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department of pharmacognosy

QUALIFICATION WORK

on the topic: «CHEMICAL STUDY THE LIPOPHILIC COMPOUNDS OF *JUNIPERUS SABINA*»

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ABSTRACT

Lakbaibi A. « Chemical study the lipophilic compounds of *Juniperus sabina*»

The qualification work is devoted to the study the lipophilic compounds of *Juniperus sabina*. Was obtained the lipophilic fraction and essential oil from needle of *J. sabina* and its chemical composition and quantitative content of biological active compounds (BAC) was established.

Key words: *Juniperus sabina*, lipophilic fraction, essential oil, chemical composition.

АНОТАЦІЯ

Лакбаібі А. «Хімічне дослідження ліпофільних сполук *Juniperus sabina*»

Кваліфікаційна робота присвячена дослідженню ліпофільних сполук *Juniperus sabina*. Одержано ліпофільну фракцію та ефірну олію з хвої *J. sabina* та встановлено їх хімічний склад та кількісний вміст біологічно активних сполук.

Ключові слова: *Juniperus sabina*, ліпофільна фракція, ефірна олія, хімічний склад.

CONTENT

LIST OF CONVENTIONAL ABBREVIATIONS

ASL - according to sea level;

BAC – biological active compounds;

ChS – chromatographic system;

DMAC – dimethylacetamide;

EFac – essential fatty acids;

GC – gas chromatogtaphy;

J. sabina – Juniperus sabina;

LF – lipophilic fraction;

MS – mass spectrum;

NUPh – National university of Pharmacy;

Rt – retentional time;

StSp – standard samples;

Syn. – synonym;

TLC – thin layer chromatography;

UV- light – ultra violet light;

СФ – spectrophotometer.

INTRODUCTION

The topic actuality. The study of plants for identification and isolation of differents classes of biological active compounds (BAC) it is one of the important task of modern Pharmacognosy. This is especially actual for the plants used in folk medicine and homeopathy for the treatment of widespread diseases. Among the plants that have significant pharmacological effect special place is occupied plants that belong to the poisonous. Such a plant is *Juniperus sabina*. Under the influence of ecological factors, new microorganisms are insensitive to traditional drugs are appear differents causative agents dermatological diseases like dermatitis, dermatitis, eczema. So, it is necessary to search for natural substances to solve this problem.

The aim of investigation was study the lipophilic compounds of *J. sabina*.

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

- To analyze the scientific sources regarding the botanical characteristics of Cupressaceae, chemical composition of juniper, use of juniper in medicine;
- To determine the loss in mass during drying and calculate the yield of raw materials;
- To obtain the lipophilic fraction and recalculated it yield;
- To investigate the qualitative composition of the lipophilic fraction by chromatographic methods;
- To establish the quantitative content of fatty acids, chlorophylls and carotenoids in the obtained lipophilic fraction;
- To obtain the essential oil of needles and establish its quantitative content;
- To establish some physico-chemical parameters of the essential oil and to establish its component composition using the chromato-mass spectrometric method.

The object of study was needles of *Juniperus sabina*.

The subject of study was estebliched the content of BAC of lipophilic

fraction and essential oil from needles of *Juniperus sabina* (fatty acids, chlorophils, carotenoids, terpenoids).

Methods of study: physical - determination of loss in mass during drying; physical and chemical - chromatography in a thin layer of sorbent; instrumental, physical and chemical – chromate-mass-spectrometry, spectrophotometry, photoelectrocolorimetry.

Practical significance of the obtained results.

The practical value of the qualification work is due to the fact that, as a result of the conducted research, the acquirer expanded the information on the chemical composition of the needles of *J. sabina*. Taking into account the spectrum of biological activity of the identified lipophilic compounds, it can be concluded that the obtained lipophilic fraction and essential oil can be used in the future for the development of external medicinal products for the treatment of skin diseases caused by *S. aureus.*

Elements of scientific research. Based on the analysis of the literature, the main issues of the research was formulated, determined the quantitative content of lipophilic fraction and essential oil from *J. sabina*.

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 38 foreign languages sources and appendices. The content of the work is laid out on 47 pages of the main text and illustrated with 17 tables and 16 figures.

CHAPTER 1. BOTANICAL CHARACTERISTIC OF FAMILY CUPRESSACEAE (LITERATURE REVIEW)

1.1 Botanical characteristic the *Cupressaceae* family and plants belongs the Juniper (*Juniperus*) genus

Cupressaceae is the most widely distributed conifer family, with a near-global range in all continents except for Antarctica. It is the most large in number of genus and the third of species number of family of conifers. Junipers are coniferous plants in the genus *Juniperus* of the familyCupressaceae. Family Cupressaceae combining together 19 genus and 130 species, widely distributed in both hemispheres [19].

Depending on taxonomic viewpoint, between 50 and 67 species of juniper are widely distributed throughout the Northern Hemisphere, from the Arctic, south to tropical Africa in the Old World, and to the mountains of Central America.

Cupressaceae, the cypress family (order Pinales), 30 genera with 133 species ofevergreen ornamental and timber shrubs and trees, distributed throughout the world. The leaves of these plants are opposite or whorled and usually paired or in threes [9].

Adult leaves are narrow, scalelike, and pressed against the branchlets, which themselves are often flattened. Awllike juvenile and transitional leaves are often present on mature trees.

