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QUALIFICATION WORK

on the topic: «**STUDY THE BIOLOGICAL ACTIVE COMPOUNDS OF
DILL HERB**»

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ABSTRACT

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The qualification work is devoted to study of chemical content the herb of dill. The quantitative and qualitative composition of biologically active compounds of plant material was established .

Key words: dill, herb, compounds, qualitative composition, quantitative analyse

АНОТАЦІЯ

Кайгін В. «Дослідження біологічно активних сполук трави кропу»

Кваліфікаційна робота присвячена дослідженню хімічного складу трави кропу. У рослинному матеріалі визначено якісний та кількісний вміст біологічно активних сполук.

Ключові слова: кріп, трава, сполуки, якісний склад, кількісний аналіз

CONTENT

INTRODUCTION	6
CHAPTER 1. THE CURRENT STATE OF THE DILL STUDY (LITERATURE REVIEW)	8
1.1. The plant origin and botanic characteristic of the <i>dill</i>	8
1.2. Growing conditions of dill and its distribution in nature	9
1.3. The rools of <i>dill</i> plant material collected	11
1.4. Biological active compounds of dill	12
1.5. Use of <i>dill</i> in medicine	13
Conclusions	16
THE EXPERIMENTAL PART	17
CHAPTER 2. INVESTIGATION OF QUALITATIVE COMPOSITION OF BAC OF <i>DILL</i> HERB	17
2.1 Object of study	17
2.2 Determination of <i>dill</i> herb loss in mass during drying	17
2.3 Polysaccharides	19
2.4 Phenolic compounds identification	19
2.4.1 Simple phenols	19
2.4.2 Coumarines	19
2.4.3 Flavonoids	21
2.4.4. Tannic compounds	24
2.4.5. Saponins	25
Conclusions	27
CHAPTER 3. QUANTITATIVE DETERMINATION OF BAC OF <i>DILL</i> HERB	28
3.1 Obtaining the lipophilic fraction	28
3.2 Research of fatty acids	28
3.3 Research the essential oil components	31
3.3.1 Obtaining and quantitative determination of essential oil from <i>dill</i> herb	31

3.3.2 Chromatography - mass - spectrometry research of components of essential oils from <i>dill</i> herb.	34
3.4 Determination the chlorophylls in lipophilic fraction of dill herb	37
3.5. Determination the free and associated amino acids in <i>dill</i> herb	38
3.6. The study of microelement composition of <i>dill</i> herb	41
3.7 Determination of phenolic compounds by HPLC method	43
Conclusions	44
General conclusions	46
References	47

LIST OF CONVENTIONAL ABBREVIATIONS

A. graveolens – *Anethum graveolens*;

BAC – biological active compounds;

EuPh – Europea Pharmacopoeia;

HPLC – high performance liquid chromatography;

HW – Hewlett-Parkard;

min. – minutes;

MPh - microphotometer

PC – paper chromatography;

PLC – thin layer chromatography

Rt – retentional time;

UV- light – ultra violet light.

INTRODUCTION

The topic actuality. The dill (*Anethum graveolens* L.) is widely cultivated agricultural plant in Ukraine and used in medicine and food. The plant growing in different conditions and the season you can collect several harvests. Pharmacopeia (*EurPh*) herbal drugs are fruits of dill, but scientific interest is the study of it herb to expand the resource base of biological active compounds (BAC). The output of dry material from one plant is quite significant, so use of agricultural herbs can expand assortment of herbal medicines provided with resource base. We must remember that the plants used as food in the increased dose exhibit specific pharmacological activity. Given the above said, we believe that *A. graveolens* herb is a promising source of BAC and interesting subject for phytochemical investigation

The aim of investigation was study the chemical composition of dill herb.

To achieve the goal, the following **tasks** should be completed:

- To compile a literature review on the given issue and analyze the level of dill herb research in various aspects;
- To determine the loss in mass during drying for dill herb;
- To obtain the extracts of dill herb and carry out its chemical screening in order to determine the composition of BAC;
- To obtain a lipophilic fraction and investigate its chemical composition;
 - To obtain the essential oil, establish its component composition and some physicochemical parameters;
 - To analyze the mineral element composition of plant material.

The object of study **was herb** of *dill*.

The subject of study was identification and determination of the content of BAC belongs different chemical groups (polysaccharides, phenolic compounds, saponins, aminoacids, fatty acids, chlorophyls, essential oils and mineral components).

Methods of study: physical - determination of loss in mass during drying;

physical and chemical - chromatography in a thin layer of sorbent, paper chromatography; chemical - BAC identification reactions, their quantitative determination; instrumental, physical and chemical – gas chromatography, chromatography-mass-spectrometry, emission spectrophotometry, photolorimetry, refractometry.

Practical significance of the obtained results.

The obtained practical results made it possible to expand information about the chemical composition of dill herb. Considering the wide range of BAC of the obtained essential oil, it can be concluded that it is a promising substance that can be used to create of various dosage forms. Considering the biological activity of essential oil components, we believe that the substance is promising for the creation of carminative and antispasmodic agents for the treatment of diseases of the gastrointestinal tract.

Elements of scientific research. The presented work is a finished fragment of scientific research. The qualitative composition and quantitative content of BAC, which have different chemical structures, were established. All obtained results are reliable and statistically verified.

Approbation of research results and publication. The results of the research are presented at the V International Scientific and Practical Internet Conference «Current achievements of pharmaceutical science in the creation and standardization of medicines and dietary supplements containing components of natural origin», April 14, 2023, National university of Pharmacy. Based on the materials of the qualification work, 1 abstract of the report was published.

Structure and scope of qualification work. The work consists of an introduction, an abstract in English and Ukrainian, a literature review, two chapters of own research, general conclusions, a list of used literature, which includes 45 foreign languages sources and appendices. The content of the work is laid out on 44 pages of the main text and illustrated with 16 tables and 10 figures.

CHAPTER 1. THE CURRENT STATE OF THE DILL STUDY (LITERATURE REVIEW)

1.1. The plant origin and botanic characteristic of the dill

Anethum graveolens has a long and ancient history in many countries as a culinary and medicinal herb [6]. The genus name Dill (*Anethum*) is derived from Greek and means strong smelling. The earliest known record of dill as a medicinal herb was found in Egypt 5000 years ago [11]. The name derives from the Greek name dill – anethon. In latin. *graveolens* - very odorous. Dill is grown as a spice and as a medicinal plant. In ancient Greece it was used for decorating bouquets. Bunches Dill gave brave soldiers. It is known that the ancient Egyptians used the plant as a remedy for headaches. In European countries with spread X century.

The Motherland of dill is Iran, India, Egypt. In the wild it found in Asia, Europe, America, European part of CIS, Cetral Asia, Siberia [13]. It grows mainly in gardens, fields, along roads. It cultivated in many countries. Synonyms of dill in different countries shown in Table 1.1.

Table 1.1

Synonyms of dill

Country	Synonyms	Country	Synonyms
Arabic	Shibit	Gaelic	Dile
Armenium	Samit	German	Dill, Gurkenkraut
Chinese	Shih lo	Hindi	Sowa, Anithi
Czech	Kopr	Indonesian	Adas manis
Danish	Dild	Italian	Aneto
Dutch	Dille, Stinkende vinke	Laotian	Phak si
Egyptian	Sjachet, Sjamar	Malay	Adas china
Esperanto	Aneto	Norwegian	Dill
French	Aneth odorant	Pashto	Shabit
Estonian	Aedtill, Till	Polish	Koper (ogrodowy)
Portuguese	Endro	Romanian	Mărar

Botanical characteristics of dill

Dill is a blue-flowering plant, about 40 - 150 cm tall. Plants form a rosette of fragrant green, filamentous leaves, a leafy, tubular stem, and then blossom into intensive green stem and seeds [16]. While it is sometimes grown as a biennial, it is most commonly grown as an annual [30]. Depending upon the variety, the plant can grow anywhere from 1 foot (for dwarf varieties) up to 4 feet in ideal conditions [42]. The branching stems have white to off-white, vertical striations that run down their length, and they are devoid of hair. Unlike fennel, which resembles dill in appearance, the stems of dill are hollow [28]. They also end with a broad, expansive inflorescence that is topped with yellow, scented, hermaphroditic flowers. Dill's inflorescences are arranged in umbels, in which the flower stalks develop from a common point [31]. It is similar in appearance to an umbrella and it is this characteristic that gives the entire Umbelliferae family its name. The flowers develop into dry fruits called schizocarps that are split into two parts, each one holding seeds. Dill fruits are oval, bright green color, compressed, winged about one-tenth inch wide, with three longitudinal ridges on the back and three dark lines or oil cells (vittae) between them and two on the flat surface. The taste of the fruits somewhat resembles caraway. Like the stems, they have light colored lines or striations that run down their length. Dill's aromatic leaves are delicate in appearance and are often described as lacy, feathery, or needle-like. Dill produces a single taproot that can reach deeply into the ground in ideal conditions [35]. The main root is thin, branched. Dill is commonly found near houses, in gardens, on the edge of fields and crops, roads and along roadside on vacant lots as weeds. It grows in open places: in the steppes, meadows, clearings of forests, fields. As a shrub garden dill occurs in small groups and as single plants.

1.2. Growing conditions of Dill and its distribution in nature

Dill has been cultivated since ancient times and use of this plant for medicinal and consumption purposes has been recorded dating back to the Greek and Egyptian civilizations. Dill is widely used to give flavor to food [34].

Dill - cold-resistant plant. Cultivation of dill possible for all types of garden

soils. Seeds are sown for permanent, leaving 20-30 cm between. The seeds begin to germinate at a temperature of 3-5 C, and the leaves can grow at low temperatures (5-8 °). The optimum temperature for growth of this culture 16-17 °, but the flowering and ripening seeds need warmer temperatures (18-20 °). It flowers in June and July - the flowering of mass - at the end of June - beginning of July [40].

Fruiting in late July. Mostly grows in well-lit areas. Refers to plant a long day. Increasing global day accelerates the transition to stem forming. At 10-12 hour days lengthening occurs leaves but stebleutvorennya happens. These features should be considered when growing plants in the northern regions, where recommended in crops in greenhouses technically create a shortened day to prevent stem forming [42].

This method increases the harvest of dill. Picky about soil moisture and air. If insufficient quantities of water quality product declines - of dill leaves are shallow, rude, but its excess negative impact on the harvest of raw materials and essential oil content. Top soil - rich in humus sandy, net of shrubs.

Place the dill on structural, light, neperezvolozhenyh soils. Preferred predecessors are row crops, under which brought organic and mineral fertilizers. Better prepare soil in the autumn and early spring - March - April - just spend sowing. Methods of sowing set to the purpose of growing production. For receipt of a dill sown green solid way of seeding rate of 20-30 g / m².

To be technical products, sow dill string method with aisles of 20-25 cm and a seeding rate of 7-10 g / m². Seeding depth is 1.5-2 cm Crops must prykochuyut and, if necessary, moisten the soil. After appearing stairs fluff piece rake across the strings - 2 times, the second time - in the phase of 2-3 true leaves. When growing tech products leave the plant in line at a distance of 7-10 cm in need of dill norm should be watered 5-7 l / m². For technical crops of dill aisle loosen every 7-10 days before flowering [40].

Dill struck blackleg, fusarium and farinaceous dew. When growing herbs in pestitsydy not apply. of dill seeds at sowing seeds mixed with lettuce or radishes

(0,3 g / m²), products that are harvested at the revelation of stairs dill.

