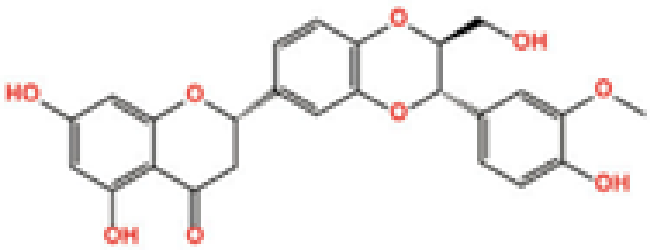

Fig.1.14. (-) Isosilandrin A

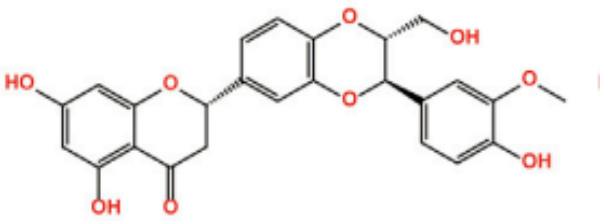

Fig.1.15. (-) Isosilandrin B

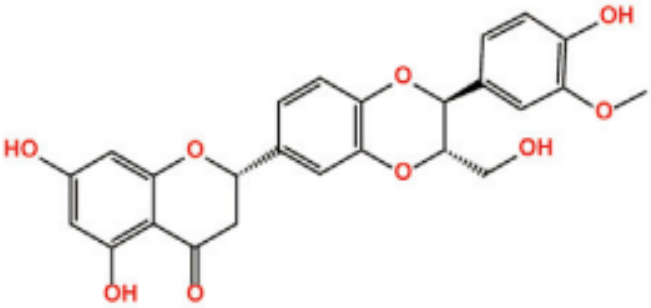

Fig.1.16. Silandrin A

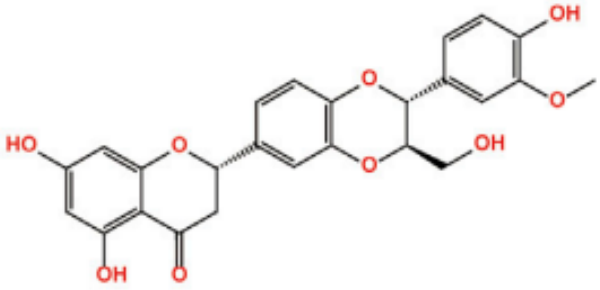

Fig.1.17. Silandrin

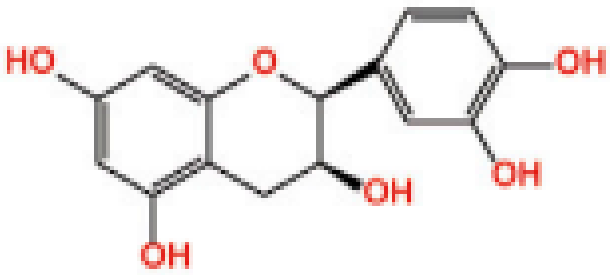

Fig.1.18. (-) Epicatechin

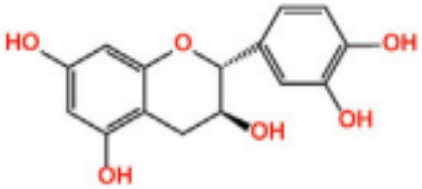

Fig.1.19. Catechin

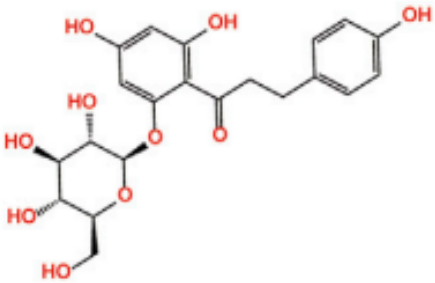

Fig.1.20. Phlorizin

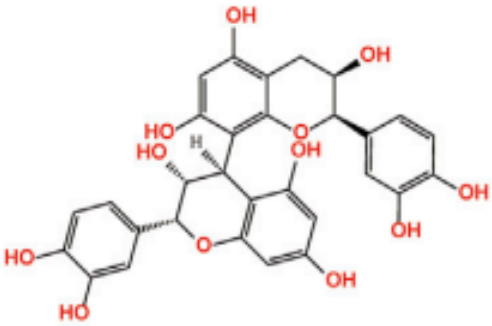

Fig.1.21. Procyanidin B_2

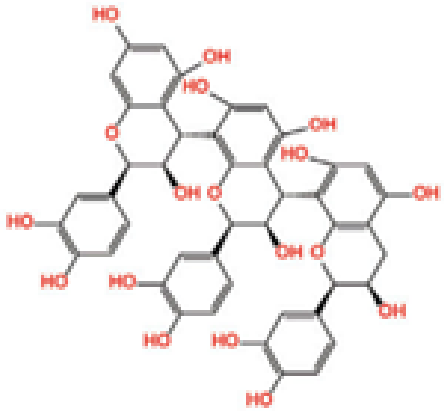

Fig.1.22. Procyanidin C_1

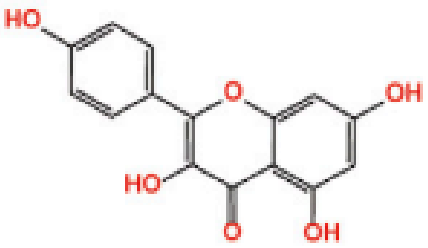

Fig.1.23. Kaempferol

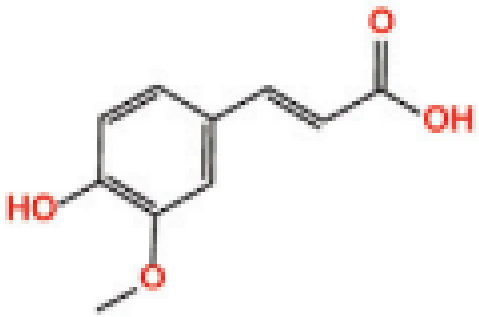

Fig.1.24. Ferulic acid

Fig.1.25. Palmitic acid

Fig.1.26. Stearic acid

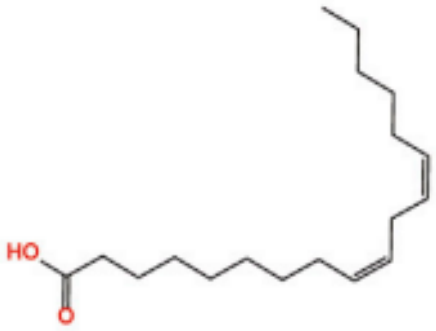

Fig.1.27. Linoleic acid

1.4 Application in medicine

This wild plant has been used as a medicinal remedy for thousands of years [4,12,13,16,17,19]. The ancient Greeks used a decoction of milk thistle fruit 2,000 years ago (It was first mentioned by Theophrastus). It is believed that the Romans were aware of its beneficial properties and used it to treat liver diseases. In India, milk thistle was used in homeopathic and folk medicine. In Europe, milk thistle extract has long been recommended for liver disorders such as hepatitis and cirrhosis [17,20,21,32,37]. Famous French physician Leclerc established the stabilizing effect of milk thistle fruit on the autonomic nervous system, and also re-recommended tincture of the fruit to improve appetite. German researchers have conducted experiments proving the protective effects of milk thistle in chronic chlorine poisoning. In addition, research in Düsseldorf has shown that milk thistle is highly effective in fatty liver dystrophy [4,20,21,36].

The seeds of milk thistle are the medicinal raw material. Milk thistle is a unique plant for the treatment of, first of all, the liver, stomach, intestines, has proven itself in diseases of the skin, cardiovascular pathology, ear, throat, nose. With the help of milk thistle can solve a number of gynecological problems. It is also used in the treatment of hemorrhoids. Milk thistle oil is widely used in cosmetology. For the recovery of the body, it can be constantly added to food in the form of oil or meal.

Such hepatoprotective preparations as Silibor, Darsil, Heparcil have been developed on the basis of Milk thistle. Also the plant is used in dietary supplements. It is used in the form of cold-pressed milk thistle seed oil, milk thistle seed powder meal. Its pharmacological effects in vivo and in vitro include anti-inflammatory, antioxidant, anti-tumor, hypoglycemic, neuroprotective and immunoregulatory properties [4,5,6,7,8,12,16,17,32,33]. Pharmacological activity of milk thistle preparations is shown in the figure 1.28.

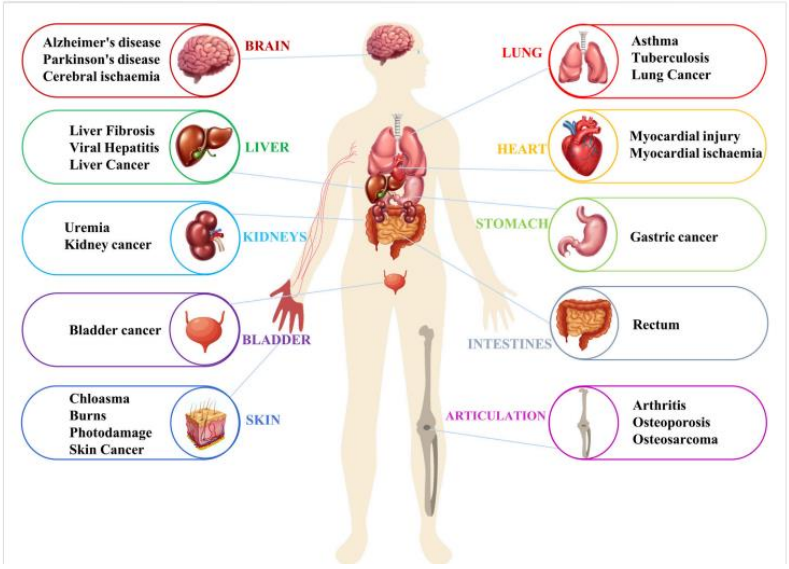

Fig.1.28. Pharmacological activity of milk thistle.