The male reproductive structures are borne at the ends of short twigs; the female structures (cones) are terminal, with opposite or whorled scales, consisting of both a fused bract (modified leaf) and a scale. The cones, usually woody, have erect ovules.

Formerly, 9 genera and about 16 species were treated as a separate family, Taxodiaceae, by most botanists. However, molecular studies have shown that these, along with the traditional Cupressaceae, form a single natural group.

The leaves are arranged either spirally, in decussate pairs (opposite pairs, each pair at 90° to the previous pair) or in decussate whorls of 3 or 4, depending on

the genus. On young plants, the leaves are needle-like, becoming small and scale-like on mature plants of many (but not all) genera; some genera and species retain needle-like leaves throughout their life.

Old leaves are mostly not shed individually, but in small sprays of foliage (cladoptosis); exceptions are the leaves on shoots, which develop into branches, which eventually fall off individually when the bark starts to flake.

Most are evergreen with the leaves persisting 2–10 years, but three genera (*Glyptostrobus*, *Metasequoia* and*Taxodium*) are deciduous or include deciduous species.

The seed cones are either woody, leathery, or (in *Juniperus*) berry-like and fleshy, with one to several ovules per scale. The bract scale and ovuliferous scale are fused together except at the apex, where the bract scale is often visible as a short spine (often called an *umbo*) on the ovuliferous scale [19].

As with the foliage, the cone scales are arranged spirally, decussate (opposite) or whorled, depending on the genus. The seeds are mostly small and somewhat flattened, with two narrow wings, one down each side of the seed; rarely (e.g.*Actinostrobus*) triangular in section with three wings; in some genera (e.g.*Glyptostrobus* and *Libocedrus*) one of the wings is significantly larger than the other, and in some others (e.g. *Juniperus*, *Microbiota*, *Platycladus* and *Taxodium*) the seed is larger and wingless [12]. Most habitats on land are occupied, with the exceptions of polar tundra and tropical lowland rainforest(though several species are important components of temperate rainforests and tropical highland cloud forests). Despite the wide overall distribution, many genera and species show very restricted relictual distributions, and many are endangered species [35]. The list of the most common species of *Juniperus* genus shown in Table 1.1.

Table 1.1

Species of *Juniperus* genus

№	English name	Latin name
1	Chinese pyramid juniper	*Juniperus chinensis*

2	Common juniper	*J. communis*
3	Old-field juniper	*J. communis var. depressa*
4	Irish juniper	*J. communis var. hibernica*
5	Mountain juniper	*J. communis var.saxatilis*
6	Shore juniper	*J. conferta*
7	Syrian juniper	*J. drupaceae*
8	Creeping juniper	*J. horizontalis*
9	Andora juniper	*J. horizontalis plumosa*
10	Plum juniper	*J. macrocarpa*
11	One-seed juniper	*J. monosperma*
12	Western juniper	*J. occidentalis*
13	Red juniper	*J. oxycedrus*
14	Alligator juniper	*J. pachyphloae*
15	Phoinician juniper	*J. phjenica*
16	Redberry juniper	*J. pinchoti*
17	Indian juniper	*J. polycarpos*
18	Needle juniper	*J. rigida*
19	Creeping juniper savin	*J. sabina*
20	RockyMountain juniper	*J. scopulorum*
21	Low juniper	*J. sibirica*
22	Incense juniper	*J. thurifera*
23	Red cedar juniper	*J. virginiana*

1.2 The morphological characteristic of *Juniperus sabina*

Juniperus sabina is a species of juniper native to the mountains of central and southern Europe and western and central Asia, from Spain to eastern Siberia, typically growing at altitudes of 1,000-3,300 m ASL.

A shrub up to 5 m high, with irregular crown, rarely erect and usually more or less prostrate. Branches with upturned ends. Bark on old trees reddish-brown.

Shoots slender, up to 1 mm in diameter, rounded to slightly angled. Shoots and leaves emit an unpleasant odour when rubbed.

Foliage dimorphic; on young plants and sterile branches leaves needle-like, in whorls, 4 mm long, pointed, glaucous above; scale leaves decussate, ovate, 1-3 mm long, with a dorsal gland [4].

The species monoecious or dioecious. Fruit globose to ovate, 5-7 mm long, bluish-black, pruinose, composed of 4 to 6 scales, on a curved petiole; ripening in the autumn of the first year or in the following spring. Seeds 1-3 to a fruit, ovate and furrowed. The fruit and leaves are poisonous.

The shrub is very variable in shape, up to 1–4 m tall. The leaves are of two forms, juvenile needle-like leaves 5–10 mm long, and adult scale-leaves 1–2 mm long on slender shoots 0.8–1 mm thick [38].

Juvenile leaves are found mainly on seedlings but mature shrubs sometimes continue to bear some juvenile leaves as well as adult, particularly on shaded shoots low in the crown. It is largely dioeciouswith separate male and female plants, but some individual plants produce both sexes.

The cones are berry-like, 5–9 mm in diameter, blue-black with a whitish waxy bloom, and contain 1-3 (rarely 4 or 5) seeds; they are mature in about 18 months. The male cones are 2–4 mm long, and shed their pollen in early spring.

There are two varieties, treated by some botanists as distinct species: *Juniperus sabina* var. *sabina*. Juvenile foliage rare in adult plants; *Juniperus Sabina* var. *davurica* (Pallas) Farjon (syn. *J. davurica* Pallas). Juvenile foliage frequent in adult plants.