In the conditions USA offer a sow crop possible in light, well-drained soil, rich in moisture, organic matter. In middle latitudes of the globe are more than 100 species of dill [42].

Table 1.2

The most spread types of dill

Type	Some characteristics
Gribovsky	The leaves are dark green with a slight bloom, high aromatic. Undemanding. Gives a good harvest seeds. Cultivation is possible in all regions.
Kibray	Leaves light - green. Impact of long-term yield. Resistant to mildew. Long time no blooms.
Bush	has a dense, fragrant herbs. Light - green with a slight bloom. Beautifully grow after stripping.
Lisnohorodskyy	The leaves are green with a purple tinge. Impact of long-term yield. When flowering is 130 cm.
Superdukat	The leaves are large-scale with low wax . Greens tender, juicy , fragrant. Country of rigin – Denmark.
Indiana	Grows in India, has fruits that compared with other varieties of dill larger, but the taste and smell of green less expressive.

1.3. The rools of dill plant material collected

Harvesting for medicinal use

The herb green part collect in spring or summer when the plants a height of 20-25 cm. Plants pull, shake the earth, bind tightly in bundles or placed in boxes with horizontal rows of roots for each other. For procurement of green mass is dried in tailored spaces. Store in a well-sealed container. Technical production cut in July, the plants in a well ventilated area and keep a few days or dried.

The herb part dried in the shade, spreading a thin layer should be turned periodically. Dried grass stored in cloth bags or cardboard boxes for a period not exceeding two years.

The fruits collection start when 60-70% of the seeds in umbels acquire a brown color. Cut florets bind together and dried. After drying threshed [14].

To reduce the loss of raw materials can be wrapped light cotton cloth, then threshed and, if necessary, the fruit is dried in an oven at a temperature of no more than 30-40 degrees. Dried fruit is stored in a glass jar with a tightly fitting lid for a term not exceeding two years [33, 40, 42].

For dill essential oil harvested in phase milky ripeness of seeds in the central umbrella inflorescence. Plants mow down at a height of 18-20 cm from the demolition and fresh way to modify the hydrodistillation [32].

The yield green mass during this period 40%. Number essential oil corresponds 1,5-2,7% of absolutely dry mass.

1.4. Biological active compounds of dill

The fruits content from 2 to 4% high of essential oil. The main components of essential oil are anethole and carvacrole [1, 5]. It also consists α -phellandrene, dihydrocarvon, carveol, dihydrocarveol, isoeugenol [27]. Fatty oil contains up to 93% of glycerides of fatty acids, including palmitic, oleic, linoleic. In fruits also found coumarins, phenolcarbonic acids, flavonoids, resins, waxes, proteins, nitrogenous compounds [2, 10]. Dill herb contains 0,65-2% essential oil with a lower content of carvone compared to the essential oil of fruits; part of it also includes vitamins C, B1, B2, PP, P, pro-vitamin A, calcium, potassium, phosphorus, iron, folic acid, flavonoids (quercetin, isorhamnetine) (Fig. 1.1).

In herb and fruits was detected carvacrole, anethol, 8-hydroxygeraniol, coumarin and furocoumarin. For the fruits the most character are dihydrocarvone, α -phellandrene, limonene, apiol. In herb was identified phenolic compounds derivatives: coffeic, chlorogenic, ferulic and cinnamic acids; quercetin, kaempferol, gallic acid, ascorbic acid [12, 36].

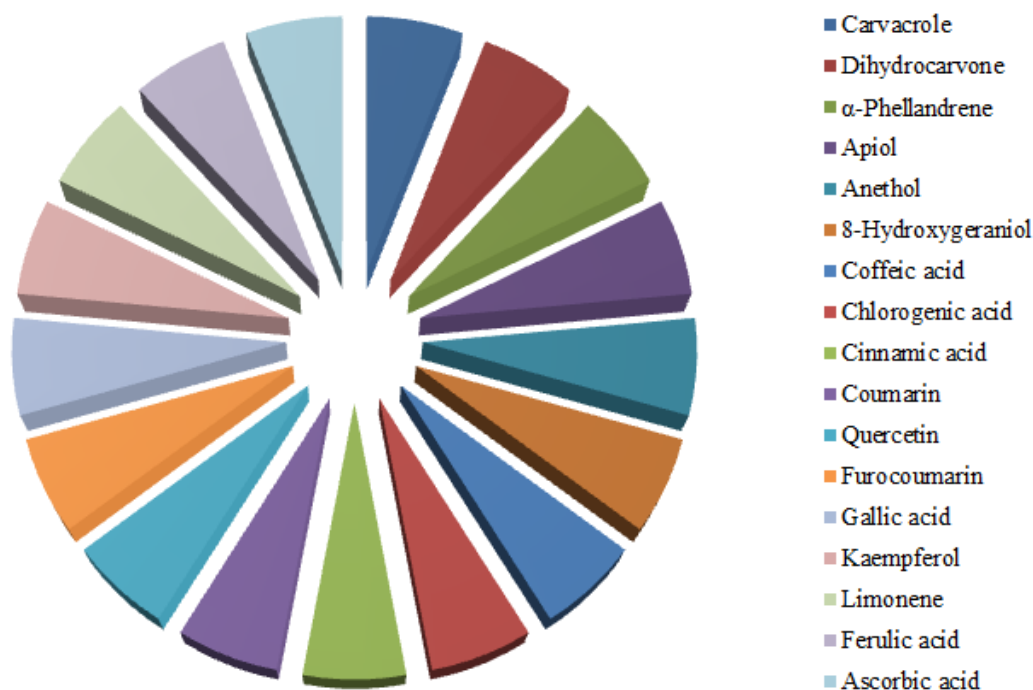


Fig. 1.1 BAC of Dill herb and fruits

1.5. Use of Dill in medicine

The Egyptian writings are some of the earliest records that we have of the dill plant being used medicinally [4]. Dill was most often associated with being an effective remedy for flatulence and as an aid for digestion [26]. Due to the content of essential oil, it has an antimicrobial and bactericidal effect [3, 8, 25].

Ancient Greeks believed that parts of the dill plant could help with the hiccoughs. Pliny and Dioscorides shared this belief and wrote that dill could “stayeth the hicketts [hiccoughs] and used for treat eye diseases. The Greeks also used dill as a sleep aid. They would cover their eyes with the plant to help them get a good night’s rest. Dill water, or “gripe water,” is an ancient remedy that has been used by mothers for centuries to calm colicky babies or to help them sleep.

Dill was even recommended in the past to help mothers increase their milk flow. Who was not fond of hiccoughs and stomach noises, insisted that bottles of dill oil be made available to his dinner guests. Nicholas Culpeper recommended dill in his writings, as a tonic “which strengthens the brain.

Today, recent studies are bearing out the belief that dill is a useful remedy for indigestion and ulcers. Researchers have found that dill inhibits the secretion of stomach acids in mice and that it may help to prevent ruptures in the stomach lining. Dill is also believed to have anti-cancer properties, hepatoprotective and nephroprotective activity against free radicals generated by paracetamol, because it is high in monoterpenes.

Official raw materials are seeds of dill - *Fructus Anethi* . K. Kant (1913) recommended the dill seeds in the treatment of chronic bronchitis, pneumonia, scabies and asthma, flatulence, dyspepsia and malaria. It useful to apply the fruits of dill as an antihypertensive, antioxydant, edative, anti-spasmodic, enhancing bile secretion, reduces flatulence and laxative agent in hypertension and atherosclerosis (especially headaches), diseases of the liver and gastrointestinal tractis [15].

Herbs contain a wide variety of antioxidant phytochemicals or bioactive molecules that can neutralize the free radicals and thus retard the progress of many chronic diseases associated with oxidative stress and reactive oxygen species [39].

A. graveolens has been reported to contain flavonoids, phenolic, and essential oil. The increased consumption of vegetables and herbs containing high levels of phytochemicals has been recommended to prevent or reduce oxidative stress in the human body [19].

Endogenous antioxidant defense mechanisms may be insufficient and hence dietary intake of antioxidant compounds is essential [7]. The intake of natural antioxidants has been associated with reduced risk for cancer, cardiovascular disease, diabetes, and diseases associated with aging .

The liver is associated with many important life functions; it has great capacity to detoxicate toxic substances and synthesize useful principles [37]. The extract of dill could protect the liver against high-fat-diet-induced oxidative damage in rats [43, 44].

The crude extract of has been reported to have antihypercholesterolemia and antihyperlipidemic properties.

The effect of dill extract on serum lipoproteins in hypercholesterolemic rats and the possible mechanism of action of a crude extract on liver enzymes activity have been studied [4]. The present study was designed to specifically investigate the antioxidant efficacy of the aqueous extract against paracetamol drug-induced oxidative stress in albino rats.

Dill is an excellent remedy for flatulence and the colic that is sometimes associated with it. It is the herb of choice for colic of children. Chewing the seeds will help clear bad breath. The tea use like carminative, aromatic, anti-spasmodic, anti-inflammatory, galactogogue, diuretic. Dill also helps stimulate appetite, and a decoction of the seed may be helpful for insomnia as well as for pains due to flatulence [19].

Galen preparations of the fruits of dill increase gastric secretion got to have antispasmodic, expectorant, calming effect, regulate motor activity of the intestine, exhibit an antibacterial effect.

Dill is one of the oldest folk medicines. Use dill in food regulates arterial pressure, improves heart and lung activity, calms the nervous system. Infusion of herbs used in hypertension first and second degree in combination with sodium bromide. Powder of dried herbs recommended for constipation and chronic colitis.

Dill fruits, infused as a tea, folk doctors of Abkhazia used as a diuretic; Armenians in the mornings pulmonary tuberculosis patients allowed to drink hot milk mixed with a pinch of of dill seeds and cinnamon.

When bowel disease dill suspends the fermentation process, so in Georgia when consumed acidic and spicy, as well as a large number of plant foods, causing increased fermentation in the gut, consume a lot of dill.

Many ancient physicians, including Ibn Sina, considered excessive and prolonged use of dill harmful to the brain. Large doses allegedly caused blurred vision. From the standpoint of modern medicine ancient physicians observed side effects of dill can be explained as follows.

Large doses of dill lower blood pressure by expanding the blood vessels, so the person consuming a lot and often dill, hypotonic state may be manifested in the

form of fainting , blackout before the eyes, temporary visual impairment , the total loss of strength , fatigue, occurs after physical or mental stress.

In order to eliminate the negative characteristics of dill ancient physicians recommended to take honey , cloves or cinnamon.

In Bulgaria, dill fruit used as an antispasmodic, tonic gastrointestinal tract, enhancing lactation sedative for insomnia and colic means of various origins.

In Germany, dill fruit used as an antispasmodic, improves digestion and lactation, a sedative. In cosmetics use leaf infusion of dill as a lotion to inflamed and reddened eye fatigue.

Conclusions

Was analyzed the current state of Dill research and completed analytical review of it phytochemical and pharmacological study. From scientific literature sources the main active compounds of Dill are terpenoids and phenolic compounds. It has been established that dill fruits are the most chemically studied, while the herb has not yet received enough attention.

The availability of the raw material base and the possibility of several harvests of dill herb per season, as well as a wide range of varieties, motivated us to conduct a study of the chemical composition of dill herb.

EXPERIMENTAL PART

CHAPTER 2. INVESTIGATION OF QUALITATIVE COMPOSITION OF BAC OF *DILL* HERB

2.1 Object of study

The object of the study was herb of dill, cultivated in Kharkiv region. Raw materials were harvested in August 2020, in the phase of milk ripeness before the formation of umbrellas. The herb was cut at a distance of 15 cm from the ground, formed into bundles and dried in a suspended state in a ventilated room. To preserve volatile compounds, plant material were dried in the shade. Dry plant materials were placed in paper bags.