1.5 Application in culinary

Milk thistle is not only a medicinal plant it is very often used in culinary recipes of various countries. The young leaves of *Silybum marianum* are tender, juicy, crisp, and refreshed, which are excellent vegetables for consume. Achenes of *Silybum marianum* are protein-rich and can be used to produce protein powder, which is characterized by high protein content, low fat content, and low cholesterol levels. There are known culinary recipes in which the combination of *Silybum marianum* oil with baked goods can increase the nutritional value of foods by providing them with additional nutritional value.

Baked goods can increase the nutritional value of the products by providing them with additional vitamins, proteins and linoleic acid [4].

CONCLUSIONS

1. A detailed literature analysis on botanical characteristics, distribution, chemical composition, use in medicine and cooking of milk thistle has been carried out.

2. The conducted literature review confirms the pharmacological prospects of milk thistle and also points to the rich chemical composition of this medicinal plant and the possibility of developing new drugs based on it.

CHAPTER 2
STUDY OF THE CHEMICAL COMPOSITION OF *SILYBUM MARIANUM* RAW MATERIALS

2.1. Characterization of the research subject

Milk thistle flowers and leaves were chosen as the objects of study, and the raw materials were harvested during flowering in June 2024. After harvesting, milk thistle was dried at room temperature from 20 to 25 degrees, crushed, sieved through a sieve, and extracts were obtained for the study. The appearance of dried milk thistle and raw materials is shown in Figure 2.1–2.2.

Fig. 2.1. Milk thistle leaves prepared for analysis and leaves ground to powder phase

Fig. 2.2. Milk thistle flowers prepared for analysis and flowers crushed to powdered form

2.2. Determination of flavolignans

1.0 g of the crushed raw material was placed in a 100-ml conical flask with a rim, 50 mL of 95% ethanol was added, connected to a reflux condenser, and heated over a water heating plate for 30 min. The resulting extract was then settled for 5-10 minutes and filtered through a paper filter into a 200-mL volumetric flask. The extraction was carried out twice more and after cooling, the volume of the solution was made up to 95% alcohol (solution A).

2 ml of the extract (solution A) was taken from the volumetric flask, transferred to a 25 mL flask and the volume of the solution was made up to the mark with 95% ethyl alcohol (solution B). The optical density of solution B was measured after 30 min on a spectrophotometer at a wavelength of 289 nm. 95% ethyl alcohol was used as a reference solution. In parallel, the optical density of the alcohol solution of silybin GSO was measured at a wavelength of 289 nm.

The content of the sum of flavolignans in terms of silybin and dry raw materials in percent (X) was calculated by the formula:

$$X = \frac{A \times V \times M_o \times 100 \times 100}{A_o \times V_o \times m \times (100-w)},$$

where: A – is the optical density of the solution under study (solution B),

A_o– is the optical density of the GSO of silybin,

m – is the mass of raw materials, g,

M_o – is the mass of GSO silybin, g,

V – is the volume of the test solution, ml,

V_o – is the volume of the standard silybin solution, ml;

W – weight loss during drying, %.

The results of quantitative determination of flavolignans in milk thistle raw material are presented in Table 2.1. and Figure 2.3.

Table.2.1

Results of quantitative determination of flavolignans in flowers and leaves of milk thistle

m	n	X_i	X mean	S^2	S mean	P	T (P, n)	Confidence interval	ε_, %
1	2	3	4	5	6	7	8	9	10
flowers									
5	4	0,72 0,73 0,75 0,72 0,74	0,72	0,0018	0,00193	0,95	2,78	0,72±0,05	0,72
leaves									
5	4	1,42 1,49 1,58 1,60 1,64	1,49	0,0014	0,0140	0,95	2,78	1,49±0,04	4,52

The quantitative content of flavolignans in the leaves of milk thistle was – 1,49±0,04% and in the flowers was – 0,72±0,05%.

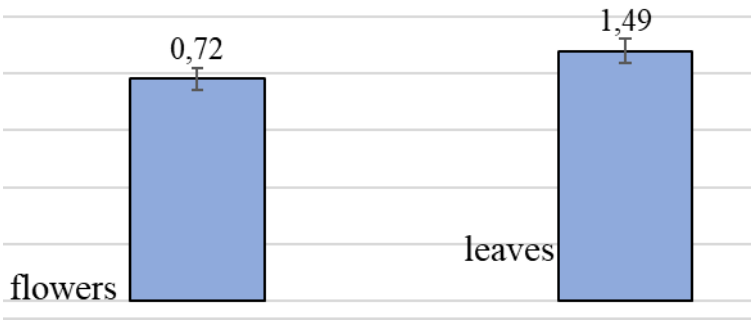

Fig. 2.3. Results of quantitative determination of flavolignans in leaves and flowers of milk thistle.

2.3. Determination of arbutin

Quantitative determination of arbutin in leaves and flowers of milk thistle was carried out by spectrophotometric method. For this purpose 0.5 g of the crushed raw material was placed in a 100 ml flask, 50 ml of water was added and heated in a water bath. The hot extract was filtered into a 100-ml volumetric flask through a paper filter. To the raw material was added 25 ml of water again and heated for 25 min. The hot extract with the raw material was transferred to the same filter and washed twice with water (10 ml each). To the filtrate was added 3 ml of basic lead acetate solution, stirred and, after cooling, made up to the mark with water. The flask was heated in a water bath until the precipitate was completely coagulated.

The hot liquid was completely filtered into a dry flask through a paper filter. After cooling, 1 ml of concentrated sulfuric acid was added to the filtrates, the flasks were weighed, and heated for 1.5 h.

The flasks were cooled, brought to their original weight with water, and the liquid was completely filtered through a paper filter. 0.1 g of zinc dust was added to the filtrates and shaken for 5 min. The liquid was then filtered into a dry flask.

To the flask was added 2 ml of 0.08 % sodium sulfacyl sulfate, 2 ml of sodium nitrite solution. After 3 min, 0.4-0.5 ml of the extract and 0.04 ml of sodium hydroxide solution were added and made up to 6 ml with water.

The tubes with the solution were heated in a water bath for one minute. After 20 minutes, the optical density of the solution was measured at 490 nm. Purified water was used as a comparison solution

The arbutin content (x, %) was calculated by the formula:

$$X = \frac{A \times 0{,}938 \times 6 \times 100}{A^{1\%}_{1\text{см}} \times A \times B},$$

where: A – is the optical density of the test solution;

0.938 – conversion factor to anhydrous arbutin;

6 – total volume of the test solution;

100 – volume of the measuring flask;

$E^{1\%}_{1cm}$ – specific absorption of arbutin at 410 nm;

A – weight of raw material;

B – volume of the extract taken for analysis.

The results of quantitative determination of arbutin in milk thistle leaves and flowers are summarized in Table 2.2 and Figure 2.4.

Table.2.2

Results of quantitative determination of arbutin in flowers and leaves of milk thistle

m	n	X_i	X mean	S^2	S mean	P	T (P, n)	Confidence interval	ε_, %
leaves									
5	4	0,37 0,36 0,37 0,37 0,37	0,37	0,00013	0,005	0,95	2,78	0,37±0,01	0,38
flowers									
5	4	0,20 0,25 0,27 0,30 0,35	0,25	0,0012	0,014	0,95	2,78	0,25±0,06	4,67

Arbutin content in milk thistle leaves was quantified – 0,37±0,01%, in flowers – 0,25±0,06 %.

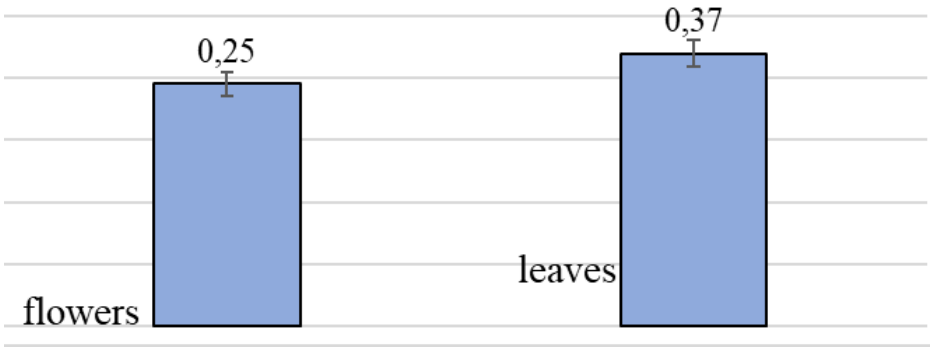

Fig. 2.4. Results of quantitative determination of arbutin in milk thistle flowers and leaves

2.4. Determination of organic acids

The study of the qualitative composition of organic acids in milk thistle flowers and leaves was carried out using chromatography. For this purpose, the mobile phase was used – 96 % ethanol-chloroform-ammonia concentrated – water (70:40:20:2). Standard samples were also used: citric acid, oxalic acid, salicylic acid, and ascorbic acid. In order to detect organic acids in milk thistle flower and leaf extracts, a pre-dried paper chromatogram was treated with bromothymol blue solution and heated in an oven at a temperature of 100 to 105 °C. The scheme of the chromatogram is shown in Fig. 2.5.

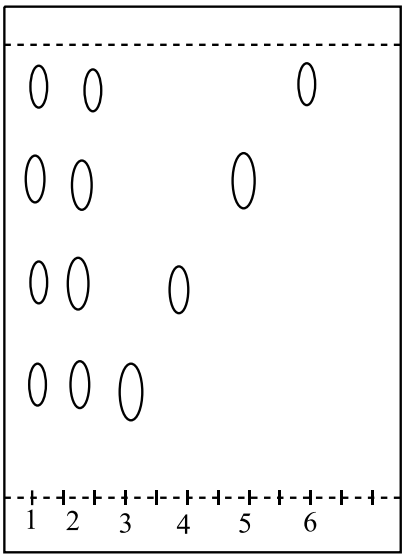

Fig. 2.5. Schematic of the chromatogram for the detection of organic acids in flowers and leaves of milk thistle: 1 – aqueous extract of milk thistle flowers, 2 – aqueous extract of milk thistle leaves; 3 – ascorbic acid; 4 – citric acid; 5 – oxalic acid; 6 – salicylic acid.