J. sabina is a popular ornamental shrub in gardens and parks, with numerous named cultivars selected.

The hybrid between *Juniperus chinensis* and *Juniperus sabina*, known as *Juniperus* × *pfitzeriana* (Pfitzer Juniper, synonym *J.* × *media*), is found in the wild where the two species meet in northwestern China, and is also very common as a cultivated ornamental plant. It is a larger shrub, growing to 3–6 m tall.

All parts of the plant are poisonous due to several toxic compounds including ethereal oils.

It grows in the undergrowth coniferous, mixed, rarely hardwood forests, forest edges, glades clearings in the thickets of heath, including the coastal dunes, sometimes on the outskirts of the marshes. Very often juniper can be found in the pine forests.

A photophilous plant, so grows at clarification of forests and cutting areas. In Siberia, distributed closest species - Siberian juniper (Juniperus sibirica Burgsd.), Characterized by much lower growth, taking the place of the form thickets [36].

This species grows on dry, open, rocky, wooded hillsides, sand terraces, maritime escarpments, and on exposed slopes and plateaus.

It is found on dunes or dune heath in coastal areas, on isolated mountains, and may spread into fields and pastures.

Establishment is more likely in open spaces between older shrubs and may be favored by grazing. *Juniperus communis* L. grows in hilly to alpine regions up to an altitude of more than 3000 m.

Common juniper is intolerant of shade, found in open environments; colonizing plants reach maximum abundance on harsh, stressed environments in which competition is lacking. It grows on poor sites, tolerates full sun and wind and is pH adaptable [11].

Generally common juniper is killed or seriously damaged by fire; the relatively long germination period and poor germination rates contribute to slow postfire reestablishment.

1.3 A brief description of some lipophilic compounds

Essential fatty acids (EFAc) - are fatty acids that humans and other animals must ingest because the body requires them for good health but cannot synthesize them. The term "essential fatty acid" refers to fatty acids required for biological processes but does not include the fats that only act as fuel.

Only two fatty acids are known to be essential for humans: alpha-linolenic acid (an omega-3 fatty acid) and linoleic acid (anomega-6 fatty acid).

Some other fatty acids are sometimes classified as "conditionally essential," meaning that they can become essential under some developmental or disease conditions; examples include docosahexaenoic acid (an omega-3 fatty acid) and gamma-linolenic acid (an omega-6 fatty acid).

Chlorophyll a green pigment found in cyanobacteria and the chloroplasts of algae and plants. It is an extremely important biomolecule, critical in photosynthesis, which allows plants to absorb energy from light. Chlorophyll absorbs light most strongly in the blue portion of the electromagnetic spectrum, followed by the red portion. Conversely, it is a poor absorber of green and near-green portions of the spectrum, hence the green color of chlorophyll-containing tissues [14].

The function of the vast majority of chlorophyll (up to several hundred molecules per photosystem) is to absorb light and transfer that light energy by resonance energy transfer to a specific chlorophyll pair in the reaction center of the photosystems.

The two currently accepted photosystem units are Photosystem II and Photosystem I, which have their own distinct reaction center chlorophylls, named P680 and P700, respectively. These pigments are named after the wavelength (in nanometers) of their red-peak absorption maximum.

Carotenoids are organic pigments that are found in the chloroplasts and plants chromoplasts and some other photosyntheticorganisms, including some bacteria and some fungi.

Carotenoids can be produced from fats and other basic organic metabolic building blocks by all these organisms.

Carotenoids generally cannot be manufactured by species in the animal kingdom so animals obtain carotenoids in their diets, and may employ them in various ways in metabolism. There are over 600 known carotenoids; they are split into two classes, xanthophylls (which contain oxygen) and carotenes

(which are purely hydrocarbons, and contain no oxygen). All carotenoids are tetraterpenoids, meaning that they are produced from 8isoprene molecules and contain 40 carbon atoms. In general, carotenoids absorb wavelengths ranging from 400-550 nanometers (violet to green light). They serve two key roles in plants and algae: they absorb light energy for use in photosynthesis, and they protect chlorophyll from photodamage.

In humans, three carotenoids (beta-carotene, alpha-carotene, and beta-cryptoxanthin) have vitamin A activity (meaning that they can be converted to retinal), and these and other carotenoids can also act asantioxidants.

In the nature, carotenoids may be in different states: free-form, they are more common in the plastids of plants, fish muscle, eggs of birds; as esters of fatty acids - in chromatophores and epidermal structures of plants; in the form of carotene-proteins - in epidermal tissue of animals and others.

Carotenoids share with chlorophyll a major role in the energy metabolism of higher plants.

By absorbing light, they are transforming the global energy of pigments to reaction centers , where it is converted into electricity , and then in chemic form of ATPh , the latter is used for the synthesis of various compounds. It is important to membrane function and carotenoids to live in an oxygen atmosphere.

Carotenoids are involved in a variety of defense mechanisms: provide protection against ultraviolet radiation, so that they can transform the energy of UV light into light we can see that manifested in the phenomena of fluorescence; act as antioxidants, protecting sensitive tissue and labile compounds from oxidation.

