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MINISTRY OF HEALTH OF UKRAINE
NATIONAL UNIVERSITY OF PHARMACY
faculty of foreign citizens' education
department of pharmacognosy and nutriciology

QUALIFICATION WORK

on the topic: **«PHYTOCHEMICAL STUDY OF *CHRYSANTHEMUM INDICUM* RAW MATERIALS»**

Prepared by: higher education graduate of group Фм19(4,10д) англ-06
specialty 226 Pharmacy, industrial pharmacy
educational program Pharmacy
Walaa AL SAYED
Supervisor: assistant professor of the higher education institution of the department of pharmacognosy and nutriciology, PhD assistant professor
Andrey POPYK
Reviewer: professor of the higher education institution of the department of pharmaceutical chemistry, DSc, professor
Sergiy VLASOV

Kharkiv – 2024 year

ANNOTATION

For the first time, the study of biologically active substances and the determination of the quantitative content of various groups of biologically active substances in the herb of *Chrysanthemum indicum* were carried out. The technological parameters of raw materials were determined. The results of the study can be used in the development of the project of quality control methods for "*Chrysanthemum indicum* Herb".

The qualification work consists of an introduction, literature review, experimental part, general conclusions, list of references, is set out on 42 pages, includes 11 tables, 31 figures, 49 references.

Key words: Chrysanthemum indicum, herb, chemical composition.

АНОТАЦІЯ

Вперше проведено дослідження біологічно активних речовин та визначення кількісного вмісту різних груп біологічно активних речовин у траві хризантеми індійської. Визначені технологічні параметри сировини. Результати дослідження можуть бути використані при розробці проекту методів контролю якості «*Chrysanthemum indicum* Herb».

Кваліфікаційна робота складається зі вступу, огляду літератури, експериментальної частини, загальних висновків, переліку використаних літературних джерел, викладена на 42 сторінках, включає 11 таблиць, 31 рисунок, 49 джерел літератури.

Ключові слова: хризантема індійська, трава, хімічний склад.

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INTRODUCTION

Rationale

In today's environment, the search for new effective natural-based medicines continues to be relevant. One of the most promising areas of search is plants, including ornamental plants. Their diverse chemical composition makes it possible to predict their versatile pharmacological activity.

Chrysanthemum indicum, which is widely grown as an ornamental plant, has been adapted for cultivation in many countries.

Given that most scientists have studied plants of the genus *Chrysanthemum*, in particular *Chrysanthemum indicum*, from the point of view of pharmaceutical use, it is important to expand this information in the direction of phytochemical research.

Thus, the expediency of phytochemical study of *Chrysanthemum indicum* as a promising plant for use in pharmacy and medicine is a relevant area.

Purpose

The purpose of the master's thesis was the phytochemical study of *Chrysanthemum indicum* herb.

Tasks of the research

To achieve this goal, it was necessary to solve the following tasks

– to study the literature sources regarding the botanical characteristics, geographical distribution, chemical composition and pharmacological activity of *Chrysanthemum indicum*;

– determine the quality indicators of *Chrysanthemum indicum* herb, the total ash, the weight loss on drying and the extractable matter;

– to study the qualitative composition of *Chrysanthemum indicum* herb;

–determine the quantitative content of biologically active compounds.

The object of the research – the phytochemical study of *Chrysanthemum indicum* herb.

The subject of the research – definition of quality indicators, the study of the qualitative composition and the quantitative content of biologically active compounds in *Chrysanthemum indicum* herb.

Methods of the research

The paper chromatography method and the well-known chemical reactions were used to identify biologically active compounds in *Chrysanthemum indicum* herb. Quantitative determination was carried out by gravimetric, spectrophotometric, and titrimetric methods.

The experiment results were processed by statistical methods according to the requirements of State Pharmacopoeia of Ukraine.

The practical significance and scientific novelty of the results

The experiments proved the prospects of continuing further pharmacognostic study of chrysanthemum *Chrysanthemum indicum* herb.

Approbation of the research results

1 abstract was published at the VI Scientific and Practical Internet Conference "Modern achievements of pharmaceutical science in the creation and standardization of medicines and dietary supplements containing components of natural origin", Kharkiv, April. 12, 2024 (Appendix).

The structure and scope of the qualification work

The qualification work consists of an introduction, a literature review, experimental part, general conclusions, list of references and appendices. references and appendices. The work is set out on 42 pages, includes 11 tables and 31 figures. The list of references includes 49 sources.

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

1.1 Botanical characteristic

Chrysanthemum indicum, sometimes called mums or chrysanths is one of the most important marketable ornamental and flower plants belonging to the family *Asteraceae*, sub-family *Asteroideae*, order *Asterales*, sub-class *Asteridae* [11, 12]. The plant is a perennial aromatic flavor, with erect hairy stem, complete shiny leaves, highly cleft from the base, 70-120 cm height and heavily branched stem [15, 16, 29]. The *Chrysanthemum indicum* stem has 76 cm long and belong to wet stems. The surface of the stem is furrowed and hairy.

The *Chrysanthemum indicum* is a complex flower, which is a cluster of small flowers joined together in a flower head, with pistils and stamens on one flower (i.e. it is a hermaphrodite) and consists of disk flowers (small flowers with stamens and pistils) and ray flowers (small flowers with a pistil but no stamens) [21, 29].

Chrysanthemum indicum flowers are compound flowers without borders (cup-shaped). The flowers are cup-shaped consisting of ribbon and tube-shaped flowers (Fig. 1.2.). The flowers grow at the end of the stem, pointing upwards. The ribbon flower filaments extend outward (straight), have a soft texture, oval shape, white color, flat edges, the tip of the ribbon flower is narrow, and the surface of the ribbon flower has clearer lines on the upper surface compared to the lower surface. Ribbon flowers have blunt ends. The color of *Chrysanthemum indicum* ribbon flowers is light purple. The flowers are fragrant, located in a corymbose inflorescence, yellow in color and tubular in structure. The appearance of the flowers and the anatomical structure are shown in figure 1.1.

The structure of *Chrysanthemum indicum* the leaf is an incomplete leaf, having only leaf blades and petioles. The *Chrysanthemum indicum* leaf blade is oval in shape (ovoid) and has an oblong shape (oval). The tip of the leaf is blunt-shaped.

The leaves of the plant are broad, deeply incised, and attached to bushy, thinly hairy stems. The leaves are usually thin and covered with small cracks. During flowering, the leaves are barely visible and are almost completely covered by the round flowers [21, 23, 32].

The *Chrysanthemum indicum* leaves are dark green, deeply cut, and are attached to the bushy shrub's fine-haired stems. When *Chrysanthemum indicum* are in bloom, the leaves are often hardly visible as they are covered up by the abundant, circular flowers.

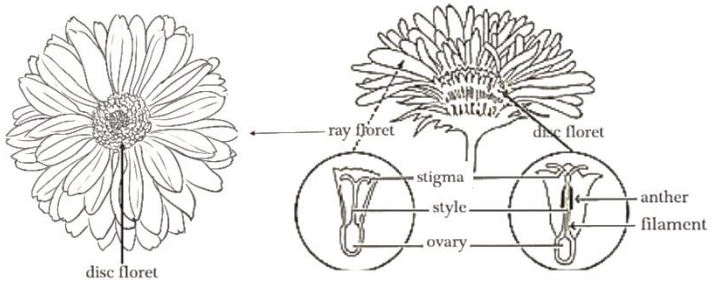

Fig. 1.1. Anatomy of a *Chrysanthemum indicum* flower

A numerous number of chrysanthemum cultivars are found in Bangladesh. Most of them are flowered in winter season.

There is a wide variety of Chrysanthemums in the world, manifested in a large number of varieties depending on the characteristics of growth, size, color and shape of the flowers, making them suitable for any decorative, medicinal purposes.