Mobile phase: 96 % ethanol – chloroform – concentrated ammonia – water in the ratio of 70:40:20:2.

Development reagent: bromothymol blue solution, when heated at 100 to 105 °C.

The chromatographic analysis revealed the presence of ascorbic, citric, oxalic and salicylic acids in milk thistle raw materials.

The quantitative content of organic acids in milk thistle flowers and leaves was determined according to the method described in SPU 2.0, Supplement 1, according to the monograph “Rosehips N” by alkalimetric titration [1].

The results of the quantitative determination of organic acids in milk thistle leaves and flowers are shown in Table 2.3 and Fig. 2.6.

Table.2.3

Results of quantitative determination of organic acids in milk thistle leaves and flowers

m	n	X_i	X mean	S^2	S mean	P	T (P, n)	Confidence interval	ε_, %
leaves									
5	4	1,70 1,90 2,15 2,50 2,70	1,87	0,0027	0,0228	0,95	2,78	1,87 ±0,12	4,37
flowers									
5	4	1,30 1,35 1,39 1,40 1,45	1,37	0,0180	0,0698	0,95	2,78	1,37±0,08	4,25

Quantitative content of organic acids in milk thistle leaves made up – 1,87±0,12 %, in flowers – 1,37±0,08%.

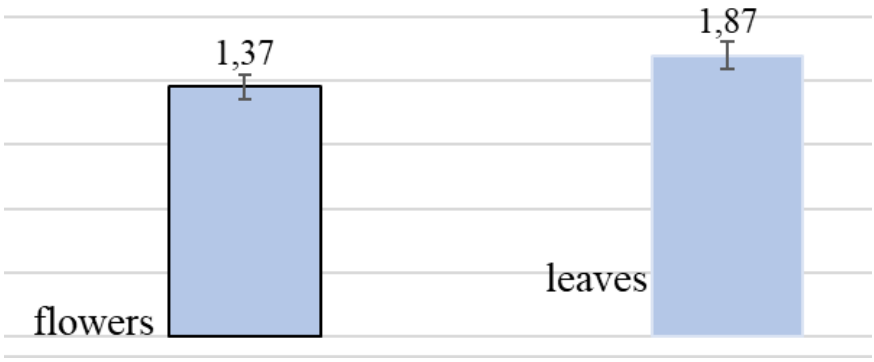

Fig. 2.6. Results of quantitative determination of organic acids in milk thistle flowers and leaves

2.5. Determination of hydroxycinnamic acids

Chromatography and aqueous extracts of milk thistle flowers and leaves were used to identify hydroxycinnamic acids.

The qualitative composition of hydroxycinnamic acids in the flowers and leaves of the plant was carried out using TLC. To do this, the analytical sample of the raw material was crushed to a particle size of 1-2 mm. Then, 10.0 g of the raw material was placed in a flask, 1000.0 ml of 70 % ethanol was added and extracted. The resulting extracts were evaporated under vacuum to a thick extract and applied to a chromatogram.

Chromatographic analysis was carried out by the method of ascending TLC in the mobile phases: 1 direction – 15 % acetic acid and 2 direction – 2 % acetic acid. Standard samples of hydroxycinnamic acids (caffeic, chlorogenic, non-chlorogenic, and p-coumaric acids) were used for comparison. The substances were determined by fluorescence in UV light at 254 nm and 365 nm before and after treatment with ammonia vapor, ferric (III) chloride solution and by comparing Rf with the valid samples of hydroxycinnamic acids. Under the action of ammonia vapor, the zones of hydroxycinnamic acids on the chromatogram acquired a blue color.

As a result of chromatographic analysis, chlorogenic, non-chlorogenic and caffeic acids were identified in the leaves and flowers of milk thistle. A diagram of the chromatogram for the detection of hydroxycinnamic acids in milk thistle raw materials is shown in Fig. 2.7.

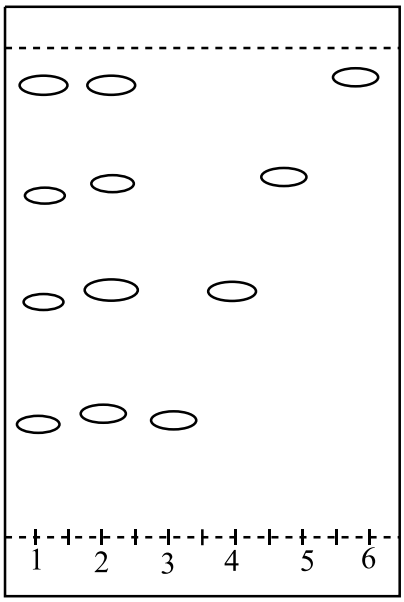

Fig. 2.7. Schematic of the chromatogram of milk thistle hydroxycinnamic acids of leaves and flowers.

1– aqueous extract of flowers, 2– aqueous extract of leaves, 3– chlorogenic acid, 4– caffeic acid, 5– non-chlorogenic acid, 6– p-coumaric acid.

Mobile phase– 15% acetic acid.

Detection reagent: alcohol solution of ferric (III) chloride followed by heating in an oven at 100-105 ^{0}C

The chromatographic analysis of the plant raw materials revealed caffeic, chlorogenic, non-chlorogenic and p-coumaric acids.

The results of the quantitative determination of hydroxycinnamic acids in milk thistle leaves and flowers are given in Table 2.4 and Fig**.** 2.8.

Table.2.4

The results of quantitative determination of hydroxycinnamic acids in milk thistle leaves and flowers, %.

m	n	X_i	X mean	S^2	S mean	P	T (P, n)	Confidence interval	ε_, %
leaves									
5	4	1,20 1,24 1,25 1,30 1,35 1,40	1,25	0,0028	0,02050	0,95	2,78	1,25±0,01	3,57
flowers									
5	4	1,40 1,48 1,57 1,60 1,65	0,70	0,0013	0,0150	0,95	2,78	0,70±0,06	4,67

The presence of hydroxycinnamic acids in milk thistle leaves was found to be –1,25±0,01 %, in flowers – 0,70±0,06 %.

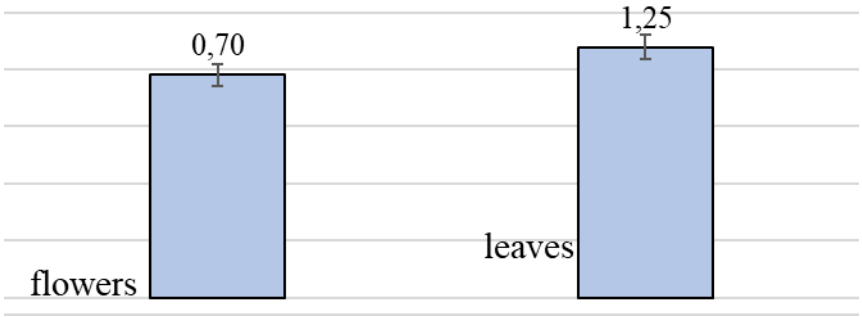

Fig. 2.8. Results of quantitative determination of hydroxycinnamic acids in milk thistle flowers and leaves.

2.6. Determination of anthocyanins

The presence of anthocyanins was detected only in milk thistle flowers, which is confirmed by the corresponding qualitative reactions.

The determination of anthocyanin content in milk thistle flowers was carried out according to the SPU 2.0, Vol. 3 presented in “Bilberry fruits, fresh”, monograph. Anthocyanins were determined using a Mecasys Optizen POP spectrophotometer [2].

The results of the determination of anthocyanins in milk thistle flowers are presented in Table 2.5 and Fig. 2.9.

Table.2.5

Results of determination of anthocyanin content in milk thistle flowers, %.

m	n	X_i	X mean	S^2	S mean	P	T (P, n)	Confidence interval	ε_, %
5	4	0,19 0,20 0,26 0,28 0,30	0,20	0,0012	0,0432	0,95	2,78	0,20±0,42	2,40

The quantitative content of anthocyanins in flowers of milk thistle is 0,20±0,42%.

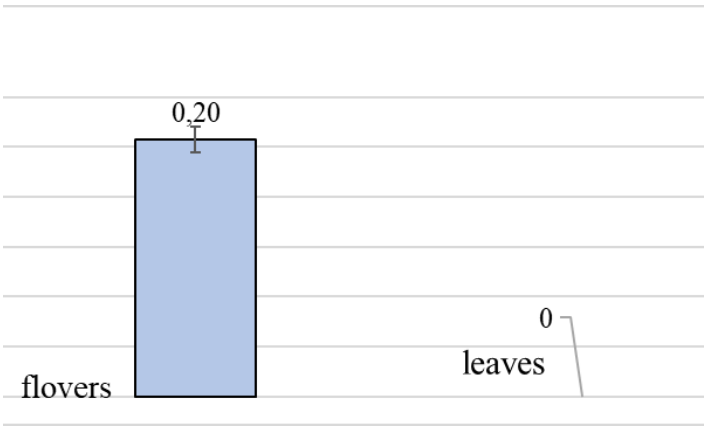

Fig. 2.9. Results of quantitative determination of anthocyanins in flowers and leaves of milk thistle.

2.7. Determination of polysaccharides

The study of polysaccharides in milk thistle flowers and leaves was carried out using a reaction with copper tartrate reagent, as a result of the reaction in two tubes, a brick-red precipitate was formed. In addition, the presence of polysaccharides was determined in aqueous extracts from milk thistle flowers and leaves by adding a threefold amount of 96 % ethanol, as a result of this reaction, the formation of white precipitates was observed.

The identification of polysaccharides in milk thistle raw materials was carried out using PC. The scheme of the chromatogram for the study of plant carbohydrates is shown in Fig. 2.10.