1.4 Chemical constituent of *Juniper* species

J. sabina. In fruits contents the essential oil (sabinen, sabinol, sabinilacetat, cedrol, α-pinene, mircen, limonen, cis-tujon, carvacrole, podophylotoxine. The autput of essential oil from leaves was 0,8 –1%. Essential oil of Europea sorts contens α-pinene, sabinene, terpineol, sabinol, cadinen. In wood – diterpens (cis-comunic, trans-comunic, isocupresic acids). In bark detected the tannic compounds

(3-5,1%). The leaves containe the essential oil 2-5% (sabinol, α-pinen, sabinen, geraniol, cadinen, α-terpinen).

J. communis. More than 200 constituents were detected and the contents of 50 compounds were reported. Dependence of the percentage yields of monoterpene, sesquiterpene, oxygenated monoterpene, and oxygenated sesquiterpene hydrocarbon groups on the extraction time was investigated, and conditions that favored the yielding of each terpene groups were emphasized.
In fruits was detected essential and fatty oil [2]. In wood - essential oil (cuparene, gumulene, cedrole, calamenene, isocadinene, δ-cadinene, β-tuyone); diterpens. In bark - steroid spigmasterine; eannic compounds (catychin, epicatechin); iesquiterpenoids. In leaves - α-pinene, β-pinene, β –mircene, cineole, Vit. C, carotene, organic acids, coumarins, flavonoids [3].

The chemical composition of the essential oil of Portuguese juniper berries *(Juniperus communis* L. ssp. *nana* Syme) was investigated by means of gas chromatography. This analysis was compared with that of an aqueous alcoholic extract of the juniper berries of similar origin. The qualitative composition of the oil and the alcoholic extract was found to be very similar. The major hydrocarbon components of the oil were α-pinene (20.0%), δ-cadinene (10.4%), limonene (8.7%), and myrcene (8.5%), whereas the alcoholic extract contained α-pinene (11.0%), δ-cadinene (12.8%), β-caryophyllene (9.8%) and germacrene D (9.3%) [1]. The main oxygenated constituent in both the oil and the extract was borneol (8.0–8.6% respectively) [1].

The foliage and the seeds contain sabinol (a terpenic alcohol) and gallic acid, which is transformed into pyrogallol. Consumption of the foliage causes a severe irritation of all mucous membranes. The sabinol attacks the nervous system, causing convulsions. The pyrogallol blocks the intestinal circuit completely.

Table 1.2

Chemical composition of some types of Juniper

Name	Fruits	Leaves	Wood
J. oxycedrus	cariophillene, abistatriens-8-ол	terpineole, pinene, mircene, squalen. β-citosterin, taxyphillin, fatty acids	cadinene, cadinole, isocupressic acid
J. conferta	sesquiterpenoids	cedrol, cedrene, sabinene, limonene	tannic compounds, diterpenoids
J. excelsa	sesquiterpenoids	sesquiterpenoids, diterpenoids.	cadinole
J.hemispharica	pinene	pinene, mircene, squalen.tannic compounds	8,14-cedranoxyde

The most common chemical components, detected in essential oils of different species of Juniper are shown in table 1.3.

Table 1.3

Most common chemical components of essential oil from Juniper species

Compound	Content, %	Compound	Content, %
α-Thujene	0.90	Limonene	5.10
α-Pinene	51.40	β-Phellandrene	0.50
α-Fenchene	0.20	γ-Terpinene	0.20
Camphene	0.80	*cis*-Sabinene	0.10
Thuja-2,4(10)-diene	0.20	Terpinolene	0.40
Sabinene	5.80	Linalool	0.10
Myrcene	8.30	Perillene	0.10

δ-3-Carene	0.20	α-Pinene oxide	0.10
α-Terpinene	0.10	*trans*-Pinocarveol	0.30
p-Cymene	0.90	*cis*-Verbenol	0.50

1.5 Using the Juniper in medicine

The ripe, unshrivelled berries (*Juniperi* fructus) should be collected in autumn and dried slowly in the shade, to avoid losing the oil [7].

First juniper berries as a diuretic used in almost all cases of diseases of the bladder and kidneys (just as anti inflammation in these organs), edema associated with impaired circulation, antimicrobic, hypoglycemic [24].

Traditional use includes cystitis, flatulence and colic. Indications: diuretic, antiseptic, aromatic, rubefacient, stomachic and antirheumatic. The oil mixed with lard is also used in veterinary practice [28]. The essential oil present is quite stimulating to the kidney nephrons and so this herb should be avoided in kidney disease and during pregnancy. Juniper tincture is applied topically for some skin donditions and baldness [8].

Various extracts of *Juniperus communis* L. have for up to 63.3 higher inhibitory effect on certain kinds of bacteria than penicillin and antioxidant properties [20, 21].

Indigenous peoples from Eurasia made tonics for kidney and stomach ailments and rheumatism. Juniper was used by Great Basin Indians as a blood tonic.

In folk medicine juniper is widely used in more than 20 cases. Native Americans from the Pacific Northwest used tonics made from the branches to treat colds, flu, arthritis, muscle aches, and kidney problems. In Sweden a beer is made that is regarded as a healthy drink. In hot countries the tree yields by incision a gum or varnish [6].

Savin is not much used internally; but, in cases of amenorrhoea and chlorosis depending on or accompanied by a torpid condition or deficient action of the uterine vessels, it may be given as a powerful uterine stimulant [27].

As a topical agent, savin is frequently employed, mostly in the form of the cerate, to make *perpetual blisters*. Equal parts of savin and verdigris, in powder, form one of the most efficacious applications for the removal of venereal warts [25].