Fig. 2.1 Appearance of dill plant material

2.2 Determination of *dill* herb loss in mass during drying

Loss in mass during drying is meant loss of mass due to hygroscopic moisture and volatile matter in the herbal drugs during drying to constant weight.

Methodic of study. The analytical samples of plant materials cutted to particle size of about 10 mm, mixed and taken two sample weight 3-5 g, with an error 0,01g. Each sample was placed in dried bottle with cover and put in heated to 100-105 ° C drying cabinet. Drying time recalculated from the time when the temperature in the closet again reached 100-105 ° C. The first weighting conducted after 2 hours.

Drying was performed to constant weight. Constant weight was considered reached when the difference between two subsequent weighting after 30 minutes of drying and cooling for 30 minutes in a desiccator did not exceed 0.01 g.

Determination of the loss in weight on drying recalculation the number of active substances on the absolutely dry raw material was carried out in a sample of 1-2 g (accurate weight) while the difference between weighing not more than 0,0005 g. A percentage calculated by the formula:

$$A = \frac{(a - a_1) \times 100}{a},$$

are: a - mass of material before drying in g;

a₁ - mass of material after drying in g.

For the determination of the final result taking the arithmetic mean of two parallel measurements, calculated to tenths fractions.

Allowable difference between the results of two parallel determinations should not exceed 0,5%.

The results of determining of loss in mass during drying in *dill* herb are shown in Table 2.1.

Table 2.1

The results of determining of loss in mass during drying in *dill* herb

№ of bottle	Mass of bottle, g	Mass of bottle with sample before drying, g	Mass of sample, g	Mass of bottle with sample after drying, g	Loss in mass during drying, %	The average value, %
1	47,5003	50,4877	2,9874	50,1757	6,17	6,14±0,19
2	47,4006	50,4131	3,0125	50,1131	5,95	
3	46,5270	49,5285	3,0015	49,2160	6,30	

The loss in mass during drying for the dill herb is $6,14\% \pm 0,19$.

2.3 Polysaccharides

For extraction of polysaccharides cutting 1.0 g of raw material to the particle size of 2 mm. Sample was placed in a flask with 50 ml shliftom. Poured raw material 10 ml of purified water. Closed flask air refrigerator and boil in water bath for 20 minutes. Cooled extract and percolated through cotton wood. To 1 ml of the filtrate add 3 ml of 96% ethanol during mixing.

As a result of the reaction we observe amorphous precipitate. It indicated the presence of polysaccharides in the plant material. As a result of the reaction, polysaccharides that do not dissolve in ethanol are precipitated and visualized in the form of a gel-like substance.

2.4 Phenolic compounds identification

For identification of BAC used qualitative chemical reactions and chromatographic methods. Chromatographic research were carried out using two-dimensional paper chromatography (PC) in solvent system: I direction - ethyl acetate-formic acid-water (10:2:3); II direction - 2% acetic acid. Thin-layer chromatography was used for chromatography with probable samples [29].

2.4.1. Simple phenols

The first reaction. 0,5 g of powdered material was placed in a flask and add 10 ml of water, boil for 2-3 minutes, filtered after cooling. To 1 ml of the filtrate add crystal of iron chloride.

The second reaction. To 2 ml of the filtrate was added 4 ml of ammonia solution and 1 ml of 10% sodium phospho-molybdenum in hydrochloric acid.

2.4.2 Coumarines

Reaction with alkali and diasoreagent: 3-5 ml alcohol solution extract was added 5 drops of 10% potassium hydroxide solution and heated in a water bath for several minutes. After we are added 3-5 drops diasosulfanilic acid.

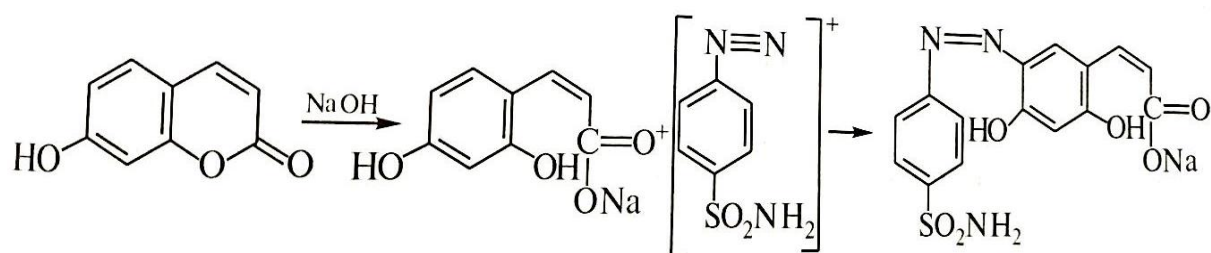


Fig. 2.2 The chemical mechanism of reaction of alkali and diasoreagent for dill herb extract

During the diazotization reaction, a red coloration of the solution is observed due to the formation of a diazo complex.

Lactone test: 3-5 ml of extract was added 5 drops of 10% alcohol solution of potassium hydroxide, heated in a water bath, was added 5.10 ml of distilled water, mixed well, add 10 drops of 10% hydrochloric acid.

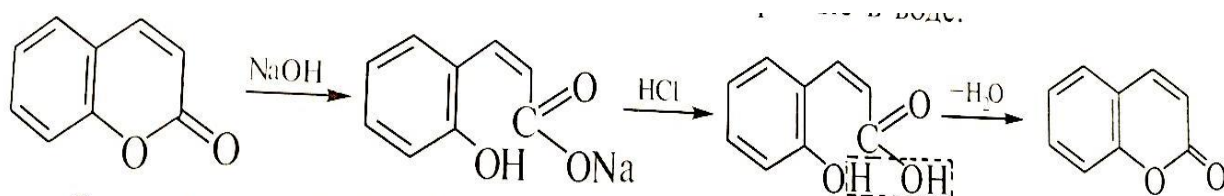


Fig. 2.3 The chemical mechanism of lactone test for dill herb extract

As a result of the lactone test, the opening of the lactone ring occurs under the influence of an alkali solution and the formation of coumarin acid.

The results of the qualitative determination some of BAC are given in Table 2.2.

Table 2.2

The results of the qualitative determination of BAS in *Anethum graveolens* herb

Class of compound	96% ethanol	FeCl ₃	sodium phospho-molybdenum in hydrochloric acid	alkali and diasoreagent	Lactone test
Polysaccharides	amorphous precipitate	-	-	-	-
Simple phenols	-	gray tint of solution	dark green solution	-	-
Coumarines	-	-	-	red color	white precipitate

Note: «-» - identification reactions are not specific for this class of compounds

2.4.3 Flavonoids

The plant extract obtaining. Cutting 3.0 g of raw material to the particle size of 2 mm. Sample was placed in a flask with 100 ml. To raw material added 35 ml of 70% ethanol. Closed flask air refrigerator and boil in water bath for 20 minutes, stirring occasionally. Conducted the filtration after cooling.

Cyanidine reaction. To 1 ml of purified extract added 2 drops of concentrated hydrochloric acid and a few pieces of metallic magnesium powder. After the end of the the allocation of gas bubbles to the colored solution was added butanol, diluted with water to the separation of layers and shaken. Fter reaction we so the pink color of organic phase and light pink color of the aqueous phase [24].

The reaction with alkali solution. To 1 ml of the extract added 2 drops of 10% alcohol-water solution of potassium hydroxide.

Reaction whith $FeCl_3$. To 1 ml of the extract added 2 drops of 10% solution of ferric chloride

Reaction with lead acetate. To 1 ml of the extract added 2 drops of lead acetate.

The results of the qualitative determination of BAC are shown in Table 2.3.

Table 2.3

Results of the qualitative determination of BAC
in *dill* herb

Compounds	Reagent			
	Cyanidine test	Solution KOH	FeCl ₃	Lead acetate
Flavonoids	pink color of the organic phase and the water phase light pink	yellow precipitate	yellow-green color of solution	yellow precipitate
Compounds	Reagent			
	96% ethanol	Sodium phospho-molybdenum in hydrochloric acid	Alkali and diasoreagent	Lactone test
Polysaccharides	amorphous precipitate	-	-	-
Simple phenols	-	dark green solution	-	-
Coumarines	-	-	red color	white precipitate

As a result of qualitative reaction, the presence flavonoid natural substances was established. Cyanidine reaction is specific for this type of compounds shown, that herb contains aglycones and glycosides derivatives of flavonoids.

For identification of phenolic compounds used chromatographic methods. Chromatograms were analyzed in daylight and UV- light after processing of ammonia pairs and an alcoholic solution of alkali [24, 29].

Chromatographic research were carried out using two-dimensional paper chromatography in solvent system: I direction - ethyl acetate-formic acid-water (10:2:3); II direction - 2% acetic acid.

Also used chromatography in compared whis standart compounds. The results of chromatographic studies are shown in Fig. 1 and Table 2.4.

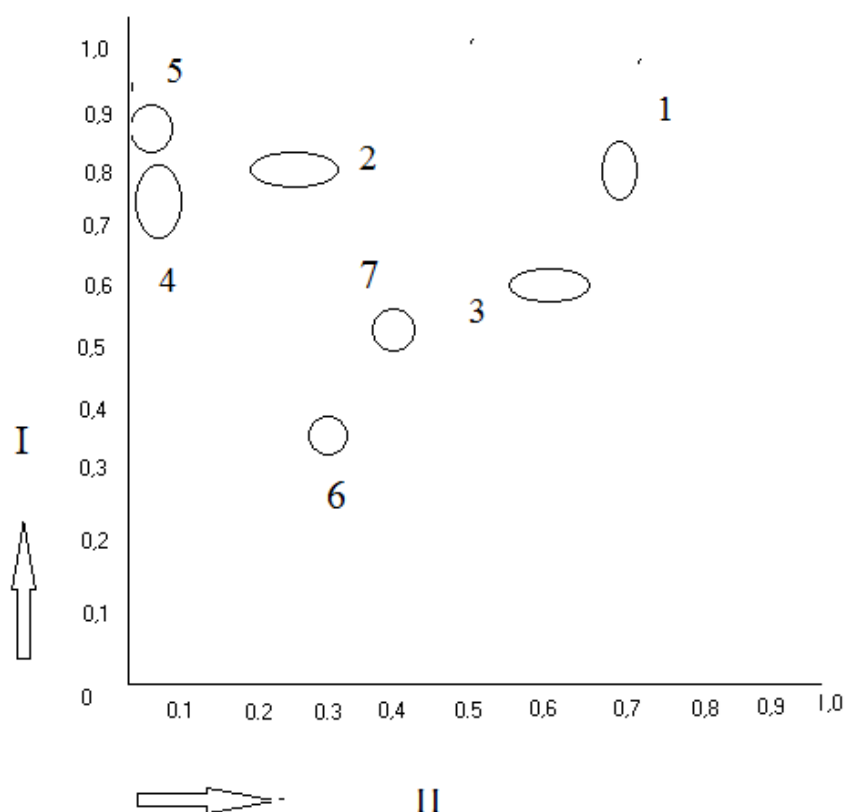


Figure 2.4 The scheme of chromatogram of phenolic compounds of *dill* herb

Chromatographic characteristic of phenolic compounds from *dill* herb

№ of compound	Rf·100		Fluorescence in UV-light	
	I direction	II direction	before processing the reagent	after processing of ammonia pairs
1	85	70	Blue	Blue
2	82	33	Light blue	Light blue
3	62	65	Light blue	Blue
4	80	10	Yellow	Yellow
5	90	7	Yellow	Yellow -green
6	40	30	Brown	Yellow-drown
7	55	42	Brown	Yellow-drown

As the shows Tab. 2.4., in *dill* herb presence of at least 7 compounds of phenolic nature, among which according Rf values compounds 4, 5, 6, 7 have flavonoid nature and compounds 1, 2, 3 are derivatives of hydroxycinnamic acids.