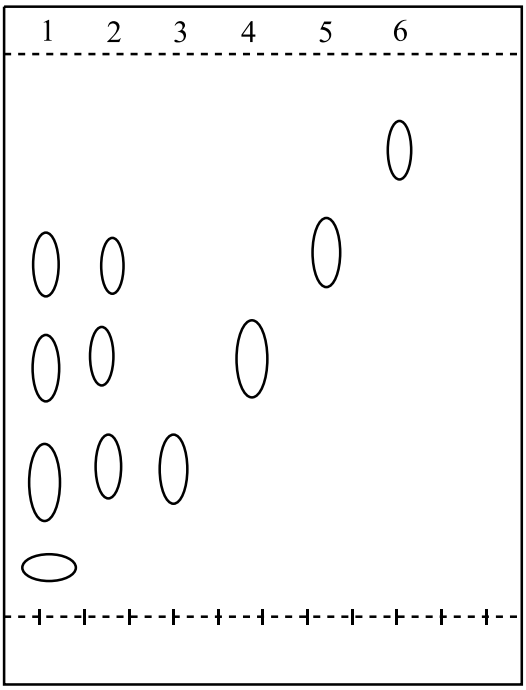

Fig. 2.10. Schematic of the chromatogram for the study of the polysaccharide composition of milk thistle flowers and leaves: 1 – water extract of flowers; 2 – water extract of leaves; 3 – ribose; 4 – glucose; 5 – galactose, 6 – arabinose

Mobile phase: acetone – butanol – water in the ratio (7:2:1).

Development reagent: aniline phthalate.

Chromatography method: descending.

As a result of the chromatographic study of the polysaccharide composition in milk thistle leaves and flowers, the following were identified: ribose (Fig. 2.11.), glucose (Fig. 2.12.), galactose (Fig. 2.13.).

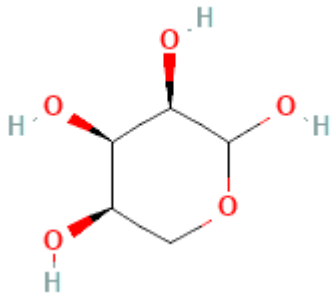

Fig. 2.11. Structural formula of ribose.

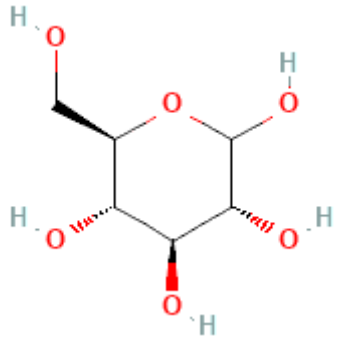

Fig. 2.12. Structural formula of glucose.

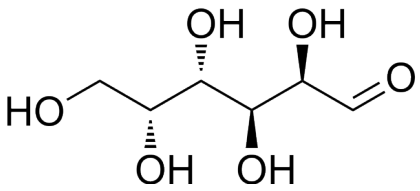

Fig. 2.13. Structural formula of galactose.

Determination of the quantitative content of polysaccharides in milk thistle flowers and leaves was carried out according to the method described in SPU 2.0, Vol. 3, “Plantain large leavesN” using the gravimetric method [3].

The results of the quantitative determination of polysaccharides in milk thistle flowers and leaves are shown in Table 2.6 and Fig. 2.14.

Table.2.6

Results of quantitative determination of polysaccharides in milk thistle leaves and flowers, %.

m	n	X_i	X mean	S^2	S mean	P	T (P, n)	Confidence interval	ε_, %
leaves									
5	4	6,40 6,80 7,70 7,80 7,90	7,70	0,0048	0,0299	0,95	2,78	7,70±0,50	4,35
flowers									
5	4	3,45 3,50 3,56 3,60 3,70	3,50	0,0013	0,0150	0,95	2,78	3,50±0,17	2,50

The presence of polysaccharides in milk thistle leaves was found to be 7,70±0,79 %, and in flowers 3,50±0,17 %.

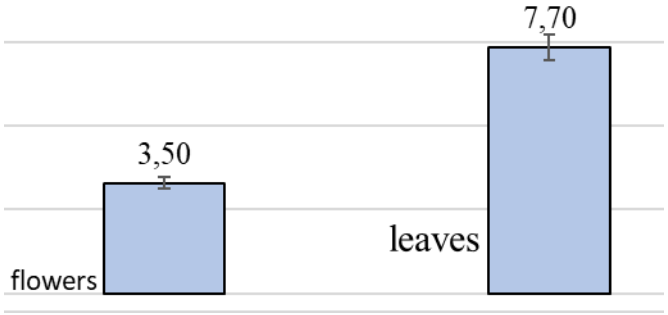

Fig. 2.14. Results of quantitative determination of polysaccharides in flowers and leaves of milk thistle.

2.8. Determination of flavonoids

Identification reactions and chromatography were used for the qualitative detection of flavonoids in milk thistle leaves and flowers. In particular, the following identification reactions were used:

1. The cyanidin reaction resulted in the formation of a red-pink color in the ethanol extract of milk thistle flowers and leaves.

2. When plumbum acetate solution was added to the tubes, a gradual formation of a yellow precipitate was observed in the tubes with flowers and leaves of the plant.

3. When adding ferric (III) chloride solution to milk thistle flower and leaf extracts, the formation of a dark green color of the plant raw material extracts was observed.

The identification reactions used indicate the presence of flavonoids in the milk thistle raw materials under study. Also, for the identification of flavonoids in the raw materials of the plant, we used TLC chromatography.

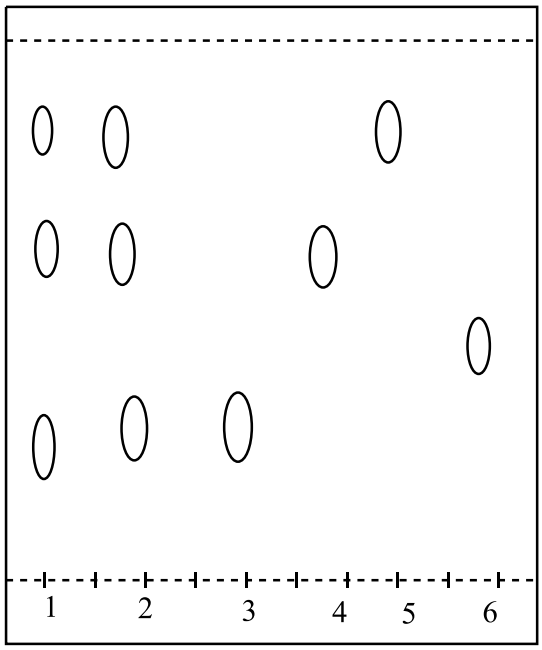

Fig. 2.15. Scheme of the chromatogram for the detection of flavonoids in milk thistle flowers and leaves: 1 – aqueous extract of milk thistle flowers, 2 – aqueous extract of milk thistle leaves; 3 – hyperoside; 4 – quercetin; 5 – rutin; 6 – myricetin.

Mobile phase: n-butanol P – acetate ice acid P – purified water P, in a ratio of 4:1:2.

Development reagent: 5% ethanol solution of $AlCI_3$.

Using the TLC method (Fig. 2.15), hyperoside (Fig. 2.16), quercetin (Fig. 2.17), rutin (Fig. 2.18), were identified in milk thistle leaves and flowers.

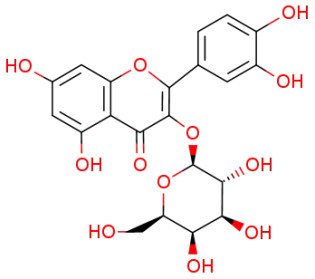

Fig. 2.16. Structural formula of hyperoside.

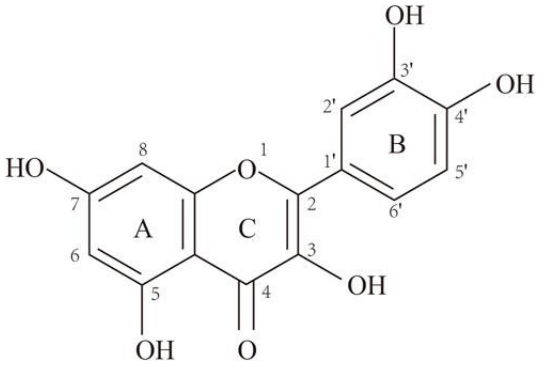

Fig. 2.17. Structural formula of quercetin.

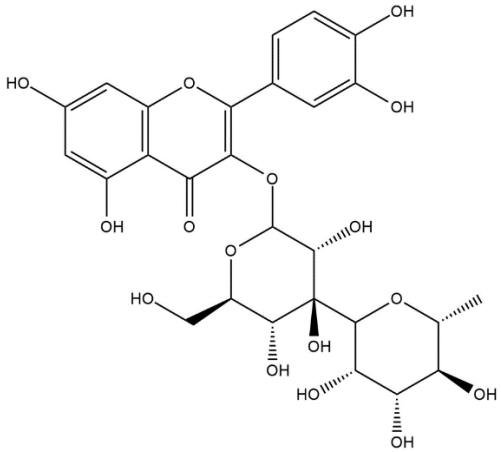

Fig. 2.18. Structural formula of rutin.

According to the methodology given in Appendix 1, ("Sophora flowers") according to the SPU 2.0, the quantitative content of flavonoids in milk thistle flowers and leaves was determined by the spectrophotometric method [1].

The results of the quantitative content of flavonoids in milk thistle raw materials are shown in Table 2.7 and Fig. 2.19.

Table.2.7

Results of quantitative determination of flavonoids in milk thistle leaves and flowers, %

m	n	X_i	X mean	S^2	S mean	P	T(P, n)	Confidence interval	ε_, %
leaves									
5	4	1,30 1,50 1,60 1,70 1,90	1,70	0,0027	0,0228	0,95	2,78	1,70 ±0,15	3,27
flowers									
5	4	0,80 0,90 1,10 1,20 1,30	1,20	0,0180	0,0698	0,95	2,78	1,20±0,10	4,15

The presence of flavanoids in milk thistle leaves was found to be 1,70±0,15 %, and in flowers 1,20±0,10 %.