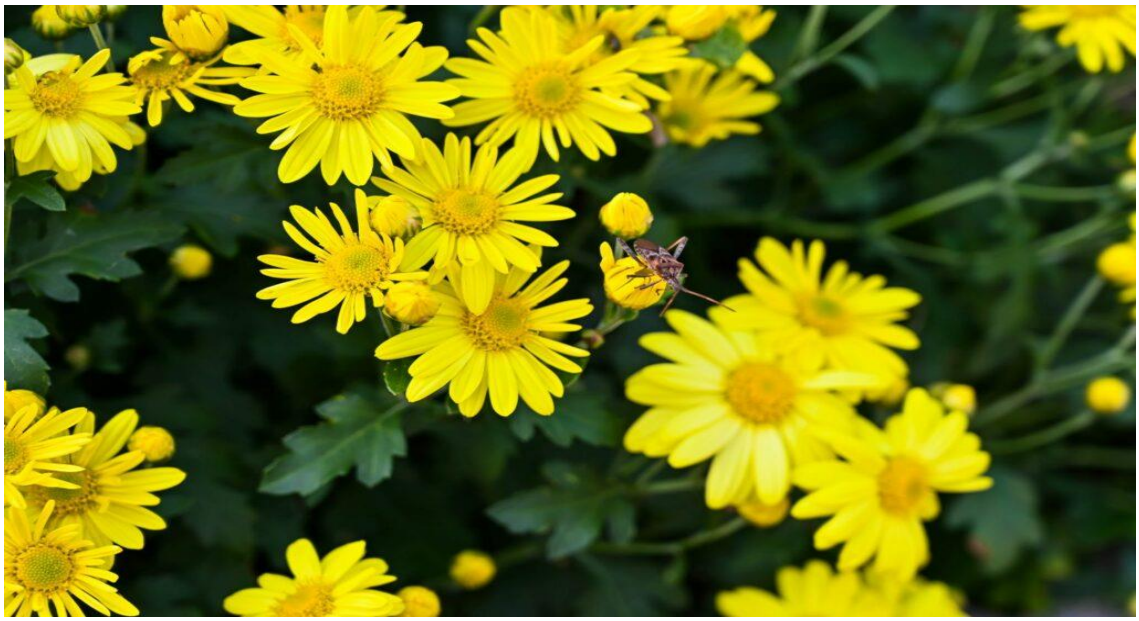

Fig. 1.2. Appearance of *Chrysanthemum indicum* flower

1.2. Geographical distribution

The *Chrysanthemum indicum* was first found back in the 15th century in China. By 1630, more than 500 varieties of chrysanthemums were recorded. By 2014, it was estimated that there were more than 20,000 cultivars in the world and about 7,000 cultivars were native to China [1, 27]. There is evidence in the literature that in Japan, the cultivation of chrysanthemums began in the Nara and Heian periods (early 8th - late 12th centuries) and gained popularity in the Edo period (early 17th - late 19th centuries). Many shapes, colors and varieties of flowers were created. The distribution of the plant is shown in the figure 1.3.

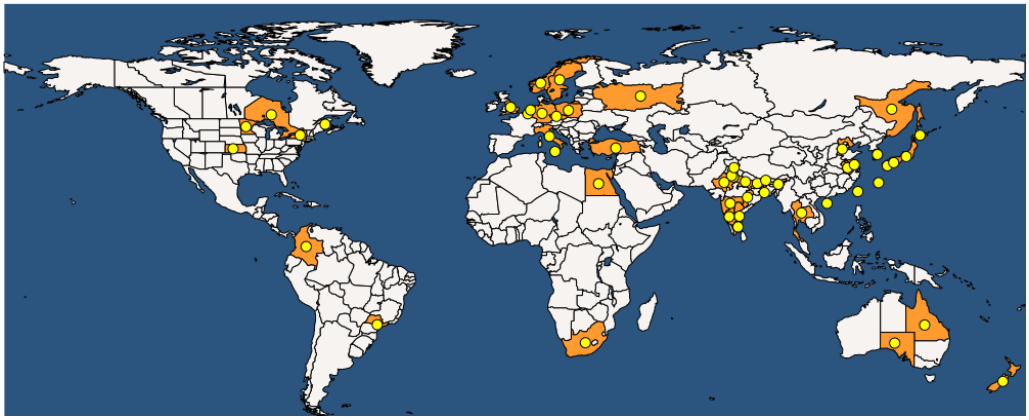

Fig.1.3. Geographical growth of *Chrysanthemum indicum*

1.2 Chemical Composition

Phytochemical investigation of *Chrysanthemum indicum* L. yielded two new quinolinone glycoalkaloids named as Chrysanthemumsides A-B (Figure 1.4.-1.5.) [31, 45, 48]. Also according to the literature, the chemical composition of the flowers of the plant includes: Chrysannol A (Figure 1.6.), Dihydrosyringin (Figure 1.7.), Syringin (Figure 1.8.), Luteoloside (Figure 1.9.), Tricin (Figure 1.10.), Cynarin (Figure 1.11.), Isorhamnetin 3-O-b-D-glucoside (Figure 1.12.), [7, 9, 17, 30, 32, 36, 37].

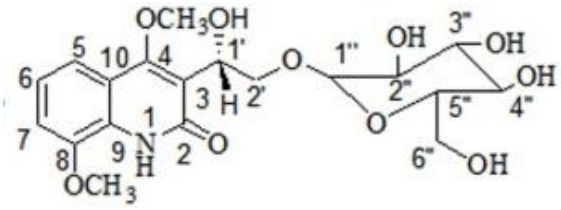

Fig. 1.4. Chrysanthemumsides A

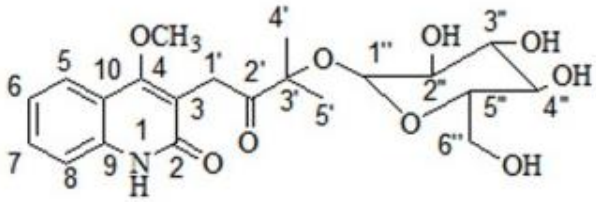

Fig. 1.5. Chrysanthemumsides B

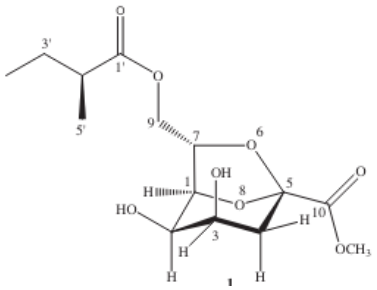

Fig. 1.6. Chrysannol A.

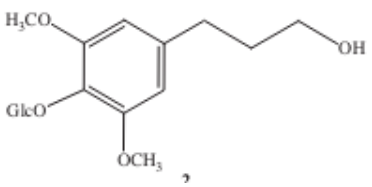

Fig. 1.7. Dihydrosyringin

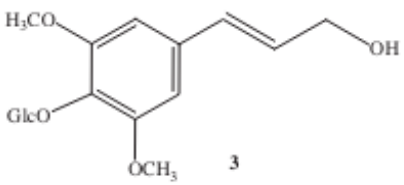

Fig. 1.8. Syringin

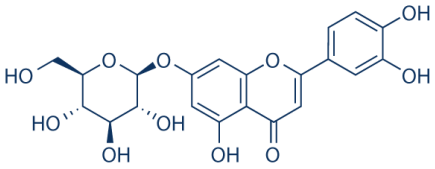

Fig. 1.9. Luteoloside

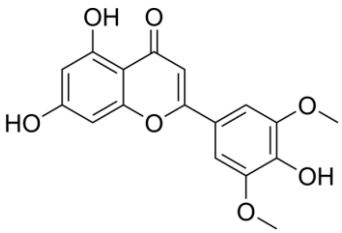

Fig. 1.10. Tricin

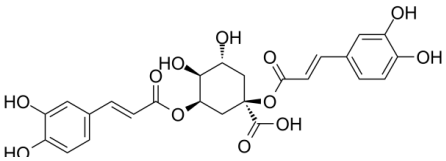

Fig. 1.11. Cynarin

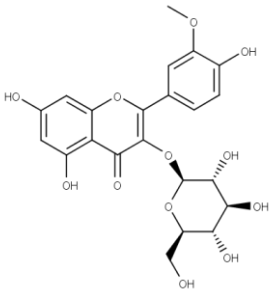

Fig. 1.12. Isorhamnetin 3-O-b-D-glucoside

The plant is rich in essential oil, the main components of which are camphor (Fig. 1.14.), borneol (Fig. 1.15.), camphene (Fig. 1.13.), α-pinene (Fig. 1.16.), p-cymene (Fig. 1.17.) and 1,8 cineole are the major constituents of the oils [32, 33]. Sixty-four compounds were tentatively identified in *Chrysanthemum indicum* with camphor (36.69%) as the major constituent followed by isoborneol (7.64%), α-terpinene (5.73%), and caryophyllene oxide (5.46%), [2, 12, 13, 16, 25, 43, 47].

The aromatic essential oil from *Chrysanthemum indicum* flower heads has a blue color.