When compared with standart sample compound 3 identified as chlorogenic acid, 4 - as quercetin, 7 like rutin.

2.4.4. Tannic compounds

Purified water was used as an extractant for the study of tanning compounds. For this crushed herbal drugs sifted through a sieve with 1 mm diameter holes. Placed 1 g of powder in a flask containing 250 ml, add 100 ml of water and heated in boiling water bath for 20 minutes. Cooled hoods and percolated cotton wool.

Reaction of tannic compounds identification

- To 2 ml of purified extract was added a few drops of 1% solution of quinine chloride.
- To 2 ml of extract was added 4 drops of iron-ammonium alum.
- To 1 ml of extract was added 2 ml of 10% acetic acid and 1 ml of salt lead acetate.

■ To 2 ml of extract was added a few crystals of sodium nitrate and 2 drops of 0.1 N hydrochloric acid.

■ Up to 2 ml of extract was added 2 drops of ferric chloride.

The results of the qualitative determination of tannic compounds are given in Table 2.5.

Table 2.5.

The results of the qualitative determination of tannic compounds in *dill* herb

Reagent				
Iron-ammonium alum	1% solution of quinine chloride	CH ₃ COOH+ (CH ₃ COO) ₂ Pb	NaNO ₂ + HCl	FeCl ₃
The dark-green color of the solution	The white precipitate	Brown precipitate	Light - yellow color	Yellow - brown color

Reactions of difference the condensed group of tannins from the hydrolyzable tannins. 2 ml of 10% acetic acid and 1 ml of a 10% solution of medium salt of lead acetate are added to 1 ml of the extract. A precipitate is formed in the presence of a group of hydrolyzing tannins. The precipitate is filtered. 5 drops of a 1% solution of ferric ammonium alum and 0.1 g of crystalline sodium acetate are added to the filtrate. In the presence of condensed tannins, a dark-green color appears.

According to the study identified the presence of oxidation of polyphenolic compounds in the tested raw materials.

2.4.5. Saponins

Obtaining extract: 50 g of powdered plant material was placed in a conical flask of 100 ml reflux, poured 50 ml of 50% alcohol.

Contents of the flask heated in a boiling water bath for 15 minutes. Extract cooled and filtered. 20 ml of the filtrate was evaporated in a water bath to 10 ml. The resulting aqueous extract was used for qualitative reactions.

- Laphone reaction (color reaction).

To 2 ml of alcohol-water extract was added 1 ml of 10% solution of copper sulfate and 1 drop of concentrated sulfuric acid and gently heated.

- To 1 ml of extract was added 3-4 drops of barite water.
- Up to 1 ml of extract was added 3-4 drops of 10% - lead acetate solution.

The results of qualitative research of saponins are shown in Table 2.6.

Table 2.6

The results of qualitative research of saponins in *Anethum graveolens* herb

Reagent/reaction		
Laphone reaction	Barite water	$(\text{CH}_3\text{COO})_2\text{Pb}$
Blue-green opalistsents	White opalistsents	Yellow precipitate

Foaming test

For the reaction are used aqueous extract. About 5 ml of water extract shaken in closed tube for 1 min. The presence of saponins in the plant material is indicated by the formation of a foam that lasts for 1 minute.

Determination of the chemical nature of saponins

Added 5 ml of 0.1 N hydrochloric acid into one of the two measuring tubes, and 5 ml of 0.1 N sodium hydroxide solution into the other. Add 3 drops of water extract to both test tubes and shake for 1 minute. With the presence of triterpene saponins in the raw material, foam of the same volume and stability is formed in both test tubes, and when saponins of the steroid group are present, the volume of foam and its stability are much greater in an alkaline environment.

The formation of stable foam, without changes in an alkaline environment, indicates the presence of saponins of the triterpene group.

As a result of qualitative reactions determined presence of saponins in *dill* herb.

Conclusions

1. For the dill herb the *EurPh* indicator called the loss in mass during drying has been established.
2. Qualitative composition BAC of *dill* herb was carried out. In plant material was determined presens of polysaccharides, phenolic compounds, saponins.
3. For investigations used qualitative chemical reactions and chromatographic methods.
4. Water and alcohol extracts of dill herb were used in the experiment.
5. Among phenolic compounds was identified simple phenols, coumarins, flavonoids, hydroxycinnamic acids, tannic compounds. According the results of cyaniding reaction in herbal drugs presens aglycone and glycoside form of flavonoids. As a result of the cyanidine reaction according to Bryant's modification, the organic and aqueous layers had a pink color of different intensity.
6. In *dill* herb presence 7 phenolic compounds, among which compounds 4, 5, 6, 7 had flavonoid nature and compounds 1, 2, 3 are derivatives of hydroxycinnamic acids.
7. By chromathographic method in compared with standart samples compound 3 identified as chlorogenic acid, 4 - as quercetin, 7 rutin.
8. During the reaction with iron-ammonium alums, a dark green precipitate was observed, indicating the presence of condensed tannin compounds.
9. The presence of saponins in raw materials was established by qualitative chemical reactions. When conducting a study on the chemical nature of saponins, it was established that the studied herbal material contain saponins of the triterpene group.

CHAPTER 3. QUANTITATIVE DETERMINATION OF BAC OF *DILL* HERB

3.1 Obtaining the lipophilic fraction

Raw materials sample crushed and sieved to the particles of 3 mm, separated from the dust through a sieve number 18, were placed in a bag of filtering paper and weighed on an analytical balance. Prepared in this way substance extracted in Soxhlet apparatus. Extractants - chloroform. Extraction was carried out in a water bath to extract discoloration. The flask receiver were weighed before and after extraction. To remove a pair of extractant flask was dried in an oven at 60 ° C for 30 minutes.

These fractions have the form of resinous liquid, dark-green color, with a characteristic odor, insoluble in water and alcohol, soluble in chloroform and ethyl acetate.

Output of the lipophilic fraction in terms of absolutely dry raw material was calculated using the formula:

$$X = \frac{m_{l.f.} \cdot 100 \cdot 100}{m_s \cdot (100 - W)}$$

are: $m_{l.f.}$ – mass of the lipophilic fraction; m_s – mass of sample; W – moisture of raw material

The output of lipophilic fraction considering moisture of raw material is 18,3%.

3.2 Research of fatty acids

To determine the qualitative and quantitative analysis of fatty acids in *dill* herb using 100 mg of sample . Extraction of the acid tests conducted by use Folch solution (chloroform -methanol 2:1) by heating to 40 ° C for 5 minutes.

Qualitative and quantitative analysis of fatty acids was determined by gas chromatography. Separation and register of fatty acids was performed in gas chromatograph "Chrome - 5" on a metal column 2.6m long , 0.32 mm in diameter

filled with sorbent " Hromaton - super" 10% polyethylenglycolsuccinate. Analysis of samples of free fatty acids was carried out in isothermal mode at 195 ° C and heated flame ionization detector to 250 ° C. Rate of carrier gas of high purity nitrogen 50 ml / min., water 30 ml / min. , air - 300 ml/min. Identification of free fatty acids was carried out by comparing the time of their exit from the known methyl esters of fatty acids. Quantitative analysis was performed by the absolute calibration of each fatty acid separately , as well as mixtures thereof with the drawing calibration curves which were determined and the concentration of each fatty acid in the sample [18, 41].

Methylation of fatty acids was performed as follows: from the centrifuge tube were collected chloroform layer was transferred into a reaction tube of 25 ml, the solution was evaporated to dryness in a stream of nitrogen gas and heating temperature of 60 ° C, was added 1 ml of 5 % sulfuric acid in methanol, placed in a test tube water bath for 30 minutes at 80 ° C; cooled tube was added 3 ml of distilled water and 5 ml of hexane -ether 1:1 , stirred and after defending selected upper phase was transferred to a centrifuge tube and evaporated solution; residue was diluted with hexane to 0.5-1 ml, 1 ml were selected for inclusion in the gas chromatograph.

Analysis of samples of free fatty acids was carried out in isothermal mode at 195 ° C and heated flame ionization detector to 250 ° C. The speed the carrier gas of high purity 50 ml / min., nitrogen 30 ml / min., air - 300 ml / min. Identification of free fatty acids was carried out by comparing the time of their exit from the known methyl esters of fatty acids [41].

Quantitative analysis was performed by the absolute calibration of each fatty acid separately, as well as mixtures thereof with the drawing calibration curves which were determined and the concentration of each fatty acid in the sample.

Fatty acid composition of *dill* herb are presented in Tab. 3.1.

Table 3.1

Fatty acid composition of *Anethum graveolens* herb

№	Name of acid	Content, mkg/100 mg
1	Decanic	2,0
2	Lauric	4,0
3	Tridecanic	3,3
4	Miristic	20,0
5	Pentadecanic	5,0
6	Palmitic	180,5
7	Geptodecinic	10,0
8	Stearinic	76,0
9	Oleic	620,0
10	Linoleic	700,0
11	Linolenic	40,0
Sum of unsaturated fatty acids		1440,0
Sum of saturated fatty acids		194,5

As shown in Tab. 3.1 in raw material founded 11 fatty acids, from which 2 (linoleic and linolenic) are essential and part of a complex of vitamin F. Dominant (mkg/100 mg) are palmitic, oleic, and linoleic acids.

Scheme of the gas chromatograms of fatty acid of *Anethum graveolens* herb shown in Fig. 3.1.