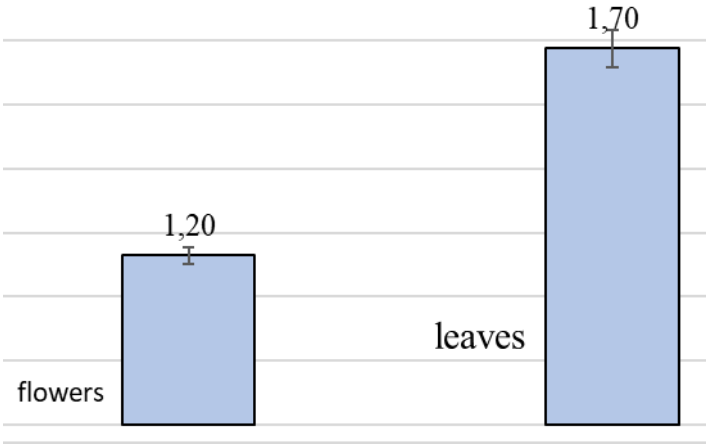

Fig. 2.19. Results of quantitative determination of flavonoids in flowers and leaves of milk thistle.

2.9. Determination of tannins

Various identification reactions were used to detect tannins in milk thistle raw materials:

1. A gelatin solution was added to the extracts of milk thistle flowers and leaves to form a cloudy precipitate in tubes of plant material.

2. With a quinine chloride solution – due to the addition of quinine chloride to the tubes, a white amorphous precipitate was formed in the tubes with flowers and leaves of milk thistle.

3. With a solution of ferric (III) ammonium sulfate – the formation of black-green color in test tubes with leaves and flowers of milk thistle was observed.

The quantitative determination of tannins in the leaves and flowers of milk thistle was determined by the spectrophotometric method [2].

The results of the quantitative determination of tannins in milk thistle flowers and leaves are shown in Table 2.8 and Fig. 2.20.

Table.2.8

Results of quantitative determination of tannins in milk thistle flowers and leaves

m	n	X_i	X mean	S^2	S mean	P	T (P, n)	Confidence interval	ε_, %
flowers									
5	4	1,99 2,01 2,04 2,11 2,15	2,07	0,0074	0,0369	0,95	2,78	2,07±0,10	4,80
leaves									
5	4	3,28 3,39 3,42 3,46 3,50	3,44	0,0064	0,0257	0,95	2,78	3,44± 0,09	2,97

It was found that the tannin content in flowers is 2,07±0,10%, and in leaves – 3,44± 0,09%.

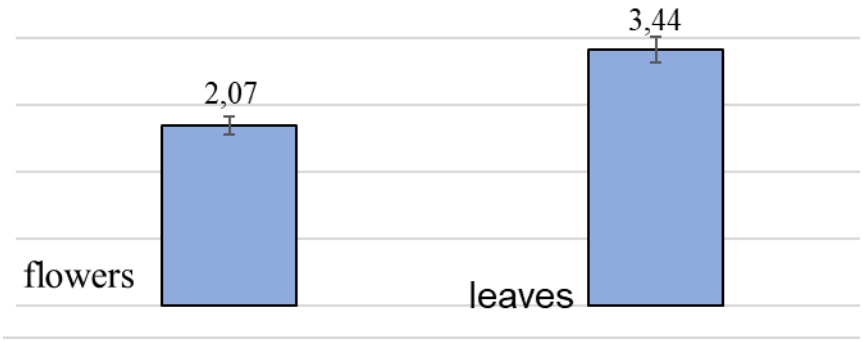

Fig. 2.20. Results of quantitative determination of tannins in milk thistle flowers and leaves.

CONCLUSIONS

The qualitative composition of milk thistle leaves and flowers was studied using identification reactions, chromatography on paper and in a thin sorbent layer.

The presence of organic and hydroxycinnamic acids, polysaccharides, flavonoids, anthocyanins, arbutin, flavolignans and tannins in the flowers and leaves of the plant was established.

The content of biologically active substances in milk thistle flowers and leaves – organic and hydroxycinnamic acids, polysaccharides, arbutin, flavolignans, flavonoids, tannins and anthocyanins for the flowers of the plant – was determined.

CHAPTER 3
DETERMINATION OF QUALITY INDICATORS OF *SILYBUM MARIANUM*

3.1. Determination of total ash content

The quantitative content of total ash in the flowers and leaves of milk thistle was studied by the method “Total ash” included in SPU 2.0, Vol. 1 [2]. The results of determination of total ash in flowers and leaves of milk thistle are presented in Table 3.1 and Fig. 3.1.

Table.3.1

Results of determination of total ash content in milk thistle leaves and flowers, %.

m	n	X_i	X med.	S^2	S mean	P	T(P, n)	Confidence interval	ε_, %
leaves									
5	4	3,10 3,20 3,30 3,40 4,05	3,30	0,0006	0,0641	0,95	2,78	3,30±0,17	3,50
flowers									
5	4	2,10 2,20 2,50 2,70 2,90	2,90	0,00210	0,0752	0,95	2,78	2,90±0,20	4,20

It was found that the mass loss during drying in milk thistle flowers is 3,30±0,17 %, in leaves it is 2.90±0,20%.

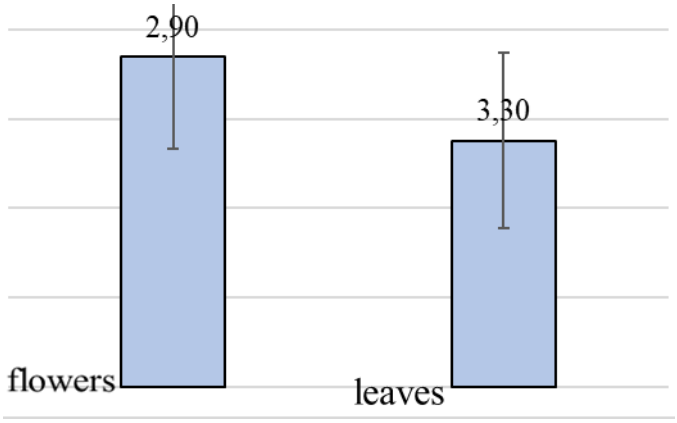

Fig. 3.1. Results of quantitative determination of total ash in milk thistle flowers and leaves.

3.2. Determination of mass loss during drying

The loss in weight during drying in milk thistle flowers and leaves was determined according to the method given in SPU 2.0, Vol. 1, according to the monograph “Loss in weight during drying” using the gravimetric method [2, 3]. The results of the study are presented in Table 3.2 and Fig. 3.2.

Table.3.2

Results of determination of weight loss during drying of milk thistle leaves and flowers, %.

m	n	X_i	X mean	S^2	S mean	P	T(P, n)	Confidence interval	ε_, %
leaves									
5	4	5,10 5,20 5,30 5,40 5,50	5,19	0,045360	0,09524	0,95	2,78	5,19±0,20	5,25

Continuation of table 3.2

m	n	X_i	X mean	S^2	S mean	P	T(P, n)	Confidence interval	ε_, %
flowers									
5	4	4,30 4,50 4,70 4,90 5,50	4,40	0,04536	0,09524	0,95	2,78	4,40±0,30	4,20

It was found that the mass loss during drying in milk thistle flowers is 4.40±0,30 %, in leaves it is 5.19±0,20 %.

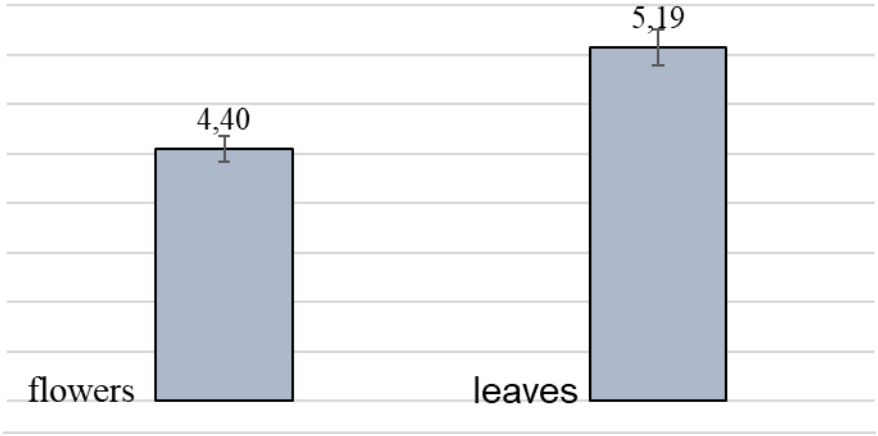

Fig. 3.2. Results of quantitative determination of loss in masa during drying of milk thistle flowers and leaves.

CONCLUSIONS

For leaves and flowers of milk thistle, we quantified the loss in mass during drying and total ash, which can be used to develop quality control methods for raw materials.

GENERAL CONCLUSIONS

1. A detailed review of the literature on the botanical characteristics, geographical distribution, chemical composition and use of milk thistle raw materials in medicine was carried out.

2. The qualitative composition of milk thistle flowers and leaves was studied.

Using chromatographic methods of analysis in milk thistle flowers and leaves, the following were determined organic acids (ascorbic, citric, oxalic and salicylic acids); hydroxycinnamic acids (caffeic, chlorogenic, non-chlorogenic and p-coumaric acids); polysaccharides (glucose, galactose, ribose); flavonoids (hyperoside, quercetin, rutin).

3. Quantitative determination of the content of hydroxycinnamic and organic acids, flavolignans, arbutin, polysaccharides, flavonoids, tannins in leaves and anthocyanins in flowers of milk thistle was carried out.

4.The quality indicators of milk thistle leaves and flowers were determined, in particular: weight loss during drying; total ash content.