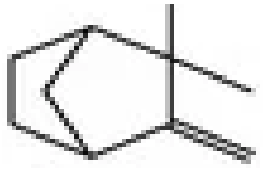

Fig. 1.13. Camphene

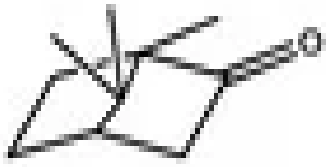

Fig. 1.14. Camphor

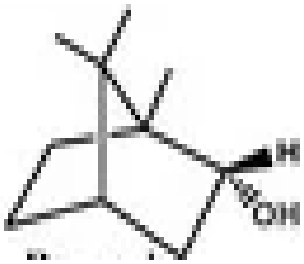

Fig. 1.15. Borneol

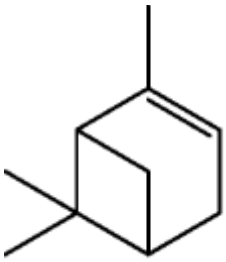

Fig. 1.16. α-pinene

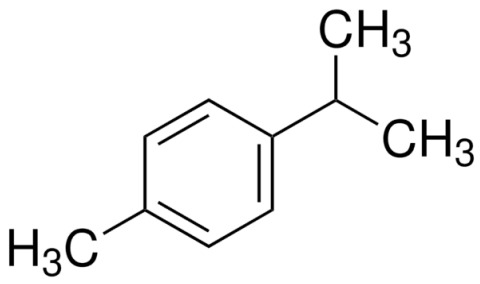

Fig. 1.17. p-cymene

However, the essential oil obtained from the Korean *Chrysanthemum indicum* flower heads revealed nearly the same major constituents in which camphor represents the major component [6, 7, 9, 18, 35, 36, 37]. This greatly highlighted the impact of geographical distribution on the volatile oil compositions.

1.4 Application in Medicine

Chrysanthemum indicum flower is a well-known edible and medicinal plant with small yellow flowers. A medicinal plant with small yellow flowers. The flowers and buds are widely used as a dietary supplement or herbal tea, which many consumers consider a health food. It is also widely used to treat a variety of immune disorders, symptoms of hypertension, and certain infectious diseases such as stomatitis and fever, in the folk medicine of China and Korea for many centuries.

A series of studies have demonstrated that this plant has strong anti-bacterial, anti-viral, anti-oxidant, anti-inflammatory, and immunomodulatory properties [1, 3, 4, 5, 15, 20, 22, 24, 26, 27, 39].

The plant due to the content of essential oil shows antiviral action. The antiviral activity of the essential oils obtained from the flower heads of *Chrysanthemum indicum* was assessed using the plaque reduction assay on Vero cells.

However, both oils in general and the *Chrysanthemum indicum* essential oil in particular, showed substantial antiviral potential in a dose dependent manner

against VSV, HAV and HSV-1. Studies prove that the activity of chrysanthemum oil is stronger than that of clove oil and eucalyptus oil, which have powerful antiviral activity [1, 4, 8, 41, 42, 43]. The significant antiviral activity of the essential oil of the plant can be attributed to the presence of camphor, isoborneol and other major components. Therefore, the essential oil of the plant can be used for the treatment of herpes. In addition, isoborneol that the plant contains was found to inhibit the replication of herpes simplex virus-1 through specific inhibition of glycosylation of its polypeptides [6, 7, 38, 39, 40, 41].

The research also revealed a high level of antibacterial activity of the plant's essential oil. Scientists used the in vitro agar diffusion method against various standard Gram-positive and Gram-negative bacteria (Bacillus subtilis, Streptococcus), measuring the average diameter of inhibition zones and determining the minimum inhibition concentration. Thus the essential oil of the plant can be used to treat fungal diseases [8, 24, 26, 38, 40].

Korean scientists conducted a study of *Chrysanthemum indicum* essential oil grown in Korea, the study demonstrated significant antimicrobial potential against all oral bacteria tested [35, 40, 44].

Eir oil of the plant exhibits high antimycobacterial and anti-Helicobacter activity against Helicobacter pylori, M. tuberculosis [14, 47, 49].

The observed antimycobacterial and anti-Helicobacter pylori potential of essential oil is mainly attributed to the presence of terpenes and fatty acids, which account for lipophilicity and deeper penetration through the cell membrane, causing massive disturbances in oxidative phosphorylation and electron transfer chain, and leading to severe interference in energy production with the concomitant evolution of auto-oxidation and peroxidative degradation of compounds and lysis of bacteria [14, 15, 16, 17, 18].

A decoction of *Chrysanthemum indicum* leaves is widely used in folk medicine to treat colds, headaches, bronchitis, rheumatism, edema and boils, as the leaf content is enriched with antibacterial properties [10, 11, 20, 22]. The plant has a strong aroma due to its essential oil, which is used in flavors and perfumes.

1.5 Application in Culinary

Chrysanthemum indicum, is very often used in cooking in China and Japan. The plant is also known as the "garland chrysanthemum" or "edible chrysanthemum" and is widely used in East Asian cooking. Its leaves are tender and have a slightly bitter taste, similar to spinach or mustard greens. They can be used fresh in salads or fried with other vegetables. In addition, chrysanthemum flower petals can be used to decorate dishes, adding color and a subtle floral aroma [11, 14, 38]**.**

Conclusions

1. The literature data on the botanical characteristics, geographical distribution, chemical composition, use in medicine and cooking of the *Chrysanthemum indicum* are analyzed.

2. According to the literature review, *Chrysanthemum indicum* is a promising source, its plant material contains various groups of biologically active compounds. A detailed study of these compounds is a relevant and promising area of phytochemistry**.**

CHAPTER 2
DEFINITION OF QUALITY INDICATORS IN *CHRYSANTHEMUM INDICUM* RAW MATERIALS

2.1. Characterization of the research subject

The object of the study was the herb *Chrysanthemum indica*, which was collected during flowering in June 2023. After harvesting, the plant was dried at room temperature at 20-25 degrees, crushed, and extracts were obtained for research. The appearance of the dried plant, raw materials and extract is shown in figure 2.1.

Fig. 2.1**.** Appearance of dried plant, raw material and extract.

2.2. Determination of Total Ash content

Total ash in the herb was investigated using the following methodology.

3 g of the chopped plant herb was placed in a pre-calcined and accurately weighed porcelain crucible, spreading the substance evenly over the bottom of the crucible. The crucible was then gently heated on an electric stove in a fume hood, allowing the substance to burn at a lower temperature first. The remaining charcoal

particles should be burned at a lower temperature; after the charcoal was almost completely burned, the flame was increased.

If the coal particles are incompletely burned, the residue is cooled, moistened with water or ammonium nitrate solution, evaporated in a water bath, and the residue is calcined. If necessary, this operation is repeated several times.

The calcination was continued in a muffle furnace at red heat (about 500 ºC) to a constant weight, preventing the ash from fusing with the crucible walls. Upon completion, the crucible was cooled in a desiccator and weighed. The calcination was carried out to a constant mass. Constant mass was considered to be achieved if the difference between two consecutive weighings after 30 minutes of calcination and 30 minutes of cooling in the desiccator did not exceed 0.0005 g.

The ash content was calculated using the formula:

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

where: X is the ash content of the feedstock, %;

m - weight of raw materials before combustion, g;

m_1 - weight of raw materials after combustion,

W - mass loss during drying of raw materials, %.

The results of determination of total ash in *Chrysanthemum indicum* herb are given in the table 2.1 below.

Table.2.1

Results of determination of total ash in *Chrysanthemum indicum* herb

m	n	X_i	X med.	S^2	S mean	P	T(P, n)	Confidence interval	ε_, %
5	4	4,33 4,30 4,34 4,35 4,40	4,36	0,020600	0,064175	0,95	2,78	4,36 ± 0,16	4,00

As a result of the determination, it was found that the total ash content in *Chrysanthemum indicum* herb – 4,36 ± 0,16 %.

2.3. Determination of Mass Loss During drying

Loss in mass during drying in *Chrysanthemum indicum* herb was determined using the following methodology.

The loss in mass during drying of raw materials is understood as the loss in mass due to hygroscopic moisture and volatile substances, which is determined in raw materials when dried to a constant mass.

The analytical sample of the raw material (grass) was crushed to a particle size of about 10 mm, mixed and a weight of 3-5 g was taken, with an error of 0.01 g. The sample was placed in a previously dried and weighed bullion bottle with a lid and placed in a drying oven heated to 100-105 ºC. The drying time was counted from the moment when the temperature in the drying oven reached 100-105 ºC again. The first weighing was performed after 2 hours.

Drying was carried out until a constant weight. Constant mass is considered to be achieved when the difference between two subsequent weighings after 30 minutes of drying and 30 minutes of cooling in the desiccator does not exceed 0.01 g. The determination of the loss in mass during drying for the conversion of the amount of active substances and ash to absolutely dry raw materials was carried out in 2.0 g (exact weighing) taken from the analytical sample intended for determining the content of ash and active substances by the method described above, but with a difference between weighings not exceeding 0.0005 g.