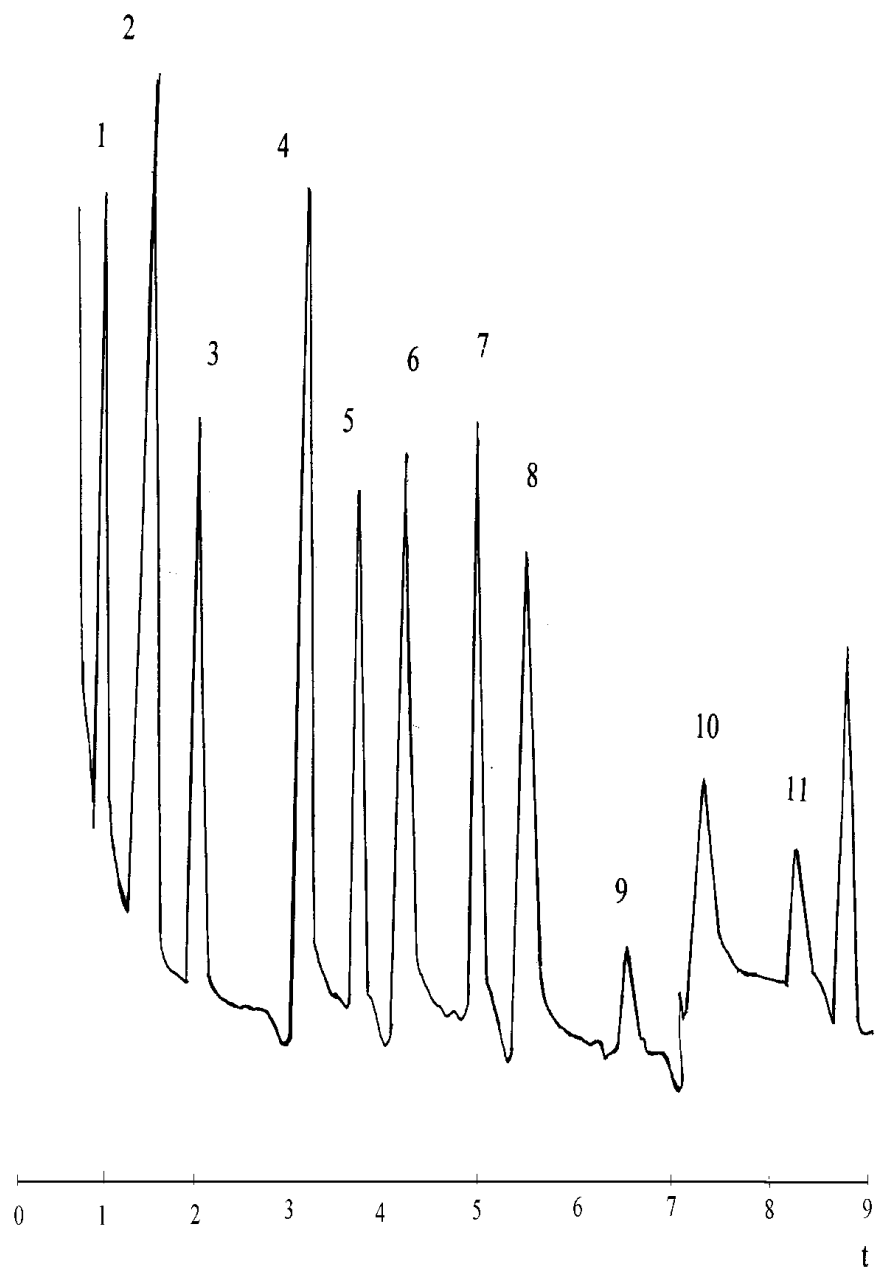


Fig. 3.1 Scheme of the gas chromatograms of fatty acid
of *dill* herb

Note: 1 – decanic, 2 – lauric, 3 – tridecanic, 4 – miristic, 5 – pentadecanic, 6 – palmitic, 7 – heptodecnic, 8 – stearinic, 9 – oleic, 10 – linoic, 11 – linoleic acids.

3.3 Research the essential oil components

3.3.1 Obtaining and quantitative determination essential oil from *dill* herb

Quantitative determination of essential oil in raw material is carried out by steam distillation in a Ginsberg's apparatus (Fig. 3.2). The distillate is collected in

the graduated tube, the aqueous phase is returned to the distillation flask [9, 23].

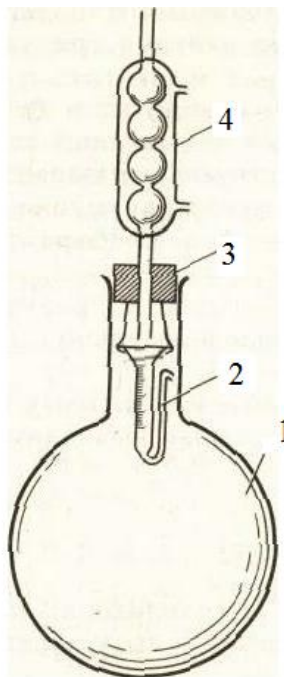


Fig. 3.2 The Ginsberg's apparatus scheme: 1 - round bottom flask; 2 - graduated receiver for essential oil; 3 – stopper; 4 - return cooler.

Place 10 g of cut crude drug (weigh out with precision $\pm 0,01$ g) into a flask and add 300 ml of water, introduce the receiver in to the flask and attach the reflux condenser, heat to the temperature of boiling and distil for the prescribed time. Stop the heating, allow to cool and read the volume of volatile oil collected in the receiver [23].

Calculate the result as millilitres per 100 g of drug from the expression:

$$X = \frac{a \times 100 \times 100}{b(100 - w)}$$

a - volume of a volatile oil, ml;

b - mass of vegetable drugs, g; w – loss on drying, %

Was obtained essential oils from *Anethum graveolens* herb. The quantitative content of essential oil was 0,9%.

For essential oil was determined the physical-chemical properties: refraction index, solubility, colour and transparency, odour, taste (Table 3.2).

Table 3.2

The physical-chemical properties of dill essential oil

The physical-chemical properties	Method of determination	Result
<i>Refraction index</i>	Get acquainted with the employment of a refractometer	Refraction index of essential oil of <i>Anethum graveolens</i> herb at 20° is 1,41
<i>Solubility</i>	2 ml of oil was added to 2 test tubes. Purified water was added to one, ethanol 96% to the second	Essential oil soluble in 96% alcohol, insoluble in water
<i>Colour and transparency</i>	Place 5 ml of a volatile oil into a cylinder (d= 2-3 cm)	It is light green liquid
<i>Odour</i>	Place two drops of a volatile oil on a strip of a filtrate paper (12x5 cm) and compare the odour with the odour of the (for 1 h in every 15 min)	Essential oil with characteristic, pleasant smell.
<i>Taste</i>	Placed one drop of a volatile oil on a sugar and with the help of tongue determined its taste	Essential oil has characteristic sweet-bitter taste

According results of study, *dill* herb essential oil is oily, light green, clear liquid, with a characteristic, pleasant smell and sweet-bitter taste.

3.3.2 Chromatography - mass - spectrometry research of components of essential oils from *dill* herb.

Qualitative and quantitative determination of essential oil components conducted chromatography-mass spectrometric method in gas chromatography-mass spectrograph company "Hewlett-Packard" (NC), United States, consisting of the brand chromatograph HP6890 GC and mass - selective detector 5973N.

The components were separated on a silica capillary column HP company (HP 19091J-433 HP-5) length of 30 m and an inner diameter of 0.25 mm, filled with 5% phenilmethylsyloxanom [45].

Column temperature programming was used: initial temperature 600 final - 2400. Distillation period (from the initial to the final temperature of isothermal sections of the program) 1 hour.

Sweep the speed 3 grad/1min. Sample volume was 0.3 ml with a coefficient of flow separation and 1:15 inlet pressure of 40 kPa column; carrier gas - helium. Recording time - 0.5 sec.

The resulting spectra are seen as based on the general laws of fragmentation of molecules of organic compounds under electron impact, or by searching mass spectral library databases «Flavor2.L.» And «NIST98 L.».

Before carrying out the search for each chromatographic peak calculated average mass spectrum from which the background subtracted spectrum [17].

The identification of compounds was performed by comparison of mass spectra of the chromatographic peak of the mass spectra of reference compounds with high probability recognition program identified in the array spectra database

The quantitative content of the compounds was calculated by the ratio of the peak component to the sum of the areas of all peaks in the chromatogram (method of normalization).

Research conducted chromatography-mass spectrometric method using the built-processing program of mass spectra.

Mass spectra corresponding chromatographic peaks were identified by comparing their mass spectra with reference compounds [22].

Results of chromat-mass-spectrometry research of *dill* herb essential oil shown in Table 3.3.

Table 3.3

Results of chromat-mass-spectrometry research
of *dill* herb essential oil

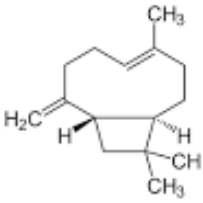
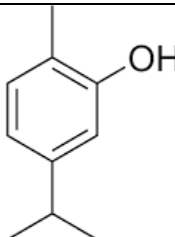
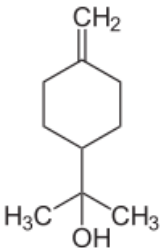
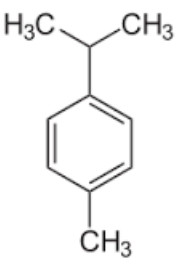
№	Compound	Rt, min.	Content, %
1	Limonene	6,67	0,15
2	α -Phellandrene	12,24	0,35
3	Linaloole	12,73	0,17
4	Eudesmol	18,03	2,34
5	<i>p</i> -Cymene	18,63	1,14
6	Eugenol	18,83	5,13
7	<i>p</i> -Cymene-8-ol	18,81	23,13
8	α -Terpineole	19,88	20,45
9	Carvacrole	21,97	25,10
10	Pentadecane	24,39	13,76
11	<i>trans</i> -Caryophyllene	25,15	10,45
12	Hexadecan	25,98	9,78

Chromatogram of essential oil components are shown in Fig. 3.3.

As seen from Tab. 3.3 in essential oil identified 12 compounds. Dominated in quantitative content are caryophyllene (10,45%), carvacrol (25,10%), α -terpineole (20,45%), *p*-cymene-8-ol (23,13%).

Table 3.4

Chemical and structural formulas of dominated in dill essential oil
components

Compound	Chemical formula	Structural formula
Caryophyllene	$C_{15}H_{24}$	
Carvacrol	$C_{10}H_{14}O$	
α -Terpineole	$C_{10}H_{18}O$	
<i>p</i> -Cymene	$C_{10}H_{14}$	

If we consider the belonging of compounds from Tab. 3.4 to chemical groups, caryophyllene belongs to bicyclic sesquiterpenes; carvacrol and *p*-cymene – aromatic compounds; α -terpineole – monoterpene alcohol.