REFERENCES

1. Державна Фармакопея України. Доповнення 1 / ДП «Український науковий фармакопейний центр якості лікарських засобів». 2–ге вид. Харків : «Український науковий фармакопейний центр якості лікарських засобів», 2016. 360 с.
2. Державна Фармакопея України : у 3 т. / ДП «Український науковий фармакопейний центр якості лікарських засобів». 2–ге вид. Харків : «Український науковий фармакопейний центр якості лікарських засобів», 2015. Т. 1. 1128 с.
3. Державна Фармакопея України : у 3 т. / ДП «Український науковий фармакопейний центр якості лікарських засобів». 2–ге вид. Харків : «Український науковий фармакопейний центр якості лікарських засобів», 2014. Т. 3. 732 с.
4. A review of the botany, phytochemistry, pharmacology, synthetic biology and comprehensive utilization of *Silybum marianum* / X. Zhuang et al. *Frontiers in Pharmacology.* 2024. Vol. 15. P. 1–22.
5. A combination of *Pueraria lobata* and *Silybum marianum* protects against alcoholic liver disease in mice / R. Feng et al. *Phytomedicine*. 2019. Vol. 30. P. 58–67.
6. A green ultrasound-assisted extraction optimization of the natural antioxidant and anti-aging flavonolignans from milk thistle *Silybum marianum* (L.) Gaertn. fruits for cosmetic applications / S. Drouet et al. *Antioxidants.* 2019. Vol. 8(8). P. 1–19.
7. Anticancer and antimutagenic activity of *Silybum marianum* L. *and Eucalyptus camaldulensis* Dehnh. against skin cancer induced by DMBA: *in vitro* and *in vivo* models / H. A. Karem et al. *Pakistan Journal of Pharmaceutical Sciences*. 2021. Vol. 34(3). P. 987–993.

8. Comparative assessment of phytoconstituents, antioxidant activity and chemical analysis of different parts of Milk Thistle *Silybum marianum* L / A. Javeed et al. *Molecules.* 2022. Vol. 27(9). P. 1–12.
9. Comparing the extraction methods, chemical composition, phenolic contents and antioxidant activity of edible oils from Cannabis sativa and *Silybum marianum* seeds / M. Kalinowska et al. *Scientific Reports*. 2022. Vol. 12. P. 120–140.
10. Chemistry of Silybin / D. Biedermann et al. *Natural Product Reports.* 2014. Vol. 31(9). P. 1077–1232.
11. Csupor D., Csorba A., Hohmann J. Recent advances in the analysis of flavonolignans of *Silybum marianum. Journal of Pharmaceutical and Biomedical Analysis.* 2016. Vol. 130. P. 301–317.
12. Co-treatment of silymarin and cisplatin inhibited cell proliferation, induced apoptosis in ovarian cancer / M. R. Karimzadeh et al. *Molecular Biology Reports.* 2024. Vol. 51(1). P. 118–128.
13. Efficacy of silymarin in patients with non-alcoholic fatty liver disease – the Siliver trial: a study protocol for a randomized controlled clinical trial / C. Ribeiro de Avelar et al. *Trials.* 2023. Vol. 24(1). P.1–11.
14. Flavonolignans of milk thistle (*Silybum marianum* L.) seeds affected by fertilization type and plant genotype / H. Yadegari et al. *International Journal of Horticultural Science and Technology.* 2021. Vol. 8, № 4. P. 371–384.
15. Gene expression analysis and metabolite profiling of silymarin biosynthesis during milk thistle (*Silybum marianum* (L.) Gaertn.) fruit ripening / S. Drouet et al. *International Journal of Molecular Sciences.* 2020. Vol. 21(13). P. 1–18.
16. Hanafy N. A., El-Kemary M. A. Silymarin / curcumin loaded albumin nanoparticles coated by chitosan as muco-inhalable delivery system observing anti-inflammatory and anti COVID-19 characterizations in oleic

acid triggered lung injury and in vitro COVID-19 experiment. *International Journal of Biological Macromolecules*. 2022. Vol. 198. P. 101–110.

17. In vitro effects of undifferentiated callus extracts from *Plantago major* L, *Rhodiola rosea* L and *Silybum marianum* L in normal and malignant human skin cells / A. G. Wingren et al. *Heliyon*. 2023. Vol. 9. P. 1–9.
18. Liu Y., Liu Z., Zheng Y. Research progress of milk thistle. *Journal of Ginseng Research.* 2016. Vol. 28(02). P. 55–58.
19. Milk thistle in liver diseases: past, present, future Ludovico / L. Abenavoli et al. *Phytotherapy. Research.* 2010. Vol. 24(10). P. 1423–1432.
20. Milk thistle (*Silybum marianum*): a concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases / L. Abenavoli et al. *Phytotherapy. Research.* 2018. Vol. 32(11). P. 2202–2213.
21. Neuroprotective potential of silymarin against CNS Disorders: insight into the pathways and molecular mechanisms of action / A. Borah et al. *CNS Neuroscience & Therapeutics.* 2013. Vol. 19(11). P. 847–853.
22. Profile of fatty acids, tocopherols, phytosterols and polyphenols in mediterranean oils (argan oils, olive oils, milk thistle seed oils and nigella seed oil) and evaluation of their antioxidant and cytoprotective activities / A. Zarrouk et al. *Current Pharmaceutical Design.* 2019. Vol. 25(15). P. 1791–1805.
23. Silybinin downregulates types I and III collagen expression via suppression of the mTOR signaling pathway / S. Choi et al. *International Journal of Molecular Sciences.* 2023. Vol. 24(18). P. 1–14.
24. Silybinin inhibits the migration, invasion and epithelial-mesenchymal transition of prostate cancer by activating the autophagic degradation of YAP / W. Dan et al. *Journal of Cancer.* 2022. Vol. 13(13). P. 3415–3426.
25. Silybin showed higher cytotoxic, antiproliferative, and anti-inflammatory activities in the CaCo Cancer cell line while retaining viability and proliferation in normal intestinal IPEC-1 Cells / D. Faixová et al. *Life.* 2023. Vol. 13 (2). P. 1–16.

26. Silymarin (milk thistle extract) as a therapeutic agent in gastrointestinal cancer / M. Fallah et al. *Biomed Pharmacother*. 2021. Vol. 142. P. 112–124.
27. Silybinin induced-autophagic and apoptotic death is associated with an increase in reactive oxygen and nitrogen species in HeLa cells / F. Simiao et al. *Free Radical Research.* 2011. Vol. 45. P. 11–12.
28. Silybinin inhibits epithelial-mesenchymal transition of renal cell carcinoma through autophagy-dependent Wnt/β-catenin signaling / Y. Fan et al. *International Journal of Molecular Medicine*. 2020. Vol. 45. P. 2–10.
29. *Silybum marianum* total extract, silymarin and silybinin abate hepatocarcinogenesis and hepatocellular carcinoma growth via modulation of the HGF/c-Met, Wnt/β-catenin, and PI_3K/Akt/mTOR signaling pathways / Y. Nour et al. *Biomedicine & Pharmacotherapy*. 2022. Vol. 145. P. 112–409.
30. Silymarin enriched extract (*Silybum marianum*) additive effect on doxorubicin-mediated cytotoxicity in PC-3 prostate cancer cells / K. Gioti et al. *Planta Medica*. 2019. Vol. 85(11/12). P. 997–1007.
31. Silymarin ameliorates metabolic dysfunction associated with diet-induced obesity via activation of farnesyl X receptor / M. Gu et al. *Frontiers in Pharmacology*. 2016. Vol. 6. P. 327–345.
32. Silymarin improved diet-induced liver damage and insulin resistance by decreasing inflammation in mice / Y. Guo et al. *Pharmaceutical Biology*. 2016. Vol. 54(12). P. 2995–3000.
33. The gastroprotective potential of silybinin against *Helicobacter pylori* infection and gastric tumor cells / M. Bittencourt et al. *Life Sciences.* 2020. Vol. 11. P. 117–977.
34. The silymarin composition and why does it matter? / C. S. Chambers et al. *Food Research International*. 2017. Vol. 100. P. 339–353.

35. The natural flavonoid silybin improves the response to photodynamic therapy of bladder cancer cells / L. Gándara et al. *Journal of Photochemistry and Photobiology B: Biology*. 2014. Vol. 133. P. 55–64.
36. Tayoub G., Sulaiman H., Alorfi M. Quantitative identification of total silymarin in wild *Silybum marianum* L. by using HPLC. *International Journal of Herbal Medicine.* 2018. Vol. 6(2). P. 110–114.
37. Valuable hepatoprotective plants – how can we optimize waste free uses of such highly versatile resources? / L. V. Krepkova et al. *Frontiers in Pharmacology*. 2021. Vol. 12. P. 738–752.

APPENDIX

Appendix A


СУЧАСНІ ДОСЯГНЕННЯ ФАРМАЦЕВТИЧНОЇ НАУКИ В СТВОРЕННІ ТА СТАНДАРТИЗАЦІЇ ЛІКАРСЬКИХ ЗАСОБІВ І ДІЄТИЧНИХ ДОБАВОК, ЩО МІСТЯТЬ КОМПОНЕНТИ ПРИРОДНОГО ПОХОДЖЕННЯ
Матеріали VII Міжнародної науково-практичної інтернет-конференції
11 КВІТНЯ 2025
м. Харків

Continued appendix A

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
МІНІСТЕРСТВО ОСВІТИ І НАУКИ УКРАЇНИ
НАЦІОНАЛЬНА АКАДЕМІЯ НАУК ВИЩОЇ ОСВІТИ УКРАЇНИ
НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ
КАФЕДРА ФАРМАКОГНОЗІЇ ТА НУТРИЦІОЛОГІЇ

MINISTRY OF HEALTH OF UKRAINE
MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
NATIONAL ACADEMY OF HIGHER EDUCATION OF SCIENCES OF UKRAINE
NATIONAL UNIVERSITY OF PHARMACY
DEPARTMENT OF PHARMACOGNOSY AND NUTRICIOLOGY

СУЧАСНІ ДОСЯГНЕННЯ ФАРМАЦЕВТИЧНОЇ НАУКИ В СТВОРЕННІ ТА СТАНДАРТИЗАЦІЇ ЛІКАРСЬКИХ ЗАСОБІВ І ДІЄТИЧНИХ ДОБАВОК, ЩО МІСТЯТЬ КОМПОНЕНТИ ПРИРОДНОГО ПОХОДЖЕННЯ

CURRENT APPROACHES OF PHARMACEUTICAL SCIENCE IN DEVELOPMENT AND STANDARDIZATION OF MEDICINES AND DIETARY SUPPLEMENTS THAT CONTAIN COMPONENTS OF NATURAL ORIGIN

Матеріали VII Міжнародної науково-практичної інтернет-конференції

The Proceedings of the VII International Scientific and Practical Internet-Conference

ХАРКІВ
KHARKIV
2025

Continued appendix A

УДК 615.1: 615.32: 615.07
С 89

Електронне видання мережне

Редакційна колегія: А. А. Котвіцька, І. М. Владимирова, В. Ю. Кузнєцова, В. С. Кисличенко, О. О. Іосипенко

Конференція зареєстрована в Українському інституті науково-технічної і економічної інформації (УкрІНТЕІ), посвідчення № 839 від 26 грудня 2024 р.