The powder, an infusion, or the expressed juice of the plant, is occasionally applied to warts, to old and indolent ulcers, and in cases of psora and tinea [33].

An *infusion* may be prepared by digesting of the fresh herb in boiling water: the dose is one or two tablespoonfuls.

The *oil* is by far the most convenient and certain preparation of savin, and is the only one which I employ. A *conserve* of the fresh leaves is sometimes used [26].

Oil of Savin and Savin Ointment. It obtained by submitting the fresh tops to distillation with water. It is a limpid, almost colourless liquid, having the unpleasant odour of the plant, and a bitter acrid taste. Its sp. gr. is 0.915 [13].

Its composition is analogous to that of oil of turpentine, being $C_{20}H_{16}$. It agrees with English oil of turpentine in its power of effecting the right-handed rotation of plane polarized light [20].

Winckler states that he dissolved one ounce of savin oil in the same quantity of concentrated sulphuric acid, and then distilled it from milk of lime (to remove the sulphurous acid), and obtained two drachms of an oil which was undistinguished from the volatile oil of thyme. The dose of oil of savin, as an emmenagogue, is from two to six drops, diffused in a mucilaginous or oleaginous mixture [16].

Savin cerate is used as a dressing to blistered surfaces, to produce what is termed a perpetual blister.

It is preferred to the ceratum cantharidis as being less acrid, and not liable to cause strangury. It is sometimes applied to seton tapes, to increase the discharge from setons [31].

Fruits of juniper prescribe in combination with other herbal preparation for the treatment of chronic respiratory disease (tracheids, laryngitis, bronchitis) and improving its expectoration [17].

In rheumatism and gout as an external rubbing it or take aromatic baths (not only berries, but the young stems). Infusion, decoction of the roots is used for the treatment of pulmonary tuberculosis, bronchitis, infections, gout, dermatitis. In clinical studies positive results for the treatment of arthritis. A decoction of the roots is bactericidal action. Junipers brushwoods are produced essential compounds – phytoncides, forming by this «antimicrobic zone»

Conclusions

After analyzing of scientific primary sources, it can be concluded that further research of representatives of the Juniper genus is relevant. The most studied are fruits whose pharmacological activity is due to the presence of essential oils, fatty acids, phenolic compounds.

According to the results, obtained during the study of the chemical composition of the needles of some species, in particular of the J. communis, it was established that this raw material is a source of lipophilic compounds such as fetty acids, essential oils, chlorophyls and carotenoids.

As it is known that *J. sabina* it is a very spread decorative plant and has a sufficient raw material base, we consider it an interesting object for in-depth research

Insufficient study of the chemical composition of the needles of *J. sabina* contributes to the further study of this plant material.

THE EXPERIMENTAL PART

CHAPTER 2. THE OBJECT OF STUDY. RECALCULATION THE OUTPUT AND LOSS IN MASS DURING DRYING FOR PLANT MATERIAL

2.1 Object of the study

The object of the study were the needle of *Juniperus sabina*. The plant material collected in September 2020 in Botanical garden of National university of M.N. Karazin. Raw materials were collected in dry weather. The raw materials were dried in a thin layer in a well-ventilated room. Then the dry raw materials were chopped with scissors and stored in tracny bags. Only needles were studied, without bark and wood.

Fig. 2.1 Appearance of *Juniperus sabina*

2.2 Determination the loss in mass during drying

Analytical samples of raw materials cuted 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.

Moisture of raw material (X) as a percentage calculated by the formula:

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

are: m - mass of material before drying in g; m_1 - mass of material after drying in g.

For the determination of the final result taking the arithmetic mean of free parallel measurements, calculated to tenths fractions. Allowable difference between the results of two parallel determinations should not exceed 0,5% (Table 2.1).

Table 2.1

Results of determination the average value moisture content of *J. sabina* needles

№ of bottle	Mass of bottle, g	Mass of bottle and sample before drying, g	Mass of sample, g	Mass of bottle and sample after drying, g	Moisture, %	The average value, %
1	44,7618	46,7602	1,9984	44,3052	5,25	5,80±0,38
2	45,2190	47,2274	2,0084	42,8075	6,18	
3	44,9018	46,9068	2,0005	44,3740	5,98	

Table 2.2

Metrological characteristic of determination

n	$\overline{X}$	S	S^2	S_x	f	P	t(P;f)	$\Delta\overline{X}$	$\overline{\varepsilon}$
3	5,80	0,232	0,2218	0,4812	2	95	4,30	2,03	1,15

As shown in Table 3.1 the average value moisture of *J. sabina* leaves (in %) is 5,80±0,38.

2.3 Calculation of raw materials output

This indicator is important when harvesting raw materials and allows you to predict how much dry raw materials can be obtained.

For research, a portion of fresh raw material was taken and weighed (100 g). Then the weighed raw materials were dried separately and weighed again. The obtained results were used to calculate the yield of raw materials taking into account the rate of its loss in mass during drying.

The output of raw materials in terms of absolutely dry raw material determined by the formula:

$$X = \frac{m1 \cdot 100 \cdot 100}{m2 \cdot (100 - W)},$$

are: m_1 – mass of dry raw material; m_2 – mass of fresh raw material; W – moisture of raw material

To obtain more reliable results, 3 raw material samples (about 100 g) were taken and the yield was determined for each sample.