The mass loss during drying in raw materials (X) in percent was calculated by the formula:

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

where: m is the mass of raw materials before drying, g

m_1 - weight of raw materials after drying, g;

X - weight loss during drying, %.

The results of determining the loss in weight during drying in the herb of *Chrysanthemum indicum* were statistically processed and reliable, presented in the table 2.2.

Table.2.2

Results of determining weight loss during drying in *Chrysanthemum indicum* herb

m	n	X_i	X mean	S^2	S mean	P	T(P, n)	Confidence interval	ε_, %
5	4	5,89 6,10 6,26 6,30 6,50	6,19	0,0453600	0,095247	0,95	2,78	6,19±0,20	4,20

As a result of the determination, it was found that the loss in weight during drying in the plant's grass is 6,19 ± 0,20 %.

2.4. Determination of Extractives

The next stage of our work was to determine the extractive substances in Indian Chrysanthemum herb.

For this purpose, the following methodology was used 1.0 g (exact weight) of chopped grass, sifted through a sieve with 1 mm holes, was placed in a 200-250 ml conical flask, 50 ml of different solvents of water and ethanol of different concentrations were added, the flask was closed with a stopper, weighed, and left for one hour. The flasks were then combined and heated for 2 hours. After cooling, the contents of the flasks were weighed again, having previously been sealed with the same stopper, and the loss in weight was made up with solvent. The contents of

the flasks were shaken thoroughly and filtered through a dry paper filter into dry 150-200 ml flasks.

A pipette was used to take 25 ml of the filtrate, transfer it to a porcelain cup 7-9 cm in diameter, dried at 100-105 °C to a constant weight and accurately weighed, and evaporated in a water bath to dryness. The cup with the residue was dried at 100-105 °C to a constant weight, then cooled for 30 minutes in a desiccator with anhydrous calcium chloride and immediately weighed.

The percentage content of extractive substances (X, %) in terms of absolute dry matter was calculated using the formula:

$$X = m \times 200 \times 100 / m_1 \times (100 - W);$$

where: m is the mass of dry residue, g;

m_1 - weight of raw materials, g;

W - weight loss during drying of raw materials, %.

The extractive substances in the herb were determined with water and mixtures of water and ethanol with increasing alcohol concentration.

The results of the determination of extractive substances in raw materials are statistically processed and reliable, presented in the table 2.3.-2.4.

Table 2.3

Results of determination of extractive substances in *Chrysanthemum indicum* herb by extraction with purified water

m	n	X_i	X mean	S^2	S mean	P	T (P, n)	Confidence interval	ε_, %
5	4	29,00	28,88	1,11250	0,46960	0,95	2,78	28,88±1,33	4,25
		28,29							
		27,94							
		29,99							
		30,80							

Table 2.4

Results of determination of extractive substances in *Chrysanthemum indicum* herb, extractant ethyl alcohol

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
Extractant 10 % ethyl alcohol									
5	4	30,99 31,71 32,03 33,44 34,15	30,20	1,479450	0,54397	0,95	2,78	30,20±1,49	4,45
Extractant 30 % ethyl alcohol									
5	4	29,34 32,93 31,80 34,10 33,78	34,30	1,54010	0,57274	0,95	2,78	34,30±1,60	4,98
Extractant 50 % ethyl alcohol									
5	4	50,29 51,85 52,50 49,48 48,73	50,70	2,92250	0,76452	0,95	2,78	50,70±2,09	4,03
Extractant 70 % ethyl alcohol									
5	4	26,00 25,80 25,94 27,09 26,11	25,31	0,71890	0,40478	0,95	2,78	25,31±1,10	4,20

Continued table 2.4

1	2	3	4	5	6	7	8	9	10
Extractant 96 % ethyl alcohol									
5	4	24,50 25,60 27,61 28,40 29,90	24,67	0,88970	0,39740	0,95	2,78	24,67±1,09	4,09

The maximum amount of extractive substances in *Chrysanthemum indicum* herb was observed when using 50 % ethyl alcohol as an extractant (50,70 ± 2,09%). The lowest amount of extractive substances was observed in the extraction with 96 % ethyl alcohol – 24,67 ± 1,09 %.

Conclusions

The main numerical parameters of the *Chrysanthemum indicum* herb were determined: total ash (4,36 ± 0,16 %), weight loss during drying (6,19 ± 0,20 %), extractive substances, the maximum extractant was set at 50 % ethanol, the amount of extractives was – 50,70 ± 2,09 %.

CHAPTER 3
STUDY OF THE CHEMICAL COMPOSITION OF THE RAW MATERIAL OF *CHRYSANTHEMUM INDIAN*

3.1 Study of Polysaccharides

The preliminary detection of polysaccharides was carried out using various qualitative methods, in particular: 96 % ethanol was added to the aqueous extract of *Chrysanthemum indicum* herb. As a result, the formation of a voluminous white precipitate of polysaccharides was observed.

The identification of polysaccharides was performed using chromatographic analysis, which is shown in Fig. 3.1.

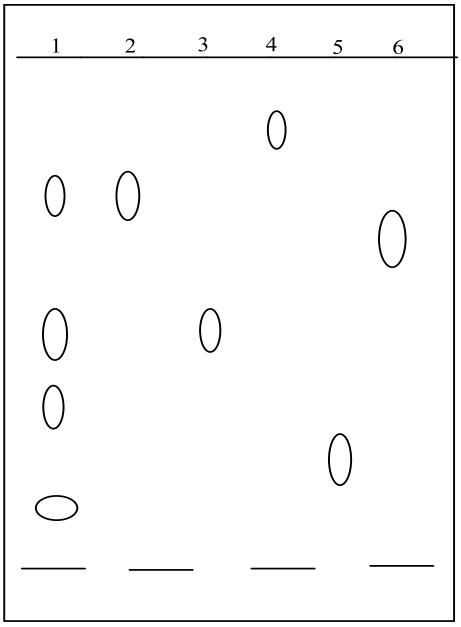

Fig. 3.1. Schematic diagram of chromatogram for determination of monosaccharide composition in herb *Chrysanthemum indian.*

1 - aqueous extraction of raw materials; 2 - glucose; 3 - galactose; 4 - ribose; 5 - rhamnose; 6 - mannose.

Chromatography method: descending.

Mobile phase: acetone-n-butanol-water (7:2:1).

Display reagent: anilinphthalate

The chromatographic study identified glucose and galactose in the aqueous extract from the herb.

The next step of our work was the quantification of polysaccharides using the following methodology: 20 g of the crushed herb is transferred to a 250 ml volumetric flask, 200 ml of water is added, and the flask is heated with stirring for 30 minutes. The extraction was carried out twice more, using 200 ml the first time and 100 ml of water the second time. The aqueous extracts were combined, centrifuged and decanted into a 500 ml volumetric flask through 5 layers of gauze,

which was inserted into a 55 ml glass funnel with a diameter of 55 ml and pre-washed with water. The filter was rinsed with water and the volume was made up to the mark (solution A).

25 ml of solution A was transferred to a centrifuge tube, 75 ml of 95 % ethyl alcohol was added, stirred, and heated in a water bath to 30 °C for 5 minutes. After 1 hour, the contents of the tube were centrifuged at 5000 rpm for 30 minutes. The supernatant from solution A was filtered under vacuum at a residual pressure of 13-16 kPa through a 40 mm diameter glass filter POR-16 dried to a constant mass at a temperature of 100-105 °C. The precipitate from the solution was quantitatively transferred to the filter, washed sequentially with 15 ml of a solution of 95 % ethyl alcohol in water (3:1 ratio), 10 ml of acetone and 10 ml of ethyl acetate. The filter with the precipitate was dried in air and then at a temperature of 100-105 °C to a constant weight.

The percentage of polysaccharides in terms of absolute dry matter (X, %) is calculated using the formula:

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

where: m_1 is the mass of the filter, g

m_2 - mass of the filter with sediment, g;

m - weight of raw materials, g;

W - weight loss during drying, %.

The results of the quantitative determination of polysaccharides in the herb of *Chrysanthemum indicum* are presented in the table 3.1.

Table 3.1

The results of the quantitative determination of polysaccharides in the herb of *Chrysanthemum indicum*

m	n	X_i	X mean	S^2	S mean	P	T (P, n)	Confidence interval	ε_, %
5	4	5,53	5,89	0,045340	0,0972707	0,95	2,78	5,89 ±0,25	4,49
		5,79							
		5,90							
		5,85							
		6,02							

The content of polysaccharides in *Chrysanthemum indicum* herb in terms of absolutely dry raw materials is 5,89 ± 0,25%.