С 89 *Сучасні досягнення фармацевтичної науки в створенні та стандартизації лікарських засобів і дієтичних добавок, що містять компоненти природного походження*: матеріали VII Міжнародної науково-практичної інтернет-конференції (м. Харків, 11 квітня 2025 р.). – Електрон. дані. – Х.: НФаУ, 2025. – 216 с. – Назва з тит. екрана.

У збірнику розглянуто теоретичні та практичні аспекти розробки, виробництва лікарських засобів рослинного походження і дієтичних добавок, контролю якості, стандартизації лікарських засобів рослинного походження та визначення безпечності дієтичних добавок, а також їх реалізації в умовах сучасного фармацевтичного ринку.

Для широкого кола науковців, магістрантів, аспірантів, докторантів, викладачів вищих фармацевтичних та медичних навчальних закладів, співробітників фармацевтичних підприємств, фармацевтичних фірм.

Друкується в авторській редакції. Автори опублікованих матеріалів несуть повну відповідальність за підбір, точність наведених фактів, цитат, економіко-статистичних даних, власних імен та інших відомостей. Матеріали подаються мовою оригіналу. Матеріали пройшли антиплагіатну перевірку за допомогою програмного забезпечення StrikePlagiarism.

УДК 615.1: 615.32: 615.07

Continued appendix A

pure MCT oil, oxidation took place in 6.52 hours, but in the sample with lyophilized extract it took up to 157.56 hours. Determination of the content of fatty acid methyl esters showed that MCT oil primarily consists of 4 fatty acids. The ascending contents of the acids were as follows: caprylic acid, decanoic acid, lauric acid and caproic acid. The percentage content of these acids is minimally affected by the extracts, so their presence does not change the chemical structure of the oils. When determining the micro and macro elements, the presence of ten elements was found, the content of which varied depending on the sample, but calcium dominated. Antimicrobial activity showed that pure MCT coconut oil has high antimicrobial activity, especially against bacterial pathogens except for *Escherichia coli* CCM 3988, which was resistant to all tested samples. Antiradical activity showed significantly increased activity after the addition of extracts. Pure MCT oil reached a value of 7.35 ±2.55 mg TEAC.l^{-1}, oil with encapsulated extract 84.59 ±7.77 mg TEAC.l^{-1}, oil with lyophilized extract 45.84 ±2.93 mg TEAC.l^{-1} and oil with encapsulated extract 31.61 ±6.75 mg TEAC.l^{-1}. Sensory analysis revealed that MCT oils are new to consumers, and they perceive them positively: from a 9-point hedonic scale, the sample with encapsulated extract received a minimum of 3.3 points and the sample with pure MCT oil received a maximum of 5.0 points from the overall impression after consumption.

Conclusions: The study concluded that the shelf life of oils is significantly influenced by the type and amount of extract added. Peroxide values were low, indicating minimal oxidation, while acid numbers varied, with encapsulated extracts leading to higher acidity. MCT oil primarily consists of four fatty acids, with the extracts not altering their chemical structure. The analysis revealed the presence of essential micro and macro elements, particularly calcium. Pure MCT oil exhibited high antimicrobial activity, although it was ineffective against *Escherichia coli*. The addition of extracts significantly increased antiradical activity. Sensory evaluation indicated a generally positive consumer perception of MCT oils, with varying scores based on the extract type.

Acknowledgements: This work was supported by the project APVV-22–0348 Potential anticancer effect of MCT coconut oil conditioned by the addition of extracts from selected *Capsicum* spp.

IDENTIFICATION OF BIOLOGICALLY ACTIVE COMPOUNDS IN RAW MATERIALS *SILYBUM MARIANUM* (L.) GAERTN.

Nouamane Nouhaila*, *Popyk A. I.
National University of Pharmacy, Kharkiv, Ukraine

Introduction. Milk thistle (*Silybum marianum* (L.) Gaertn.) is a well-known medicinal plant of the *Asteraceae* family, whose fruits are widely used in many countries as a hepatoprotective, choleretic, anti-inflammatory, antioxidant and immunomodulatory agent [1]. They contain flavolignans, flavonoids, fatty oils, tannins and biogenic amines. However, the leaves and flowers of the plant have been little studied, so a detailed phytochemical study of them is relevant.

Continued appendix A

The aim of our work was to identify the main groups of biologically active compounds in *Silybum marianum* flowers and leaves.

Materials and methods. To prepare the aqueous extracts, 5.0 g of the studied raw materials were poured with water in a ratio of 1:5 and heated in a water bath for 60 minutes, shaking occasionally. The obtained extracts were filtered through a pleated filter into a 200 ml flask. The extraction of raw materials was repeated twice more with new portions of extractant under the conditions described above. The combined extracts were concentrated and used for the determination of polysaccharides, amino acids, and tannins. Aqueous-ethanol extracts from *Silybum marianum* flowers and leaves were obtained with 70% ethanol according to the method described above. The obtained extracts were used for the detection of flavonoids. For the identification of polysaccharides, a fourfold volume of 96% ethanol was used, which was added to the extracts from *Silybum marianum* flowers and leaves, and the formation of an amorphous precipitate was observed. The presence of flavonoids and tannins in the extracts was determined using well-known chemical reactions: cyanidin reaction (pink color), with 10% solution of ferric (III) chloride (black-green color), with 2% solution of aluminum chloride (green-yellow yellow-green color), with 10% potassium hydroxide solution (yellow-green color) and 1% quinine hydrochloride solution (amorphous precipitate), with 1% gelatin solution (turbidity appeared), with ferric (III) ammonium sulfate (black-green color). The detection of amino acids was performed by reaction with a freshly prepared 0.2% ninhydrin solution in isopropyl alcohol (violet-red color) [2].

Results and discussion. The results of the experiment confirmed the presence of polysaccharides, amino acids, flavonoids and tannins in the flowers and leaves of *Silybum marianum*. The data obtained will be used for further phytochemical study of this raw material.

References:

1. Milk thistle in liver diseases: past, present, future / L. Abenavoli, R. Capasso, N. Milic, F. Capasso. *Phytotherapy Research.* 2010. Vol. 24(10). P. 1423–1432.
2. Pharmacognosy: textbook for students of higher schools / V. S. Kyslychenko, L. V. Lenchyk, I. G. Gurieva, et al. Kharkiv: NUPh: Golden Pages, 2019. 584 p.

DETERMINATION OF DRY RESIDUE IN PHYTOSUBSTANCES FROM THE HERB OF *ZINNIA ANGUSTIFOLIA* KUNTH.

Ohar V.R., Budniak L.I.
Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, Ternopil, Ukraine

Introduction. Species of the genus *Zinnia* exhibit a range of biological activities, including antioxidant, antibacterial, antifungal, antiviral, hepatoprotective, antimalarial, cytotoxic, and insecticidal properties [3].

Zinnia angustifolia Kunth. (also known as *Zinnia linearis*) is less commonly found in gardens compared to *Zinnia elegans* Jacq., but it is gradually gaining popularity. Additionally, this plant has smaller solitary flowers and narrower leaves [2].

Continued appendix A

Continued appendix A

MINISTRY OF HEALTH OF UKRAINE
MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
NATIONAL ACADEMY OF HIGHER EDUCATION
OF SCIENCES OF UKRAINE
NATIONAL UNIVERSITY OF PHARMACY
DEPARTMENT OF PHARMACOGNOSY
AND NUTRICIOLOGY

CERTIFICATE

№ 231

This is to certify that

Nouamane Nouhaila

has participated in the VII International Scientific and Practical Internet-Conference

"CURRENT APPROACHES OF PHARMACEUTICAL SCIENCE IN DEVELOPMENT AND STANDARDIZATION OF MEDICINES AND DIETARY SUPPLEMENTS THAT CONTAIN COMPONENTS OF NATURAL ORIGIN"

(Duration - 6 hours)
April, 11, 2025, Kharkiv, Ukraine

Acting Rector of the NUPh, prof. — Alla KOTVITSKA

Vice-Rector for scientific and pedagogical work of the NUPh, prof. — Inna VLADIMIROVA

Head of the department of pharmacognosy and nutriciology of the NUPh, prof. — Viktoriia KYSLYCHENKO

Ф А 2.2.1–32-366

National University of Pharmacy

Faculty Pharmaceutical
Department of pharmacognosy and nutriciology

Level of higher education master

Specialty 226 Pharmacy, industrial pharmacy
Educational and professional program Pharmacy

APPROVED
The Head of Department
of pharmacognosy and nutriciology
Prof. Viktoria KYSLYCHENKO
"02" _September_ 2024

ASSIGNMENT

FOR QUALIFICATION WORK

OF AN APPLICANT FOR HIGHER EDUCATION

Nouhaila NOUAMANE

1. Topic of qualification work: «Phytochemical study of Holy thistle flowers and leaves (*Silybum marianum* (L.) Gaertn)», supervisor of qualification work: Andrey POPYK, PhD

approved by order of NUPh from "27th" of September _2024 № 237

2. Deadline for submission of qualification work by the applicant for higher education: May, 2025

3. Outgoing data for qualification work: Phytochemical study of Holy thistle flowers and leaves (*Silybum marianum* (L.) Gaertn).