Then the arithmetic mean was obtained, using the yield of raw materials in three samples. The obtained data are listed in Table 2.3.

Table 2.3

The results of determining the yield of *J. sabina* needles, taking into account the moisture content of the raw material

№	Mass of fresh raw materials, g	Mass of raw materials after dring, g	The output of raw materials, %	Average value the output of raw materials, %
1	100,25	50,18	54,82	53,47±0,62
2	100,11	49,75	52,75	
3	100,15	49,86	52,85	

The output of raw materials in terms of absolutely dry raw material (in %) is 53,47±0,62.

It was established that the yield of dry raw materials is more than 50%. The obtained data can be used when planning the procurement of raw materials and drawing up a plan for its procurement.

Conclutions

1. Determined loss in mass during drying for raw materials which consisted (in %) of 5,80±0,38. To obtain a more reliable result, the study was conducted in three repetitions and the average value of the determination was taken as the indicator.

2. For the purpose of further planning of procurement of raw materials, the output of the raw material during drying, taking into account its moisture content, is determined.

3. The obtained data will be used in future for calculations of the quantitative content of BAC in raw material.

CHAPTER 3. OBTAINING AND PHYTOCHEMICAL STUDY THE LIPOPHILIC FRACTION OF *J. SABINA*

3.1 Obtaining the lipophilic fraction of *Juniperus sabina*

Dry raw materials were used to obtain 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 (100 g) in a bag of filtering paper and weighed on an analytical balance. Prepared in this way substance extracted in Soxclet apparatus. Extractant - chloroform. Extraction was carried out in Socsclet apparat 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 º C for 30 minutes.

The Soxhlet apparatus consists of a solid mixture tank, a solvent tank and a reflux condenser. When the flask is heated, the solvent vapor rises and condenses in the refrigerator. The resulting steam-condensate enters the extractor, into which the substance to be extracted is preliminarily placed. When the liquid in the extractor reaches a certain level, it flows back into the flask and the process continues (Fig. 3.1). This method is suitable for efficient extraction with a small amount of solvent. Such a rank is the circulation extraction.

The process was continued until the raw materials were completely exhausted. It was possible to understand this by the fact that during the next washing of the raw material, chloroform did not have a green color.

It possible to determine the degree of purification of the solvent in another way: take part of the solvent with a glass tube and evaporate on a glass slide, if there is no sediment left on the glass surface, extraction can be completed. If colorless substances were used during extraction, then you can find out about the completion of the process by comparing the refractive indices of light of a pure solvent and extract - they should be equal.

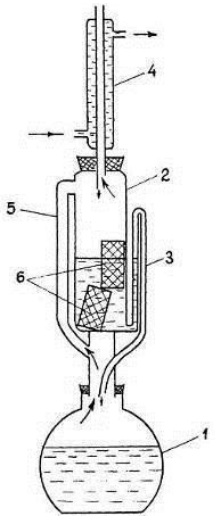


Fig. 3.1 Socsclet apparat construction

Note: 1 - flat-bottomed flask; 2 – extractor; 3 - siphon through which the saturated chloroform flows back into the flask; 4 – refrigerator; 5 – a glass tube through which solvent vapors rise; 6 - bags with raw materials.

The Soxhlet device belongs to complex chemical installations that require increased attention and caution. Before starting work, it is necessary to carefully check the used glass objects for cracks, chips, dirt, in order to avoid accidents. Since the extraction process often uses aggressive chemicals (solvents), following fire safety rules will save you from unpleasant consequences.

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

The 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–loss in mass during drying.

Table 3.1

Results of recalculation the output of LF of *J. sabina*

№	Mass of raw material, g	Mass of LF, g	The autput of LF, (%)
1	50,15	14,1573	28,23
2	50,10	14,0530	28,05
3	50,07	14,0095	27,98

The output of *J. sabina* lipophilic fraction is 28,08 %.

3.2 Research the components of lipophilic fraction

3.2.1 Qualitative identification the chlorophylls and carotenoids

To identify this compounds using one-dimensional and two-dimensional chromatography in a thin layer of sorbent (TLC). The following ratios of reagents were used in solvent systems: hexane-acetone (8:2, 6:3, 6:4). The most optimal systems were hexane:acetone ratios of 6:3 and 6:4.

As a chromogenic compound used a 10% solution of phosphor molybdic acid. The color of spots seen in daylight and UV-light before and after processing of chromogenic reagent (Fig. 3.2, Tab. 3.2) [3, 10].