3.2 Study of Organic acids

For the detection of organic acids in the herb of *Chrysanthemum indica* used chromatography on paper in mobile phases 96% ethanol - chloroform - ammonia concentrated - water (70:40:20:2) in comparison with standard samples carried out detection and identification of free organic acids in the herb of the plant. To detect organic acids, the dried chromatogram was treated with bromthymol blue solution followed by heating at 100–105 °C until the appearance of yellow or white (ascorbic acid) zones on the blue background. A schematic of the chromatogram is shown in Fig. 3.2.

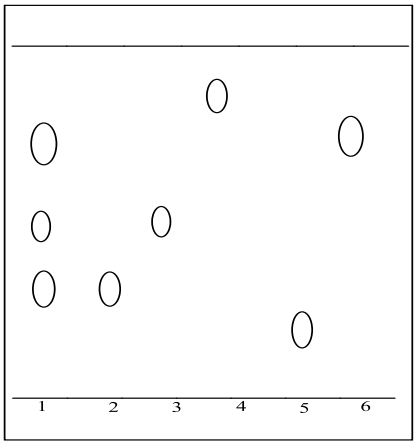

Fig. 3.2. Schematic diagram of chromatogram of free organic acids detection in *Chrysanthemum indicum* herb: 1 - aqueous extraction of the herb, 2 - oxalic acid 3 - ascorbic acid; 4 - tartaric acid; 5 - citric acid; 6 - malic acid. Mobile phase: 96 % ethanol - chloroform - concentrated ammonia - water (70:40:20:2). Showing reagent: bromthymol blue solution, heating at 100 °C.

As a result of chromatographic analysis, the following were identified in *Chrysanthemum indicum* herb: ascorbic acid, oxalic acid and malic acid.

The next stage of our work was the quantitative determination of organic acids, for this purpose the following methodology was used.

5.0 g (exact weight) of the crushed herb was transferred to a 250 ml flask, poured with 200 ml of water and kept in a water bath for 2 hours, then cooled, quantitatively transferred to a 250 ml volumetric flask, brought to the mark and stirred.

10 ml of the extract was taken, transferred to a 500 ml flask, 200-300 ml of freshly boiled water, 2 drops of 1% alcohol solution of phenolphthalein, 2 drops of 0.1% methylene blue solution were added and titrated with sodium hydroxide solution (0.1 mol/l) until the foam turned purple-red.

The content of free organic acids in terms of malic acid in absolutely dry raw materials in percent (X. %) was calculated by the formula:

$$X = \frac{V \cdot 0{,}0067 \cdot 250 \cdot 100 \cdot 100}{m \cdot 10 \cdot (100 - W)};$$

where: 0.0067 is the amount of malic acid corresponding to 1 ml of sodium hydroxide solution (0.1 mol/L), g;

V – is the volume of sodium hydroxide solution used for titration, ml;

m – is the mass of raw materials, g;

W - weight loss during drying of the raw material, %.

The results of the quantitative determination of free organic acids in the herb are presented in the tables 3.2.

Table 3.2

The results of quantitative determination of free organic acids in the herb of *Chrysanthemum indicum*

m	n	X_i	X mean	S^2	S mean	P	T (P, n)	Confidence interval	ε_, %
5	4	2,40 2,39 2,45 2,49 2,60	2,39	0,008399800	0,03849	0,95	2,78	2,39 ±0,09	4,30

The content of free organic acids in the herb in terms of malic acid and absolutely dry raw materials is 2,39 ± 0,09%.

3.3 Study of Hydroxycinnamic acids

Aqueous extract of *Chrysanthemum indicum* herb was used for the identification of hydroxycinnamic acids. The study of hydroxycinnamic acids in the herb was carried out by chromatography on FN-1 paper in the solvent system 15 % acetic acid in comparison with pharmacopoeial standard samples of hydroxycinnamic acids. After chromatography, the chromatogram was dried in a fume hood at room temperature and viewed in UV light before and after treatment with ammonia vapor. When analyzing the chromatogram, the zones of hydroxycinnamic acids were detected, which fluoresced blue in ultraviolet light. After treatment of the chromatogram with ammonia vapor their fluorescence increased, with chlorogenic and non-chlorogenic acids acquiring green-blue coloring, and caffeic acid having bright blue fluorescence.

The chromatogram was also treated with alcoholic 1% iron (III) chloride solution followed by heating in a desiccator at 100-105 ^{0}C for 5 minutes to detect

gallic acid. As a result, gallic acid in UV light had no fluorescence, and after treatment with the reagent appeared on the chromatogram as a dark gray zone.

A schematic diagram of the chromatogram for the detection and identification of hydroxycinnamic acids in *Chrysanthemum indicum* herb is shown in Fig.3.3.

As shown in Figure 3.3, the chromatographic study of hydroxycinnamic acids in the herb of the plant identified caffeic acid, chlorogenic acid, and neochlorogenic acid. The structural formulae of the identified hydroxycinnamic acids are shown in Fig. 3.4–3.7.

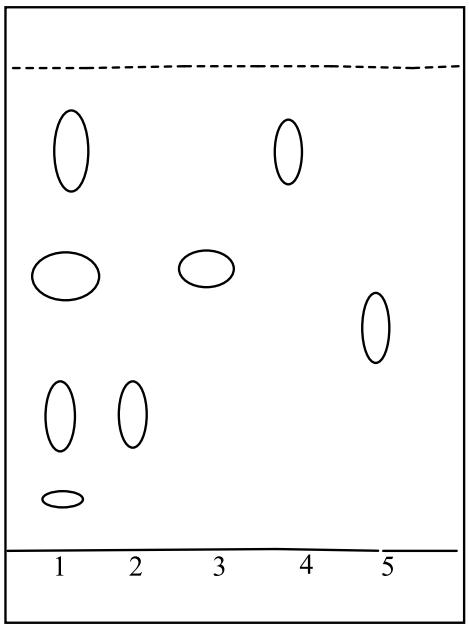

Fig. 3.3. Schematic diagram of the chromatogram of hydroxycinnamic acids in *Chrysanthemum indicum* herb:

1 - aqueous extract of herb, 2 - caffeic acid, 3 - chlorogenic acid, 4 - non-chlorogenic acid, 5 - ferulic acid.

The mobile phase is 15% acetic acid.

The manifestation reagent is 1 % iron (III) chloride solution.

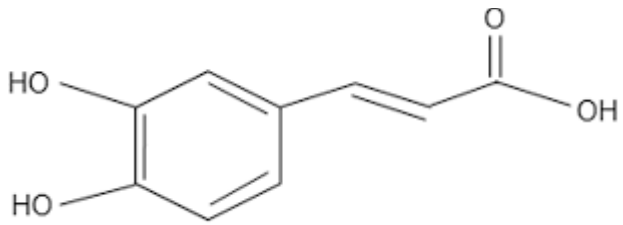

Fig. 3.4. Structural formula of caffeic acid

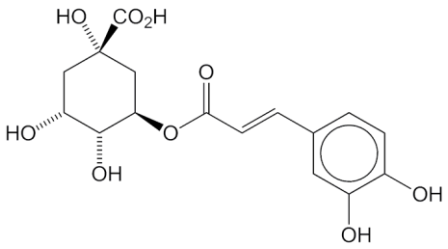

Fig. 3.5. Structural formula of chlorogenic acid

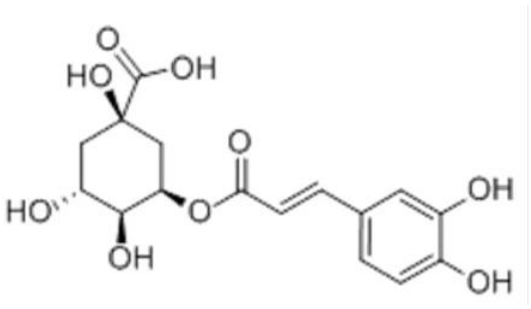

Fig. 3.6. Structural formula of non-chlorogenic acid

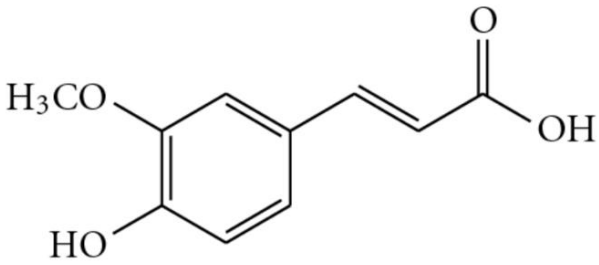

Fig. 3.7. Structural formula of ferulic acid

The next stage of our work was the quantitative determination of hydroxycinnamic acids, which was carried out using the spectrophotometric method.