4. Contents of the settlement and explanatory note (list of questions that need to be developed): Review of the literature on botanical description, chemical composition, use of Holy thistle flowers and leaves; determination of the qualitative composition and quantitative content of the main biologically active substances

5. List of graphic material (with exact indication of the required drawings):
Tables – 10, pictures – 50.

6. Consultants of chapters of qualification work

Chapters	Name, SURNAME, position of consultant	Signature, date	
		assignment was issued	assignment was received
1.	Andrey POPYK, assistent of the department of pharmacognosy and nutriciology, PhD	September 2024	September 2024
2.	Andrey POPYK, assistent of the department of pharmacognosy and nutriciology, PhD	December 2024	December 2024
3.	Andrey POPYK, assistent of the department of pharmacognosy and nutriciology, PhD	April 2025	April 2025

7. Date of issue of the assignment: "02" _September_ 2024

CALENDAR PLAN

№ з/п	Name of stages of qualification work	Deadline for the stages of qualification work	Notes
1.	Botanical characteristic, geographical distribution, chemical composition, application in medicine and culinary of *Silybum marianum* raw materials.	September 2024	**done**
2.	Study of the chemical composition of *Silybum marianum* raw materials	December 2024	**done**
3.	Determination of quality indicators of *Silybum marianum*	March 2025	**done**
	Registration of work and submission to the Examination commission	May 2025	**done**

An applicant of higher education _____ Nouhaila NOUAMANE

Supervisor of qualification work _____ Andrey POPYK

ВИТЯГ З НАКАЗУ № 237

По Національному фармацевтичному університету

від 27 вересня 2024 року

Затвердити теми кваліфікаційних робіт здобувачам вищої освіти 5-го курсу Фм20(4,10д) 2024-2025 навчального року, освітньо-професійної програми – Фармація, другого (магістерського) рівня вищої освіти, спеціальності 226 – Фармація, промислова фармація, галузь знань 22 Охорона здоров'я, денна форма здобуття освіти (термін навчання 4 роки 10 місяців), які навчаються за контрактом (мова навчання англійська та українська) згідно з додатком № 1.

Прізвище, ім'я здобувача вищої освіти	Тема кваліфікаційної роботи		Посада, прізвище та ініціали керівника	Рецензент кваліфікаційної роботи
• **по кафедрі фармакогнозії та нутриціології**				
Нуаман Нухаіла	Фітохімічне вивчення квіток та листя розторопші плямистої (*Silybum marianum* (L.) Gaertn)	Phytochemical study of Holy thistle flowers and leaves (*Silybum marianum* (L.) Gaertn)	доц. Попик А.І.	проф. Колісник С.В.

Ректор

Вірно. Секретар

ФА2.8-03-317

ВИСНОВОК

експертної комісії про проведену експертизу
щодо академічного плагіату у кваліфікаційній роботі

здобувача вищої освіти

«21» квітня 2025 р. № 331030701

Проаналізувавши кваліфікаційну роботу здобувача вищої освіти Нуаман Нухаіла, групи Фм20(4,10д) англ-03, спеціальності 226 Фармація, промислова фармація, освітньої програми «Фармація» навчання на тему: «Фітохімічне вивчення квіток та листя розторопші плямистої (*Silybum marianum (L.) Gaertn*)/ Phytochemical study of Holy thistle flowers and leaves (*Silybum marianum (L.) Gaertn*)», експертна комісія дійшла висновку, що робота, представлена до Екзаменаційної комісії для захисту, виконана самостійно і не містить елементів академічного плагіату (компіляції).

Голова комісії,
проректор ЗВО з НПР,
професор **Інна ВЛАДИМИРОВА**

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

Nouhaila NOUAMANE

on the topic: **«Phytochemical study of Holy thistle flowers and leaves (*Silybum marianum* (L.) Gaertn)»**

Relevance of the topic. Nouamane Nouhaila's work is devoted to the phytochemical study of milk thistle (*Silybum marianum*). Since this plant is widely cultivated in many countries and in Ukraine as a well-known medicinal plant, since the study of the chemical composition of the leaves and flowers of the plant is poorly understood, the topic of the of the qualification work is relevant.

Practical value of conclusions, recommendations and their validity. Nouamane Nouhaila's conducted a review of the scientific literature on the botanical characteristics, chemical composition and use of milk thistle. In the practical part, the master's student carried out a considerable amount of work - studied the qualitative composition, determined the quantitative content of BAS and established the quality indicators of raw materials according to the requirements of the State Pharmacopoeia of Ukraine of the raw materials under study.

Assessment of work. The qualification work of Nouamane Nouhaila's was performed at a high scientific level with the use of modern methods of analysis: chemical reactions, chromatographic and instrumental methods research. The results of the quantitative content of biologically active substances are statistically processed in accordance with the requirements of the State Fiscal Service of Ukraine.

General conclusion and recommendations on admission to defend. The qualification work Nouamane Nouhaila's “Phytochemical study of Holy thistle flowers and leaves (*Silybum marianum* (L.) Gaertn)” can be submitted for defense to the Examination commission.

Scientific supervisor _____ Andrey POPYK

«13» of May 2025

Ф А 2.2.1-32-356

REVIEW

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

226 Pharmacy, industrial pharmacy

Nouhaila NOUAMANE

on the topic: «**Phytochemical study of Holy thistle flowers and leaves (*Silybum marianum* (L.) Gaertn)»**

Relevance of the topic. Phytochemical study of wild plants used in folk medicine used in official and folk medicine is a promising and relevant area in phytochemistry. Milk thistle belongs to such plants, and the study of this plant is devoted to.

Theoretical level of work. Nouhaila NOUAMANE analyzed and summarized the sources of literature on the botanical characteristics, chemical composition composition, use in medicine of milk thistle (*Silybum marianum*).

Author's suggestions on the research topic. Nouhaila NOUAMANE conducted phytochemical analysis of the leaves and flowers of milk thistle, which can be further used in the development of relevant sections of quality control methods for this type of raw material.

Practical value of conclusions, recommendations and their validity. Nouhaila NOUAMANE determined the quality indicators of the raw materials under study, analyzed the following classes of BAS: polysaccharides, organic acids, phenolic compounds, in particular hydroxycinnamic and organic acids, flavolignans, flavonoids, arbutin, anthocyanins, and tannins.

Disadvantages of work. In the work there are bad expressions, spelling mistakes.

General conclusion and assessment of the work. The proposed work is of practical importance and meets the requirements for qualification work. Qualification work of Nouhaila NOUAMANE on the topic: “Phytochemical study of Holy thistle flowers and leaves (*Silybum marianum* (L.) Gaertn)” can be submitted for defense to the Examination Commission

Reviewer _____ prof. Sergii KOLISNYK

«15» of May 2025

Ф А2.2.1-36-230

ВИТЯГ

з протоколу засідання кафедри фармакогнозії та нутриціології

№ __12__ від 16 травня 2025 р.

Присутні: Бородіна Н.В., Бурда Н.Є., Гонтова Т.М., Гончаров О.В., Журавель І.О., Кисличенко В.С., Комісаренко М.А., Машталер В.В., Новосел О.М., Попик А.І., Процька В.В., Романова С.В., Скребцова К.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 тему «Phytochemical study of Holy thistle flowers and leaves (*Silybum marianum* (L.) Gaertn)» здобувачки вищої освіти випускного курсу Фм20(4,10д) англ-03 групи Нуаман Нухаіли.

Науковий керівник: доцент Андрій ПОПИК.

Рецензент: завідувач кафедри загальної хімії, д.фарм.н., професор Сергій КОЛІСНИК.

УХВАЛИЛИ: рекомендувати до захисту в Екзаменаційній комісії кваліфікаційну роботу здобувачки вищої освіти Фм20(4,10д) англ-03 групи Нуаман Нухаіли на тему «Phytochemical study of Holy thistle flowers and leaves (*Silybum marianum* (L.) Gaertn)».

Завідувачка кафедри фармакогнозії
та нутриціології, професор Вікторія КИСЛИЧЕНКО

Секретар кафедри, професор Надія БУРДА

Ф А2.2.1-32-042

НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

ПОДАННЯ
ГОЛОВІ ЕКЗАМЕНАЦІЙНОЇ КОМІСІЇ
ЩОДО ЗАХИСТУ КВАЛІФІКАЦІЙНОЇ РОБОТИ

Направляється здобувачка вищої освіти Нуаман Нухаіла до захисту кваліфікаційної роботи за галуззю знань 22 Охорона здоров'я
спеціальністю 226 Фармація, промислова фармація
освітньо-професійною програмою Фармація
на тему: «Фітохімічне вивчення квіток та листя розторопші плямистої (*Silybum marianum* (L.) Gaertn)».

Кваліфікаційна робота і рецензія додаються.

Декан факультету _____ / Микола ГОЛІК /

Висновок керівника кваліфікаційної роботи

Здобувачка вищої освіти Нуаман Нухаіла може бути допущена до захисту даної кваліфікаційної роботи в Екзаменаційній комісії.

Керівник кваліфікаційної роботи

_____ Андрій ПОПИК

«13» травня 2025 р.

Висновок кафедри про кваліфікаційну роботу

Кваліфікаційну роботу розглянуто. Здобувачка вищої освіти Нуаман Нухаіла допускається до захисту даної кваліфікаційної роботи в Екзаменаційній комісії.

Завідувачка кафедри
фармакогнозії та нутриціології _____ Вікторія КИСЛИЧЕНКО

«16» травня 2025 року

Qualification work was defended

of Examination commission on

« ___ » _____ 2025

With the grade _____

Head of the State Examination commission,

DPharmSc, Professor

_____ / Volodymyr YAKOVENKO /