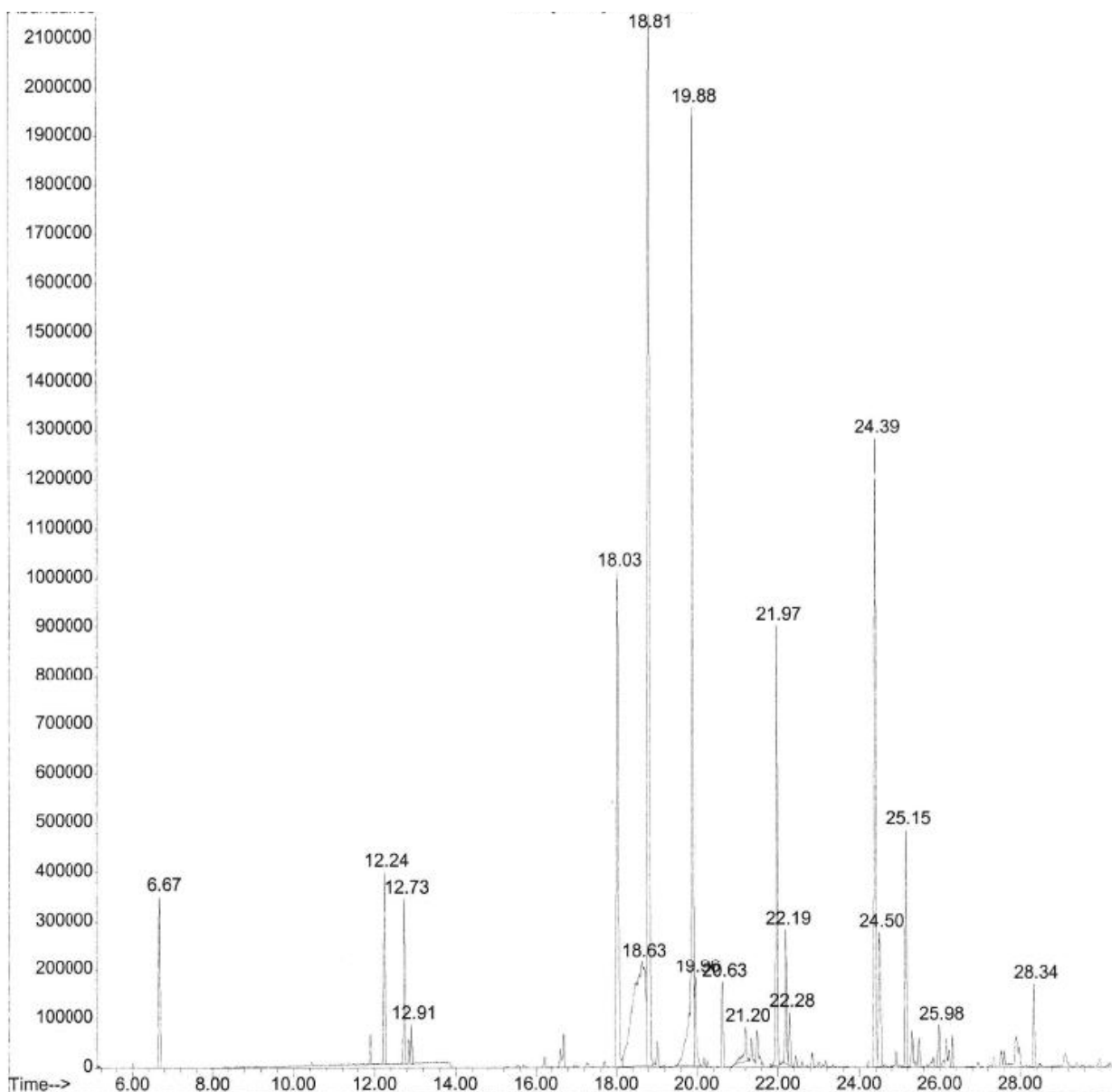


Fig. 3.3 Chromatogram of essential oil components of dill herb

3.4 Determination the chlorophylls in lipophilic fraction of dill herb

Quantitative determination of chlorophyll

For quantitative analysis of chlorophylls used photometric determine the amount of pigment. For chlorophyll extraction using 96% ethanol [21].

Methods. Sample of the lipophilic fraction leaves 0.1569 g dissolved in 10 ml of 96% ethanol. The solution was filtered, the volume of solution driven to 10 ml of 96% ethanol. From obtained solution taken away by pipette 5 ml, driven to 10 ml of 96% ethanol, were double dilution [38]. Optical density determined in

photoelectric colourimeter with a red filter with a thickness of 10 mm absorbent filter. Reference solution was 96% ethanol. At the same time was measured optical density of the standard solution Guthrie under the same conditions. To prepare the standard solution Guthrie used 4% solution of potassium dichromate - 50 ml; 1% solution of copper sulfate – 28,5 ml; ammonium hydroxide - 10 ml; distilled water - 100 ml. 1 ml of obtained solution corresponds to 0.000085 g color of chlorophyll. Chlorophyll content in lipophilic fraction in percentage (x) in terms of absolutely dry raw material was calculated using the formula:

$$X = \frac{D_1 \cdot P \cdot A \cdot 100}{D_2 \cdot a},$$

D_1 – optical density of the investigated extract; D_2 – optical density of standart; P – dilution; A – amount of chlorophyll, g in 1 ml, according in color 1 ml of standard solution of Guethrie; a - the mass of raw materials, g; $a = 0,1569$; $p = 10$ ml; $D_1 = 0,20$; $D_2 = 0,59$

Quantification of chlorophyll content in lipophilic fraction was 0,16 %.

Table 3.5

Metrological characteristics average results of chlorophyll determination

n	f	X_i	X_{cep}	S^2	$S_{cep.}$	P	t(P,f)	Interval	ϵ
1	2	3	4	5	6	7	8	9	10
5	4	0,1620	0,1654	0,00004070	0,003462	95%	2,78	0,1654±0,0049	1,24%
		0,1660							
		0,1680							
		0,1700							
		0,1720							

3.5. Determination the free and associated amino acids in *dill* herb

This group of compounds was determined on paper chromatography with the subsequent processing 10% solution of ninhydrin in acetone and heated in an oven at 80-100 ° C.

The appearance of red and red-purple of spots indicating the presence of amino acids in herb.

Qualitative and quantitative analysis of amino acids in the samples were determined using amino acid analyzer T339M Mikrotechna-Praha.

For this sample (100 mg) was dissolved in alcohol and were placed in the reaction vessel volume of 50 ml, was added an equal amount of concentrated hydrochloric acid, nitrogen blowing air removal, closed hermetically ground glass stopper and placed in the heating Thermostat with a temperature of 120 ° C for 24 hours.

Then the sample was filtered, transferred to a porcelain cup in which the solution was evaporated in a stream of nitrogen gas to remove hydrochloric acid and a pH of the solution within 1,6-2,0. After the sample is again filtered through filter paper and adjusted with a solution of sodium hydroxide to pH 2.2. To the amino acid analyzer was administered 50 mcl sample. Qualitative analysis was carried out by comparing the exit time of standard amino acids with amino acids in the sample.

Quantitative determination of amino acids in the samples conducted using the formula:

$$C = \frac{S \cdot C_1}{S_1}$$

where: C – the concentration of amino acids in the sample (mg);

C₁ – concentration of amino acids in the standard;

S – peak area of amino acids in the sample;

S₁ – peak area of amino acids in the standard.

The content of free and associated amino acids in *Anethum graveolens* herb presented in Table 3.6 and Fig. 3.4. In associated condition most accumulated (in

% of absolutely dry raw material) histidine and asparagine. Among free amino acids are dominated – isoleucine, tyrosine.

Table 3.6

The content of free and associated amino acids in
dill herb

Name of acid	The content of associated amino acids (mkg/100 mg)	Name of acid	The content of free amino acids (mkg/100 mg)
Asparagine	13,5	Asparagine	5,0
Threonine	2,2	Threonine	2,7
Serine	5,0	Serine	0,3
Glutaminic acid	6,0	Glutaminic acid	1,0
Proline	1,4	Proline	1,0
Glutamine	6,0	Glutamine	3,5
Alanine	2,7	Alanine	2,0
Valine	1,7	Valine	0,8
Methionine	10,8	Methionine	1,2
Isoleucine	6,0	Isoleucine	0,9
Leucine	1,35	Leucine	4,5
Thyrosine	8,23	Thyrosine	11,0
Phenylalanine	5,25	Phenylalanine	10,5
Histidin	15,3	Histidin	7,0
Lysine	12,5	Lysine	0,67
Arginine	8,0	Arginine	3,65
The sum of associated amino acids	105,93	The sum of free amino acids	55,72

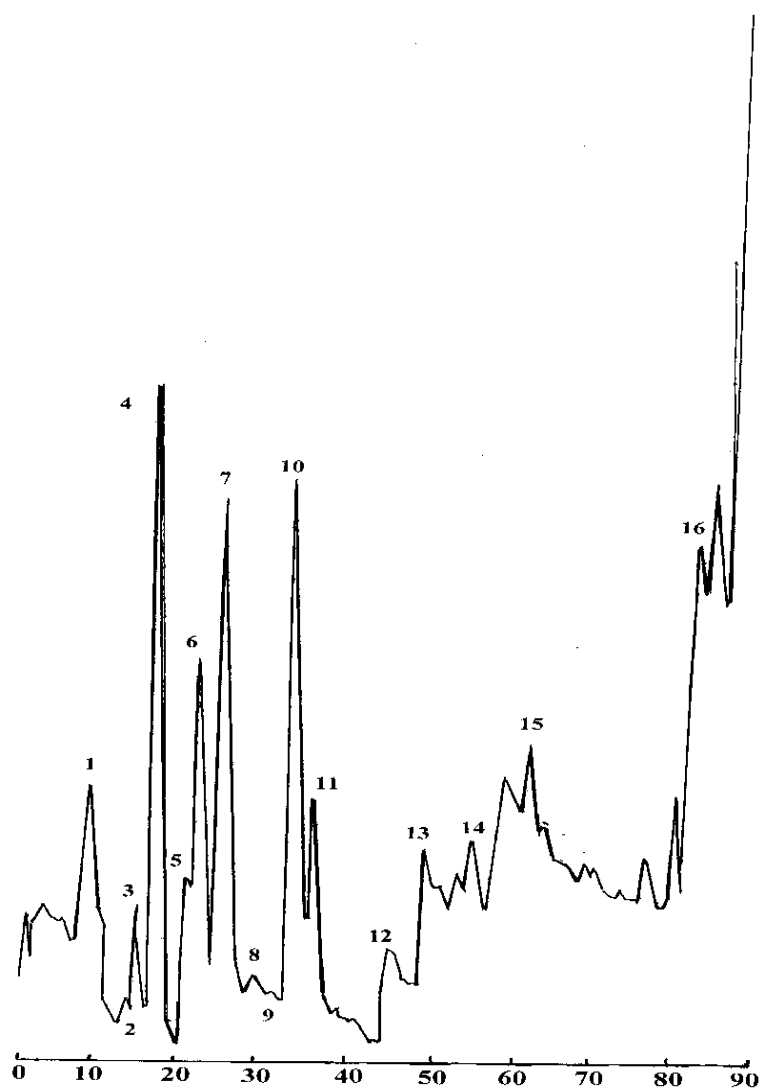


Fig. 3.4 The scheme of the gas chromatograms of amino acids of dill herb

Note: 1– asparagine, 2 – threonine, 3– serine, 4 – glutaminic acid, 5 – proline, 6 – glutamine, 7 – alanine, 8 – valine, 9 – methionine, 10 – isoleucine, 11 – leucine, 12 – thyrosine, 13 – phenylalanine, 14– histidin, 15– lysine, 16 – arginine

3.6. The study of microelement composition of *dill herb*

Study of qualitative and quantitative elements was performed using the method of atomic emission spectrophotometry. Samples were evaporated to craters of graphite electrodes in the discharge the arc alternating current power at 16 and 60 seconds of exposition. As a source of excitation of spectra using IBC-28. The spectra were recorded on film using a spectrograph DFS-8 diffraction grating 600

str / mm and threelens lighting slit. Conducted photometry line of spectra at wavelengths from 240 to 347 nm in the samples compared with standard samples of mixture of mineral elements with mikrophotometr MF-4. The results of elemental analysis of *dill* herb shown in Table 3.6.

Table 3.6.

The results of elemental analysis of *Anethum graveolens* herb

Element	The quantitative contents (mg/100 g)
Macroelements	
Potassium (K)	850
Sodium (Na)	45
Calcium (Ca)	260
Phosphorus (P)	40
Magnesium (Mg)	100
Silicon (Si)	80
Microelements	
Iron (Fe)	70
Manganese (Mn)	4
Aluminium (Al)	15
Lead (Pb)	<0,03
Strontium (Sr)	0,5
Nickel (Ni)	0,06
Molybdenum (Mo)	<0,03
Copper (Cu)	0,3
Chromium (Cr)	<0.03
Cobalt (Co)	<0.01
Cadmium (Cd)	<0.01
Arsenic (As)	<0.01
Surma (Sb)	<0.01

Detected 6 macro – (K, Na, Ca, P, Mg, Si,) and 9 microelements (Fe, Mn, Al, Pb, Sr, Ni, Mo, Cu, Cr). The results of definition are presented in Table 2.8.

In herb are missing or are outside possibilities of the method of determining emission spectrometry microelements: cobalt (<0.03), cadmium (<0.01), arsenic (<0.01) and antimony (<0.01). Are most accumulate (mg/100 g) macroelements: potassium, calcium, magnesium, phosphorus, silicon and microelements: copper, manganese, aluminum.