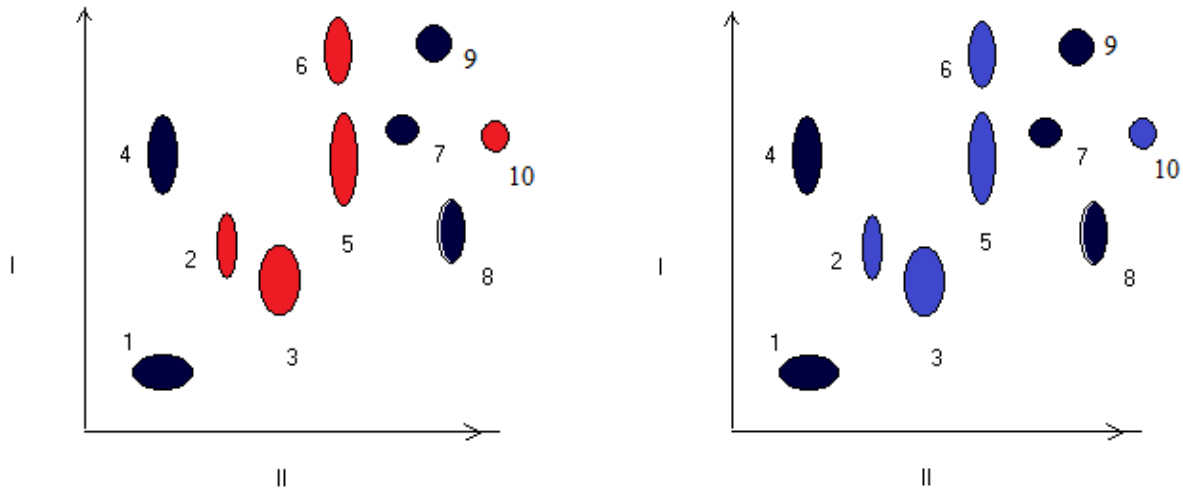
Table 3.2

The results of chromatographic analysis of chlorophylls and carotenoids in Savin leaves

№ of spot	Color before processing	Color after processing	Rf I	Rf II
1	Dark	Dark	0,15	0,20
2	Red	Blue	0,45	0,35
3	Red	Blue	0,40	0,50
4	Dark	Dark	0,75	0,22

5	Red	Blue	0,76	0,60
6	Red	Blue	0,92	0,61
7	Dark	Dark	0,75	0,70
8	Dark	Dark	0,50	0,75
9	Dark	Dark	0,93	0,74
10	Red	Blue	0,76	0,80

According to the results of fluorescence and Rf values spots 1, 4, 7-9 had dark color it chromatographic behavior character for carotenoids. Compounds 2, 3, 5, 6, 10 before processing of 10% solution of phosphor molybdic acid had red color, after – blue, it indicating in presence of chlorophyll.



Before processing the chromogenic solution

After processing 10% solution of phosphor molybdic acid

Fig. 3.2 Scheme of the two-dimensional chromatograms of chlorophylls and carotenoids the lipophilic fraction of *J. sabina*

Note: I - system hexane-acetone (6:4); II - system hexane-acetone (6:3)

3.2.2 Quantitative determination of chlorophylls

A sample of lipophilic fraction (0.1569 g) dissolved in 10 ml of 96% ethanol. The solution is filtered, the volume of solution brought to 10 ml of 96%

ethanol. From obtained solution selected pipette 5 ml, brought to 10 ml of 96% ethanol, were double dilution.

Optical density determined in photocolorimetr with a red filter absorbing filter thickness 10 mm. Reference solution was 96% ethanol.

At the same time measured the optical density of the standard solution Getry in the same conditions.

To prepare the standard solution Getry used 4% solution of potassium dichromate - 50 ml; 1% solution of copper sulfate - 28.5 ml; solution of ammonium hydroxide - 10 ml; distilled water - 100 ml.

1 ml of obtained solution corresponds to 0.000085 g of chlorophyll coloration.

The content of chlorophyll in the lipophilic fraction in percentage (x) in recalculation of absolutely dry raw materials calculated by the formula:

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

D_1 – optical density of the studied extract; D_2 – optical density of the standart; P – dilution; A – amount of chlorophyll, g in 1 ml respectively coloration 1 ml of standard solution Getry; a – mass of raw material, g.

Table 3.3

Metrological characteristic of chlorophylls recalculation

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	0,1470 0,1498 0,1500 0,1501 0,1489	0,1492	0,00002075	0,001482	95%	2,78	0,1492±0,0091	1,15

Quantitative content of chlorophylls was 0,14 %.

3.2.3 Quantitative determination of carotenoids

Accurate weight of the lipophilic fraction (m = 0,0185 g) was dissolved in a

volumetric flask of 25 ml (V) in hexane. To 2 ml (V1) of this solution was added 2 ml of hexane, thus obtaining 4 ml (V2) solution. Optical density was determined on a spectrophotometer СФ-46. The indicator of optical density for leaves - 0,762.

Extinction $E^{1\%}_{1cm}$ for the amount of carotenoids in hexane at the wave length 453 nm is assumed to be 2592. The content of carotenoids amount determined by the formula:

$$X = \frac{10 \cdot D \cdot V \cdot V_2}{E^{1\%}_{1cm} \cdot V_1 \cdot a}$$

are: D – optical density of the test solution at a specified wavelength (453 nm); $E^{1\%}_{1см}$ – extinction for β-carotene at 453 nm wavelength equals 2592; 10 – carotene content in 1 ml of 1% solution, mg; V – the total volume of extract, ml; V_1 - volume of extract that was taken for chromatography, ml; V_2 - volume of eluate, ml; a – sample mass, g.

Metrological characteristics of the average results determination of carotenoids presented in the Tab. 3.4.

Table 3.4

Metrological characteristic of carotenoids recalculation

n	f	X_{cep}	S^2	$S_{cep.}$	P	t(P,f)	Interval	ε, %
1	2	3	4	5	6	7	8	9
5	4	1,15	0,05	0,0025	95%	2,78	1,15±0,0023	1,76

Quantitative content of carotinoids (mg) 1,15±0,0023

3.2.4 Quantitative determination of fatty acids

Obtaining the extract. 5 mg of LF was placed in a vial, an internal standard (50 μg of tridecane in hexane) and 1.0 ml of a methylating agent (14% BCl_3 in methanol, Supelco 3-3033) were added. The mixture was kept in a hermetically sealed vial for 8 hours at 65° C. The reaction mixture was drained from the

sediment of the plant material and diluted with 1 ml of distilled water [29].