To do this, 2.0 g of the chopped herb of the plant was taken, passed through a sieve with 1 mm holes, placed in a 200 ml flask and 70 ml of water was added. The flask was then heated for 15 min. The extraction was performed twice more. The extracts were cooled and filtered through paper filters on a Büchner funnel. The extracts were quantitatively transferred to a 200 mL volumetric flask and the volume of the solution was made up to the mark with water (solution A).

Into a 50-ml volumetric flask, 3 ml of solution A was added and the solution was made up to 20% with ethyl alcohol. The optical density of the resulting solution was measured on a spectrophotometer at a wavelength of 327 nm. The reference solution was 20% ethanol.

The content of the sum of hydroxycinnamic acids in terms of chlorogenic acid and absolutely dry raw materials in percent (X, %) was calculated by the formula:

$$X = \frac{A \cdot 200 \cdot 50 \cdot 100}{E_{1см}^{1\%} \cdot m \cdot 1 \cdot (100 - W)}$$

where: A – is the optical density of the solution under study, nm;

m – is the weight of raw materials, g;

W – weight loss during drying, %;

$E_{1см}^{1\%}$ – specific absorption coefficient of chlorogenic acid, equal to 531.

The results of the quantitative determination of the amount of hydroxycinnamic acids in the plant herb are shown in the table 3.3.

Table 3.3

The results of quantitative determination of the amount of hydroxycinnamic acids in the herb of *Chrysanthemum indica*

m	n	X_i	X mean	S^2	S mean	P	T (P, n)	Confidence interval	ε_, %
5	4	0,48	0,48	0,0024400	0,023046	0,95	2,78	0,48 ± 0,02	1,09
		0,50							
		0,55							
		0,49							
		0,51							

According to the results of the study, the content of the sum of hydroxycinnamic acids in the herb of *Chrysanthemum indicum* in terms of chlorogenic acid and absolutely dry raw materials is 0,48 ± 0,02 %.

3.4 Study of Amino acids

Detection of amino acids in *Chrysanthemum indicum* herb was carried out using 0.2% ethanol ninhydrin solution. When the reagent was added to the aqueous extract of the herb, the formation of red-violet coloration was observed.
followed by heating at 100-105°C until the violet and red-violet zones, which corresponded to amino acids, were detected.

Chromatographic study of amino acids in *Chrysanthemum indicum* herb was also carried out. Detection and identification of free amino acids in the herb were carried out by chromatography on paper with standard samples. The mobile phase was a mixture of n-butanol - glacial acetic acid - water (4:1:2). To detect amino acids, the dried chromatogram was treated with 0.2 % ethanol ninhydrin solution followed by heating at 100-105°C until the violet and red-violet zones, which corresponded to amino acids, were detected (Fig.3.8.).

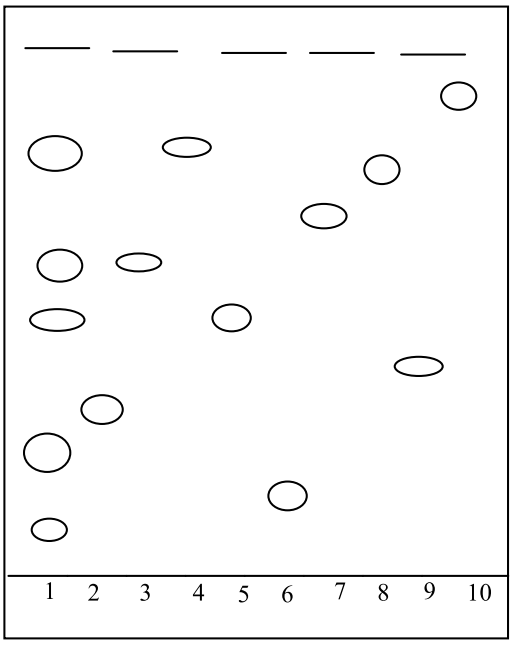

Fig. 3.8. Scheme of chromatogram for detection of free amino acids in *Chrysanthemum indicum* herb: 1 - aqueous extraction of leaves, 2 - cysteine; 3 - leucine; 4 - phenylalanine; 5 - glutamic acid; 6 - valine; 7 - methionine; 8 - threonine; 9 - lysine; 10 - tryptophan.
Mobile phase: n-butanol - glacial acetic acid - water (4:1:2).
Display reagent: 0.2% ethanol solution of ninhydrin, heating at 100-105°C.

By comparing Rf values and staining zones with standard samples of amino acids in aqueous extracts of herbs, the following were identified: glutamic acid, leucine, phenylalanine.

The next stage of our work was quantitative determination of free amino acids, which was carried out by spectrophotometric method. Purified water was

used for extraction of raw materials. Extraction was carried out under heating. The cooled extracts were filtered into a measuring flask and brought to 50 ml with purified water. 1 ml of the solution was placed in a 10 ml flask, 8 ml of 0.2 % ninhydrin solution in isopropyl alcohol was added and heated for 5 min. Quantitatively transferred to a 25 mL volumetric flask and brought to the mark with isopropyl alcohol. The optical density was measured at a wavelength of 573 nm. The compensation solution consisted of 8 mL of 0.2 % ninhydrin solution in isopropyl alcohol, brought to the mark in a 25 mL volumetric flask with the same solvent. The content of the sum of amino acids (X, %) in *Chrysanthemum indicum* herb in terms of leucine and total dry matter was calculated according to the formula:

$$X = \frac{A \cdot 50 \cdot 25 \cdot 100}{825 \cdot m \cdot 1 \cdot (100 - W)};$$

where: A - optical density of the test solution; m - weight of the raw material suspension, g; 862 - specific absorption index of the complex of leucine with ninhydrin at a wavelength of 573 nm; W - loss in mass during drying of raw materials, %.

The results of determination of amino acids in the herb of the plant are given in Tab.3.4.

Table 3.4

Results of quantitative determination of amino acids in *Chrysanthemum indicum* herb

m	n	X_i	X mean	S^2	S mean	P	T (P, n)	Confidence interval	ε_, %
5	4	0,031	0,03	0,000080	0,0003841	0,95	2,78	0,03± 0,00	4,70
		0,033							
		0,032							
		0,033							
		0,030							

According to the results of the study, the content of amino acids in in *Chrysanthemum indicum* herb in terms of absolutely dry raw materials is – 0,03± 0,002%**.**

3.5 Study of Flavonoids

Various chemical reactions were used to detect the flavonoid content of *Chrysanthemum indicum* herb.

For this purpose, 10.0 g of crushed herb was extracted with 100 ml of 70% ethyl alcohol for 30 minutes. Then the extract was cooled and filtered. The presence of this group of substances was determined using well-known qualitative reactions

When cyanidine reaction is performed - red coloration appears in the ethanol extract from the herb of the plant. The cyanidine reaction was also performed according to Briant, and a red coloration of the aqueous layer was observed, which was more intense compared to the organic one, which confirmed the presence of flavonoids.

When the reaction with iron (IIII) chloride solution is carried out, dark green coloring is formed.

When potassium hydroxide solution is added to the test tube with herb extract, yellow-brown coloring is formed.

Addition of aluminum chloride solution to the extract of *Chrysanthemum indicum* herb results in yellow-green staining.

When 2 ml of lead acetate solution was added to the test tube with the herb extract, a yellow precipitate was observed to form.

The qualitative reactions carried out confirm the presence of flavonoids in the herb of the plant.

The next stage of our work was the identification of flavonoids in the herb of *Chrysanthemum indicum* (Fig. 3.9)

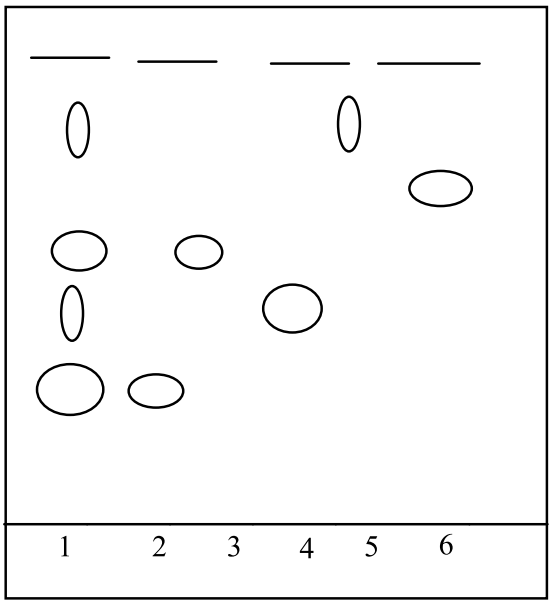

Fig. 3.9. Schematic diagram of chromatogram identification of flavonoids in *Chrysanthemum indicum* herb.