3.7 Determination of phenolic compounds by HPLC method

The qualitative composition and quantitative content of phenolic compounds in the obtained extracts were determined by high performance liquid chromatography (HPLC).

The analysis was carried out in the following chromatography mode: mobile phase supply rate - 0.25 ml/min.; the working pressure of the eluent is 240-300 kPa; the temperature of the column thermostat is 32° C; sample volume – 5 µl; gradient mode of chromatography (Table 3.7). Detection parameters: measurement scale – 1.0; scanning time – 0.5 sec.; parameters of spectrum extraction – each peak is 190-600 nm.

Phenolic compounds were identified by retention time of standards and spectral characteristics.

Table 3.7

Gradient chromatography mode

Time, min.	A% (0,2% TFA)	B% 70 %MeOH (0,2% TFA)	C% 100 %MeOH
0	92	8	0
8	62	38	0
24	0	100	0
29	0	0	100

HPLC chromatogram of phenolic compounds is given on Fig. 3.5. In the raw material 4 phenolic compounds were identified: 2 hydroxycinnamic acids

(chlorogenic and ferulic) and 1 glycoside of flavonoids (rutin) and 1 aglicon (quercetin).

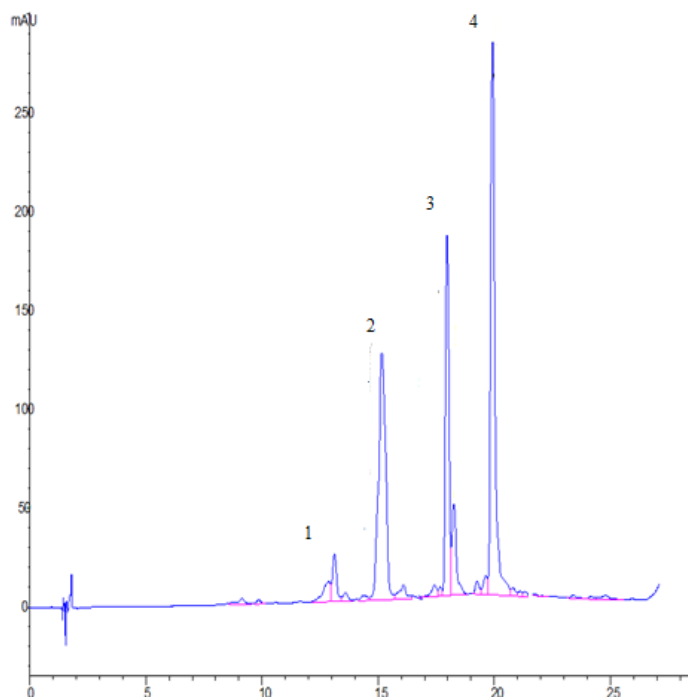


Fig. 3.5 HPLC chromatogram of phenolic compounds from Dill herb

Table 3.8

The content of phenolic compounds in dill herb

Compound	Rt., min.	Content, mg/100 g
Chlorogenic acid	13,00	55,70
Quercetin	17,10	81,25
Ferulic acid	18,50	135,80
Rutin	19,20	172,12

Conclusions

1. Quantitative determination of BAC content in dill herb was carried out. The loss in mass during drying was determined, it equal in % to $6,14 \pm 0,19$.
2. Was obtained the lipophilic fraction and its output was recalculated (18,3%).
3. By use a gas chromatography method in raw material founded 11

fatty acids, from which 2 (linoleic and linolenic) are essential and part of a complex of vitamin F. Dominant (mkg/100 mg) are palmitic, oleic, and linoleic acids.

4. By steam distillation was obtained the essential oil from *dill* herb and recalculated its quantitative content, it was 0,9%.

5. Was determined some physical-chemical properties of dill herb essential oil: refraction index, solubility, colour and transparency, odour, taste.

6. By use chromat-mass-spectrometric method identified 12 compounds of essential oil. Dominated in quantitative content are carvacrol (25,10%), α -terpineole (20,45%), p-cymene (23,13%).

7. Quantification of chlorophyll content in lipophilic fraction of *dill* herb was 0,16%.

8. By use a gas chromatography in Dill herb was identified 16 amino acids.

9. Detected 6 macro – (K, Na, Ca, P, Mg, Si,) and 9 microelements (Fe, Mn, Al, Pb, Sr, Ni, Mo, Cu, Cr).

10. By used HPLC method determined the content of 4 compounds – chlorogenic and ferulic acid, quercetin and rutin.

GENERAL CONCLUSIONS

1. Analyzed the current state of *dill* research and completed analytical review of phytochemical and pharmacological study.
2. The main active compounds of Dill are terpenoids and phenolic compounds.
2. Established that Dill it is undemanding plant that is widely cultivated in Ukraine and other countries.
3. Pharmacopeia raw material is fruit of Dill but herb is used in folk medicine.
4. The chemical composition of dill herb insufficiently studied that makes this a promising raw material for further study.
5. Qualitative composition of BAC of *dill* herb was carried out.
6. In herbal drugs was determined presens of polysaccharides, phenolic compounds, saponins. For the investigations used qualitative chemical reactions and chromatographic methods.
7. Amongst the phenolic compounds was identified simple phenols, coumarins, flavonoids, hydroxycinnamic acids, tannic compounds. According results of cyaniding reaction in herbal drugs presens different forms of flavonoids. In herb presence 5 phenolic compounds, among which compounds 4 and 5 with flavonoid nature and compounds 1, 2, 3 are derivatives of hydroxycinnamic acids.
8. By chromathographic method in compared with standart samples compound 3 identified as chlorogenic acid, 4 - as quercetin, 7- rutin.
9. By method of chlorophorm extraction was obtained lipophilic fraction of *dill* herb and recalculated its output. In raw material founded 11 fatty acids.
11. Was obtained the essential oil from *dill* herb and determined it refraction index, solubility. colour and transparency, odour and taste. By use chromato-mass-spectrometric method in essential oil identified 12 compounds.
12. Quantification the content of chlorophyll, phenolic compounds, amino acids and mineral elements was established.

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APPENDICES



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 CHEMISTRY OF NATURAL
COMPOUNDS AND NUTRICOLOGY

CERTIFICATE

№ 29

This is to certify that

Kaygin B.

has participated in the V 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, 14, 2023, Kharkiv, Ukraine

Rector of the NUPh,
prof.

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

Head of the department of chemistry
of natural compounds and nutricaoology
of the NUPh, prof.



Alla KOTVITSKA

Inna VLADIMIROVA

Viktoriia KYSLYCHENKO

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
МІНІСТЕРСТВО ОСВІТИ І НАУКИ УКРАЇНИ
НАЦІОНАЛЬНА АКАДЕМІЯ НАУК ВИЩОЇ ОСВІТИ УКРАЇНИ
НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ
КАФЕДРА ХІМІЇ ПРИРОДНИХ СПОЛУК І 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 CHEMISTRY OF NATURAL COMPOUNDS 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 V Міжнародної науково-практичної
інтернет-конференції

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

ХАРКІВ
KHARKIV
2023

STUDY THE LIPOPHILIC FRACTION OF DILL HERB

Kaygin B., Mashtaler V.V., Sydora N.V.*

National university of Pharmacy, Kharkiv, Ukraine

*University of Turku, Turku, Finland

Introduction. Family *Apiaceae* is widely used in the official and folk medicine around the world. *Anethum graveolens* is a plant that is widely cultivated and used in medicine and food as a spice [1, 4]. The plant perfect growing in different conditions and the season you can collect several harvests [2].

Pharmacopeia herbal drugs are fruits of dill – *Anethi graveolentis fructus*, but scientific interest is the study of above-ground parts - herb dill to expand the resource base of biological active substances (BAS).

Materials and methods. The object of the study was the lipophilic fraction of dill herb, extractants – chloroform. To determine the qualitative and quantitative analysis of fatty acids in *Anethum graveolens* herb using 100 mg of sample. Extraction of the acid tests conducted by use Folch solution (chloroform -methanol 2:1) by heating to 40 ° C for 5 minutes.

Qualitative and quantitative analysis of fatty acids was determined by gas chromatography (gas chromatograph "Chrome - 5") on a metal column 2.6 m long, 0.32 mm in diameter filled with sorbent "Hromaton - super" 10% polyethylenglycolsuccinate. Analysis of samples of free fatty acids was carried out in isothermal mode at 195 ° C and heated flame ionization detector to 250 ° C. Rate of carrier gas of high purity nitrogen 50 ml / min., water 30 ml / min., air - 300 ml / min. Identification of free fatty acids was carried out by comparing the time of their exit from the known methyl esters of fatty acids. Quantitative analysis was performed by the absolute calibration of each fatty acid separately, as well as mixtures thereof with the drawing calibration curves which were determined and the concentration of each fatty acid in the sample [3].

Results and discussion. Identified (mcg/100 mg) 11 fatty acids: decanic (2,0), lauric (4,0), tridecanic (3,3), meristic (20,0), pentadecanic (5,0), palmitic (180,5), heptodecnic (10,0), stearinic (76,0), oleic (620,0), linoleic (700,0), linolenic (40,0). Linoleic and linolenic acids are essential and part of a complex of vitamin F. The sum (mcg/100 mg) of unsaturated fatty acids 1440,0, saturated – 194,5. By quantitative content are dominant palmitic, oleic and linoleic acids, the content of which in (%) of the sum of acids was 11%, 37,9% and 42,8% respectively.

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ЗМІСТ	CONTENT
VIBRATION SPECTRA OF MIXED CHLOROFORMATE COMPLEXES OF MOLYBDENUM Mo ₂ ⁴⁺ <i>Akhmedov E.L., Akberova S.Sh., Huseynquliyeva K.F.</i>	4
DETERMINATION OF THE TOTAL PHENOLIC, FLAVONOID AND HYDROXYCINNAMIC ACID DERIVATIVES CONTENT IN <i>LONICERA CAERULEA</i> L. LEAVES SAMPLES <i>Burneikaitė Dovilė, Liaudanskas Mindaugas, Viškelis Jonas, Kviklys Darius</i>	5
TOTAL PHENOLIC COMPOUNDS CONTENT AND PROFILE OF RASPBERRY (<i>RUBUS IDAEUS</i> L.) ROOTS <i>Butkevičiūtė G., Raudonė L.</i>	6
BIOMARKERS OF OXIDATIVE STRESS IN THE EQUINE BLOOD AFTER <i>IN VITRO</i> TREATMENT WITH EXTRACTS DERIVED FROM PSEUDO-BULBS OF <i>COELOGYNE PANDURATA</i> LINDL. (<i>ORCHIDACEAE</i>) PLANTS <i>Буґаєн Людмила, Ткаченко Халіна, Курчалук Наталія, Ковальська Людмила, Гуренко Олександр</i>	8
<i>TANACETUM CORYMBOSUM</i> – NEW SOURCE WITH POTENTIAL APPLICABILITY IN HERBAL MEDICINES <i>Ciocarlan N. G.</i>	11
PHYTO SUPPLEMENTS AS A MEANS OF IMPROVING THE RHEOLOGICAL PROPERTIES OF BREAD <i>Dolidze M., Epitashvili T.</i>	12
PROSPECTS FOR PHARMACOGNOSTIC STUDY OF REPRESENTATIVES OF THE GENUS VIOLET FOR THE TREATMENT OF SKIN DISEASES <i>Gontova T., Ibraghazala A.</i>	14
IDENTIFICATION AND QUANTITATIVE DETERMINATION OF ORGANIC ACIDS IN CARAMBOLA FRUITS <i>Imane Malhi, Tartynska G. S.</i>	16
BENEFICIAL FEATURES OF MEDICINAL WINES <i>Kacharava T., Geliashvili Z., Isakadze N.</i>	17
GENETIC RESOURCES OF MEDICINAL AND MELLIFEROUS PLANTS IN WEST GEORGIA <i>Kacharava T., Gogoladze Kh.</i>	19
STUDY THE LIPOPHILIC FRACTION OF DILL HERB <i>Kaygin B., Mashtaler V.V., Sydora N.V.</i>	21
ESSENTIAL OIL COMPOSITION OF CARROT HERB <i>Korkmaz F., Mashtaler V.V., Sydora N.V.</i>	22
ISOLATION OF MAGNOFLORINE FROM THE ROOT OF <i>BERBERIS VULGARIS</i> USING CENTRIFUGAL PARTITION CHROMATOGRAPHY <i>Koval Maryna, Kukula-Koch Wierginia</i>	24

National University of Pharmacy

Faculty for foreign citizens' education
Department pharmacognosy

Level of higher education master

Specialty 226 Pharmacy, industrial pharmacy
Educational program Pharmacy

APPROVED
The Head of Department
Pharmacognosy

Olga MALA

«28» of September 2022

**ASSIGNMENT
FOR QUALIFICATION WORK
OF AN APPLICANT FOR HIGHER EDUCATION**

Bensu KAYGIN

1. Topic of qualification work: «Study the biological active compounds of dill herb», supervisor of qualification work: Victoria MASHTALER, PhD, assoc. prof. approved by order of NUPh from «6» of February 2023 № 35
 2. Deadline for submission of qualification work by the applicant for higher education: April 2023.
 3. Outgoing data for qualification work: the work is devoted to study of biological active compounds composition of dill herb.
 4. Contents of the settlement and explanatory note (list of questions that need to be developed): study of qualitative content of BAC – polysaccharides, phenolic compounds, saponins; quantitative determination of fatty acids, essential oils, chlorophylls, amino acids, microelements.
 5. List of graphic material (with exact indication of the required drawings):
Tables – 16, figures – 10.
-

6. Consultants of chapters of qualification work

Chapters	Name, SURNAME, position of consultant	Signature, date	
		assignment was issued	assignment was received
1	Victoria MASHTALER, PhD, assoc. prof., associate professor of higher education institution of department pharmacognosy	Victoria MASHTALER 9.2022-10.2022	Bensu KAYGIN 9.2022-10.2022
2	Victoria MASHTALER, PhD, assoc. prof., associate professor of higher education institution of department pharmacognosy	Victoria MASHTALER 10.2022-11.2022	Bensu KAYGIN 10.2022-11.2022
3	Victoria MASHTALER, PhD, assoc. prof., associate professor of higher education institution of department pharmacognosy Amzhad I. ABU SHARK, PhD, assoc. prof., associate professor of higher education institution of department pharmaceutical chemistry	Victoria MASHTALER 1.2023 Amzhad I. ABU SHARK 1.2023	Bensu KAYGIN 1.2023 Bensu KAYGIN 1.2023

7. Date of issue of the assignment: «28» of September 2022

CALENDAR PLAN

№ з/п	Name of stages of qualification work	Deadline for the stages of qualification work	Notes
1	Writing a review on a given issue	September-December 2022	done
2	Conducted of qualitative identification of BAC	January 2023	done
3	Conducted of quantitative study of fatty acids and essential oil	February 2023	done
4	Study of amino acids content	March 2023	done
5	Study of content of minerals component	April 2023	done
6	Preparation of a master's thesis for official defense	May 2023	done

An applicant of higher education

Bensu KAYGIN

Supervisor of qualification work

Victoria MASHTALER

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

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

Прізвище студента	Тема кваліфікаційної роботи		Посада, прізвище та ініціали керівника	Рецензент кваліфікаційної роботи
• по кафедрі фармакогнозії				
Кайгін Бенсу	Дослідження біологічно активних речовин трави кропу.	Study the biological active compounds of Dill herb.	доцент Машталер В.В.	доцент Абу Шарк Амжад Ібрагім

Підстава: подання декана згода ректора

Ректор

Вірно. Секретар



ВИСНОВОК

**Комісії з академічної доброчесності про проведену експертизу
щодо академічного плагіату у кваліфікаційній роботі
здобувача вищої освіти**

№ 112772 від « 30 » квітня 2023 р.

Проаналізувавши випускну кваліфікаційну роботу за магістерським рівнем здобувача вищої освіти денної форми навчання Кайгін Бенсу, 5 курсу, _____ групи, спеціальності 226 Фармація, промислова фармація, на тему: «Дослідження біологічно активних речовин трави кропу / Study the biological active compounds of Dill herb.», Комісія з академічної доброчесності дійшла висновку, що робота, представлена до Екзаменаційної комісії для захисту, виконана самостійно і не містить елементів академічного плагіату (компіляції).

**Голова комісії,
професор**



Інна ВЛАДИМИРОВА

4%
27%

REVIEW

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

Bensu Kaygin

on the topic: «Study the biological active compounds of dill herb»

Relevance of the topic. *EurPh* herbal drugs are fruits of dill, but scientific interest is the study of it herb to expand the resource base of biological active compounds. The output of dry material from one plant is quite significant, so use of agricultural herbs can expand assortment of herbal medicines provided with resource base.

Practical value of conclusions, recommendations and their validity. The obtained practical results made it possible to expand information about the chemical composition of dill herb. Considering the wide range of BAC of the obtained essential oil, it can be concluded that it is a promising substance that can be used to create of various dosage forms.

Assessment of work. The work was performed at a high scientific level, the performer showed himself as a disciplined, erudite person. All conclusions are logical, the work is well illustrated and structured.

General conclusion and recommendations on admission to defend. The obtained research results in terms of relevance, scientific and practical significance meet the requirements for qualification works of this level and therefore work of Bensu Kaygin «Study the biological active compounds of dill herb» can be presented for official defense in the State Examination Commission of National University of Pharmacy.

Scientific supervisor
«7th» of April 2023

_____Victoria MASHTALER

REVIEW

for qualification work of the master's level of higher education, specialty 226
Pharmacy, industrial pharmacy

Bensu Kaygin

on the topic: «Study the biological active compounds of dill herb»

Relevance of the topic. The dill is widely cultivated agricultural plant in Ukraine and used in medicine and food. The plant growing in different conditions and the season you can collect several harvests. The output of dry material from one plant is quite significant, so use of agricultural herbs can expand assortment of herbal medicines provided with resource base. Given the above said the *dill* herb is a promising source of BAC and interesting subject for phytochemical study.

Theoretical level of work. The qualification work was performed at a high scientific level, modern methods and techniques of phytochemical research (chromato-mass spectrometry, gas chromatography) were used in the research. Scientific primary sources are well developed and structured by the acquirer. The presented work is performed at a high theoretical and practical level, well illustrated. Based on the research materials, 1 theses of reports was published.

Author's suggestions on the research topic. Considering the wide range of BAC of the obtained essential oil, it can be concluded that it is a promising substance that can be used to create of various dosage forms.

Practical value of conclusions, recommendations and their validity. The obtained practical results made it possible to expand information about the chemical composition of dill herb.

Disadvantages of work. Some materials should have been presented in the form of a table for greater clarity.

General conclusion and assessment of the work. The material of the qualification work of Bensu Kaygin is logically and consistently, which testifies to the author's ability to structure the received information, use scientific primary

sources and summarize experimental data. The presented work meets the requirements for qualifying papers and can be recommended for defense at the State Examination Commission of National University of Pharmacy.

Reviewer _____ assoc. prof. Amzhad I. ABU SHARK

«10th» of April 2023

**МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ**

**ВИТЯГ З ПРОТОКОЛУ №13
засідання кафедри фармакогнозії**

«19» квітня 2023 року
м. Харків
засідання кафедри
фармакогнозії

Голова: завідувач кафедри, канд. фарм. наук, доцент Мала О.С.

Секретар: канд. фарм. наук, ас. Комісаренко М. А

Присутні: зав. каф. доц. Мала О.С., проф. Ковальова А. М., проф. Гонтова Т.М., проф. Кошовий О.М., проф. Криворучко О.В., доц. Бородіна Н.В., доц. Демешко О.В., доц. Очкур О.В., доц. Машталер В.В., ас. Гончаров О.В., ас. Комісаренко М.А.

ПОРЯДОК ДЕННИЙ:

1. Представлення кваліфікаційних робіт до захисту в Екзаменаційній комісії НФаУ.

СЛУХАЛИ: Про представлення до захисту в Екзаменаційній комісії НФаУ кваліфікаційної роботи здобувача вищої освіти Фм18(5.0д)англ-01 групи Кайгін БЕНСУ на тему «Study the biological active compounds of Dill herb».

Науковий керівник : к.фарм.н., доц. Вікторія МАШТАЛЕР.

Рецензент: к.фарм.н., доц. Амжад Ібрагім АБУ ШАРК.

В обговоренні кваліфікаційної роботи брали участь зав. каф. доц. Мала О.С., доц. Машталер В.В., проф. Кошовий О.М., проф. Криворучко О.В., доц. Демешко О.В., ас. Гончаров О.В.

УХВАЛИЛИ: Рекомендувати до захисту у Екзаменаційній комісії НФаУ кваліфікаційну роботу здобувача вищої освіти Кайгін БЕНСУ на тему «Study the biological active compounds of Dill herb», науковий керівник: к.фарм.н., доц. Вікторія МАШТАЛЕР.

Голова

Завідувачка кафедри фармакогнозії

Секретар

Ольга МАЛА

Микола КОМІСАРЕНКО

НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

ПОДАННЯ ГОЛОВІ ЕКЗАМЕНАЦІЙНОЇ КОМІСІЇ ЩОДО ЗАХИСТУ КВАЛІФІКАЦІЙНОЇ РОБОТИ

Направляється здобувач вищої освіти Бенсу Кайгін до захисту кваліфікаційної роботи за галуззю знань 22 Охорона здоров'я спеціальністю 226 Фармація, промислова фармація освітньою програмою Фармація на тему: «Study the biological active compounds of dill herb».

Кваліфікаційна робота і рецензія додаються.

Декан факультету _____ / Світлана КАЛАЙЧЕВА /

Висновок керівника кваліфікаційної роботи

Здобувачка вищої освіти Бенсу Кайгін успішно виконала поставлені завдання, засвоїла роботу з науковими першоджерелами, методами та методиками фармакогностичного аналізу рослинної сировини, які вона застосовувала при виконанні своєї роботи.

Отримані результати досліджень за актуальністю, науковим та практичним значенням відповідають вимогам, які висуваються до кваліфікаційних робіт, тому представлена робота може бути рекомендована до публічного захисту у Екзаменаційну комісію Національного фармацевтичного університету.

Керівник кваліфікаційної роботи

Вікторія Машталер

«7» квітня 2023 року

Висновок кафедри про кваліфікаційну роботу

Кваліфікаційну роботу розглянуто. Здобувач вищої освіти Бенсу Кайгін допускається до захисту даної кваліфікаційної роботи в Екзаменаційній комісії.

Завідувач(ка) кафедри
фармакогнозії

Ольга МАЛА

«19» квітня 2023 року

Qualification work was defended

of Examination commission on

« ____ » of June 2023

With the grade _____

Head of the State Examination commission,

DPharmSc, Professor

_____ / Oleh SHPYCHAK /