1 – alcoholic extract of Chrysanthemum indicum herb; 2 – rutin; 3 – quercetin; 4 – apigenin; 5 – hyperoside; 6 – luteolin.

Movable phase: formic acid – anhydrous – water – methyl acetate (10:10:80).

As a result of chromatographic analysis, the following were identified in *Chrysanthemum indicum* herb: rutin, quercetin, apigenin, hyperoside.

The formulas of the identified flavonoids are shown in Fig.3.10.

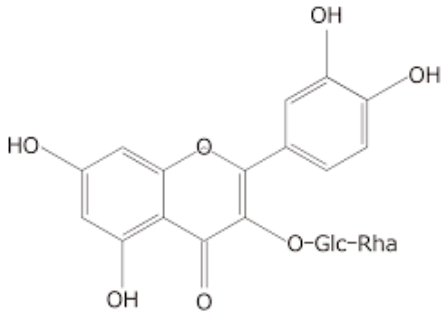

Fig. 3.10. Structural formula of rutin

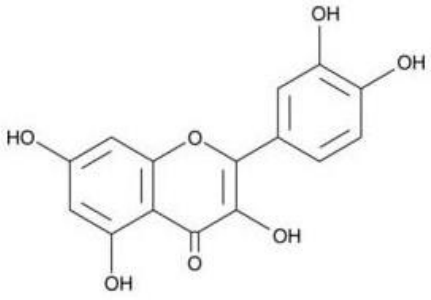

Fig. 3.11. Structural formula of quercetin

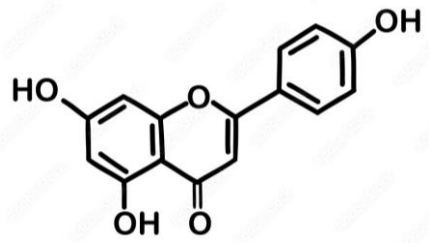

Fig. 3.12. Structural formula of apigenin

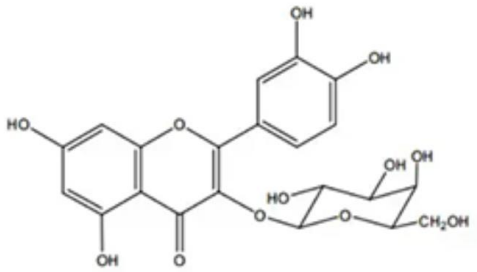

Fig. 3.13. Structural formula of hyperoside

The next stage of our work was the quantification of flavonoids in *Chrysanthemum indicum* herb.

To do this, 1.0 g of chopped grass passing through a sieve with 2 mm diameter holes was weighed, placed in a 150 ml flask with a grinder, and 30 ml of 50 % ethyl alcohol was added. The flask was heated for 30 minutes, periodically shaken to wash off the raw material particles from the flask walls. The hot extract was filtered into a 100 mL volumetric flask, the extraction was repeated twice more under the conditions described above, filtering the extract into the same volumetric flask. After cooling, the volume of the extract was made up to the mark with 50 % ethyl alcohol and mixed (solution A).

In a 25-ml volumetric flask, 1 ml of solution A, 1 ml of aluminum chloride solution in 95 % alcohol were transferred to a volumetric flask and the volume of the solution was made up to the mark with 95 % ethyl alcohol. After 40 minutes, the optical density of the solution was measured on a spectrophotometer at a wavelength of 415 nm in a cuvette with a layer thickness of 10 mm. A solution consisting of 1 ml of the herb extract, 1 drop of diluted acetic acid and adjusted to the mark with 95 % ethyl alcohol in a 25 ml volumetric flask was used as a comparison solution.

In parallel, the optical density of the State Standard Reference Material (SRM) of rutin, which is prepared similarly to the solution under study, was measured.

The content of the sum of flavonoids in the herb of *Chrysanthemum indicum* in terms of rutin and absolutely dry raw materials in percent (X, %) was calculated by the formula:

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

where: A – is the optical density of the solution under study;

A_0 – is the optical density of the DSA of the rutin;

m – is the mass of raw materials, g;

m_0 – is the mass of rutin DSO, g;

W – is the mass loss during drying of the raw material, %.

The results of the quantitative determination of the amount of flavonoids in the herb of *Chrysanthemum indicum* are presented in the table 3.5.

Table 3.5

Results of quantitative determination of flavonoids in *Chrysanthemum indicum* herb

m	n	X_i	X mean	S^2	S mean	P	T (P, n)	Confidence interval	ε_, %
5	4	3,14	3,19	0,0026500	0,0226932	0,95	2,78	3,19 ± 0,05	1,99
		3,18							
		3,20							
		3,17							
		3,33							

According to the results of the study, the content of the sum of flavonoids in terms of rutin and absolutely dry raw materials in the herb of *Chrysanthemum indicum* is 3,19 ± 0,05 %.

3.6. Study of Tannins

Various qualitative reactions were used to determine the presence of tannins in *Chrysanthemum indicum* herb. To do this, 5,0 g of crushed herb was weighed, placed in a 250 ml flask, and poured with 100 ml of hot water. It was heated for 30 minutes. Then the contents of the flask were cooled and filtered. Detection of tannins was performed using general precipitation and color reactions.

General precipitation reactions : 1. An equal amount of fresh 0,5 % gelatin solution and one drop of 10 % hydrochloric acid solution were added dropwise to 2 ml of the herb extract to increase the sensitivity of the reaction. 2. A few drops of a 1% quinine hydrochloride solution were added to 2 ml of the herbal extract. The reaction resulted in the formation of an amorphous precipitate, which confirmed the presence of tannins. 3. To 1 ml of the extract was added 2 ml of 10 % acetic acid and 1 ml of 10 % medium salt of lead acetate. No changes occurred as a result of the reaction.

Color reaction: 1. A solution of ferric ammonium alum was added dropwise to 2 ml of the herb extract, as a result of the reaction, a dark green color appeared in the test tube.

The conducted qualitative reactions indicate the overwhelming majority of tannins of condensed nature in the herb of *Chrysanthemum indicum*.

The next stage of our work was the quantitative determination of tannins in *Chrysanthemum indicum* herb using the spectrophotometric method.

To do this, 1,0 g of raw material was taken, placed in a 100-ml conical flask with a lapped stopper, and poured with 30 ml of 70% ethanol. The flask was then heated in a water bath at 50-60 °C for 30 minutes. The extraction was performed three times. The resulting extract was filtered through a dry paper filter into a 100 ml dry flask, and made up to the mark with 70% ethanol (solution A).

A pipette was used to take 3 ml of the filtrate, transfer it to a 25 ml volumetric flask, and bring it to the mark with 95% ethanol. The optical density of the resulting solution was measured on a spectrophotometer SF-46 at a wavelength of 271 nm in a cuvette with a layer thickness of 10 mm. The reference solution was 95% ethanol. In parallel, the optical density of the solution of the Pharmacopoeial Standard Reference Material (PRM) of gallic acid was measured.

The content of the sum of polyphenols (X, %) in terms of gallic acid and absolutely dry raw materials was calculated by the formula:

$$X = \frac{A \cdot m_{\underline{0}} \cdot 100 \cdot 25 \cdot 0.25 \cdot 100 \cdot 100}{A_0 \cdot m \cdot 3 \cdot 25 \cdot 25 \cdot (100 - W)};$$

where A – is the optical density of the solution under study;

A_o – is the optical density of the FSS of gallic acid;

m_o – is the weight of the standard solution, g;

W – weight loss during drying, %;

m – is the weight of the raw material sample, g.

The results of the quantitative determination of tannins in the herb of *Chrysanthemum indicum* are shown in table 3.6.

Table 3.6

Results of quantitative determination of tannins in *Chrysanthemum indicum* herb

m	n	X_i	X mean	S^2	S mean	P	T (P, n)	Confidence interval	ε_, %
5	4	1,30 1,33 1,40 1,35 1,37	1,33	0,000340000	0,0094877	0,95	2,78	1,33±0,04	1,94

The content of the sum of polyphenolic compounds in *Chrysanthemum indicum* herb in terms of gallic acid and absolutely dry raw materials is 1,33 ± 0,04 %.

3.7. Research on Chlorophylls and Carotenoids

Quantitative determination of chlorophylls and carotenoids in *Chrysanthemum indicum* herb was carried out by spectrophotometric method. 96% ethanol was used for extraction. Extraction was carried out until the extractant was completely discolored. The extracts were filtered into a measuring flask and brought up to 25 ml with 96 % ethanol. The optical density of chlorophylls *a* and *b* and carotenoids was determined by spectrophotometric method at a wavelength of 665 nm for chlorophyll a, 649 nm for chlorophyll b, and 441 nm for carotenoids.

Compensation solution was 96 % ethanol. The concentration of chlorophylls *a* (Ca, mg/l) and *b* (Cb, mg/l) and their total content (Ca+b, mg/l) were calculated according to the formula:

$$C_a = 13{,}70 \times A665 - 5{,}76 \times A649;$$

$$C_b = 25{,}80 \times A649 - 7{,}60 \times A665;$$

$$C_{(a+b)} = 6{,}10 \times A665 + 20{,}04 \times A649 = 25{,}1 \times A654;$$

where:

A665 - optical density of the solution at a wavelength of 665 nm;

A649 - optical density of the solution at a wavelength of 649 nm.

Carotenoids concentration (Scar, mg/l) was calculated according to the formula:

$$C_{car} = 4{,}695 \times A441 / 0{,}268 \times (C_a + C_b)$$

where:

A441 - optical density of the solution at a wavelength of 441 nm;

$C_a + C_b$ - total content of chlorophylls a and b in the solution, mg/ml.

The content of chlorophylls and carotenoids (X, mg/g) in recalculation on absolutely dry raw material was calculated according to the formula:

$$X = \frac{C \times V \times 100}{m \times 1000 \times (100 - W)},$$

where:

C – concentration of pigment in the studied extract, mg/ml;

V – volume of alcoholic extract, ml;

m – weight of the test raw material, g;

W – loss in mass during drying of raw materials, %.

The results of chlorophylls and carotenoids quantification are presented in Table. 3.7.

Table 3.7

Results of quantitative determination of chlorophylls and carotenoids in *Chrysanthemum indicum* herb

m	n	Xi	X mean.	S2	Scp.	P	t (P,n)	Confidence interval	ε, %
Chlorophyll a									
5	4	0,85 0,88 0,90 0,82 0,83	0,81	0,0010	0,0135	0,95	2,78	0,81±0,03	4,50
Chlorophyll b									
5	4	0,50 0,52 0,53 0,54 0,56	0,53	0,0004	0,0098	0,95	2,78	0,53±0,02	4,47
Carotenoids									
5	4	2,10 2,11 2,13 2,14 2,16	2,11	0,0055	0,0350	0,95	2,78	2,11±0,12	4,40

As a result of quantitative analysis of pigments, the content of chlorophyll *a* in chrysanthemum herb was found to be 0,81±0,03 %, chlorophyll *b* was found to be 0,53±0,02 %, the amount of carotenoids was also determined, the total amount of carotenoids amounted to – 2,11±0,12 %.

Conclusions

1**.** The chemical composition of the *Chrysanthemum indicum* herb was studied.

2. Using various chemical reactions, polysaccharides, flavonoids, amino acids, and tannins were identified.

3. The quantitative content of various biologically active substances in the grass of *Chrysanthemum indicum* was determined.

4. Using the chromatographic method of analysis in the grass of *Chrysanthemum indicum,* the following species were identified: polysaccharides identified glucose and galactose; identified organic acids in *Chrysanthemum indicum* herb: ascorbic acid, oxalic acid and malic acid; identified hydroxycinnamic acids in *Chrysanthemum indicum* herb such as caffeic acid, chlorogenic acid, neochlorogenic acid; identified amino acid acids in *Chrysanthemum indicum* herb such as glutamic acid, leucine and phenylalanine; identified flavonoids in *Chrysanthemum indicum* herb such as rutin, quercetin, apigenin, hyperoside.

GENERAL CONCLUSIONS

1. The literature data regarding the botanical characteristic, geographical distribution, chemical composition, application in medicine and culinary of *Chrysanthemum indicum* were analyzed.

2. The new promising source of plant raw material – contains various groups of biologically active compounds. The detailed study of these compounds is a relevant and modern direction of pharmacognosy.

3. The main numerical parameters of the *Chrysanthemum indicum* herb were determined: total ash (4,36 ± 0,16 %), weight loss during drying (6,19 ± 0,20 %), extractive substances, the maximum extractant was set at 50 % ethanol, the amount of extractives was – 50,70 ± 2,09 %.

. 4.Quantitative study of the content of various biologically active compounds in the herb of Indian chrysanthemum, in particular polysaccharides (5,89±0,25 %); organic acids (2,39±0,09 %); hydroxycinnamic acids (0,48±0,02 %); amino acids (0,03±0,002 %); flavonoids (3,19±0,05%); tannins (1,33±0,06 %); and the amount of chlorophyll a (0,81±0.03 %) and chlorophyll b (0,53±0,02 %) and carotenoids (2,11±0,12 %) was determined.

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APPENDIX

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

Матеріали VI Міжнародної науково-практичної інтернет-конференції

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НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ
КАФЕДРА ФАРМАКОГНОЗІЇ ТА НУТРИЦІОЛОГІЇ

MINISTRY OF HEALTH OF UKRAINE
MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
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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 VI Міжнародної науково-практичної інтернет-конференції

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

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Конференція зареєстрована в Українському інституті науково-технічної і економічної інформації (УкрІНТЕІ), посвідчення № 600 від 11.12.2023 р.

С 89 *Сучасні досягнення фармацевтичної науки в створенні та стандартизації лікарських засобів і дієтичних добавок, що містять компоненти природного походження*: матеріали VI Міжнародної науково-практичної інтернет-конференції (м. Харків, 12 квітня 2024 р.). – Електрон. дані. – Х.: НФаУ, 2024. – 212 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.

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IDENTIFICATION OF BIOLOGICALLY ACTIVE COMPOUNDS IN CHRYSANTHEMUM INDIAN HERB

Al Sayed Walaa, Tartynska G.S.
National University of Pharmacy, Kharkiv, Ukraine

Introduction. Indian chrysanthemum (*Chrysanthemum indicum* L.) is a species of perennial herbaceous plants of the *Asteraceae* family. Plants reach 0.5-1 m in height, the stems are usually simple. The leaves are deeply divided into lobes; the edge of the leaf is serrated. Inflorescence is a yellow apical, rarely axillary basket. Marginal flowers are ligulate, usually golden yellow, central tubular. The fruit is a small seed. The chemical composition of Indian chrysanthemum presented by essential oil, vitamins, phenolic compounds, anthocyanins. In folk medicine, flowers are used as hypotensive and antipyretic, leaves are used for migraine [2]. Therefore, itis urgent to carry out more detailed phytochemical study of Indian chrysanthemum.

Purpose. The purpose of our work was to identify the main groups of biologically active compounds in the Indian chrysanthemum herb.

Materials and methods. 5.0 g of the researched crushed raw material was used to prepare water extracts from Indian chrysanthemum herb, which was poured with water in a ratio of 1:5 and heated in a boiling water bath for 60 minutes, periodically shaking. The resulting extract was filtered through a pleated filter into a 200 ml flask. The extraction of raw materials was repeated two more times in the conditions described above with new portions of the extractant. The combined extract was concentrated and used to determine polysaccharides, amino acids, tannins. The water-alcohol extract was obtained according to the method described above. Extraction of raw materials was carried out with 70% ethanol, concentrated water- alcohol extraction for the detection of flavonoids. For the detection of polysaccharides, four times the volume of 96% ethanol was used, which was added tothe extract from the Indian chrysanthemum herb (formation of an amorphous precipitate). The presence of flavonoids and tannins was determined using well- known chemical reactions: cyanidin reaction (pink color), with 10% solution of iron
(III) chloride (black-green color), 2% solution of aluminum chloride (green-yellow color), 10% solution potassium hydroxide (yellow-green color) and 1% quinine hydrochloride solution (amorphous precipitate), 1% gelatin solution (turbidity appeared), iron (III) ammonium sulfate (black-green color). Detection of amino acids was carried out using a reaction with 0.2% freshly prepared solution of ninhydrin in isopropyl alcohol (purple-red color) [1].

Results and their discussion. The results of the experiment confirmed the presence of polysaccharides, amino acids, flavonoids, tannins in the Indian chrysanthemum herb. The obtained data can be used for further phytochemical study of raw material of Indian chrysanthemum (*Chrysanthemum indicum* L.).

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