To extract methyl esters of fatty acids, 0.2 ml of methylene chloride was added and stirred from time to time for an hour. The obtained extract of methyl ethers was chromatographed.

The research was carried out by the method of chromatography-mass spectrometry on an Agilent Technologies 6890 chromatograph with a 5973 mass spectrometric detector. Chromatography parameters: sample introduction (2 μl) into the chromatographic column was performed in splitless mode [34].

Sample injection speed 1.2 ml/min for 0.2 min; chromatographic column – capillary INNOWAX with an outer diameter of 0.25 mm and a length of 30 m; velocity of carrier gas (helium) 1.2 ml/min; the temperature of the heater is 250°C.

For the identification of components, the NIST05 and WILEY 2007 mass spectra library with a total number of more than 470000 spectra were used in combination with AMDIS and NIST identification programs. The internal standard method was used to quantitatively calculate the content of fatty acids.

Fatty acid composition are presented in Tab. 3.5, the MS-spectra of identificated compounds are shown in Fig. 3.4-3.7.

Table 3.5

Fatty acid composition of *J. sabina*

№	Name of acid	Chemical formula	Index	Content, mkg/100 mg
1	Lauric	$C_{12}H_{22}O_2$	C_{12}: 0	112,4
2	Myristic	$C_{14}H_{28}O_2$	C_{14} : 0	81,6
3	Palmitic	$C_{16}H_{32}O_2$	C_{16} : 0	17,4
4	Stearinic	$C_{18}H_{36}O_2$	C_{18} : 0	12,3
5	Oleic	$C_{18}H_{34}O_2$	C_{18} : 1	6,4
6	Linoleic	$C_{18}H_{22}O_2$	C_{18} : 2	13,5
7	Linolenic	$C_{18}H_{30}O_2$	C_{18} : 3	43, 6
8	Arachinic	$C_{20}H_{34}O_2$	C_{20} : 0	42,9
Total: 328,6		saturated		263,3
		unsaturated		63,5

General scheme of the gas chromatograms of fatty is shown in Fig. 3.3.

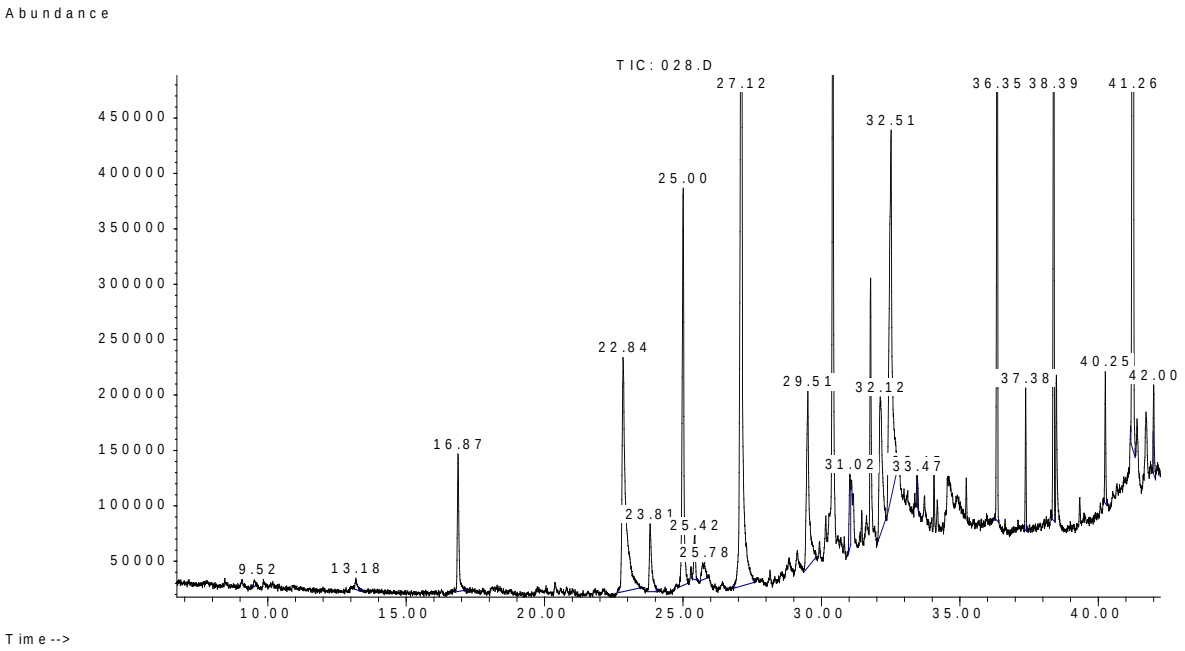


Fig. 3.3 Scheme of the general chromatogram of lipophilic compounds of *J. sabina*

As shown in Tab. 3. 6 was founded 8 fatty acids, from which 3 (oleic, linoleic and linolenic) are essential. Are dominant (mkg/100 mg) saturated fatty acids, among saturated – lauric acid (112,4) (Fig. 3.4).

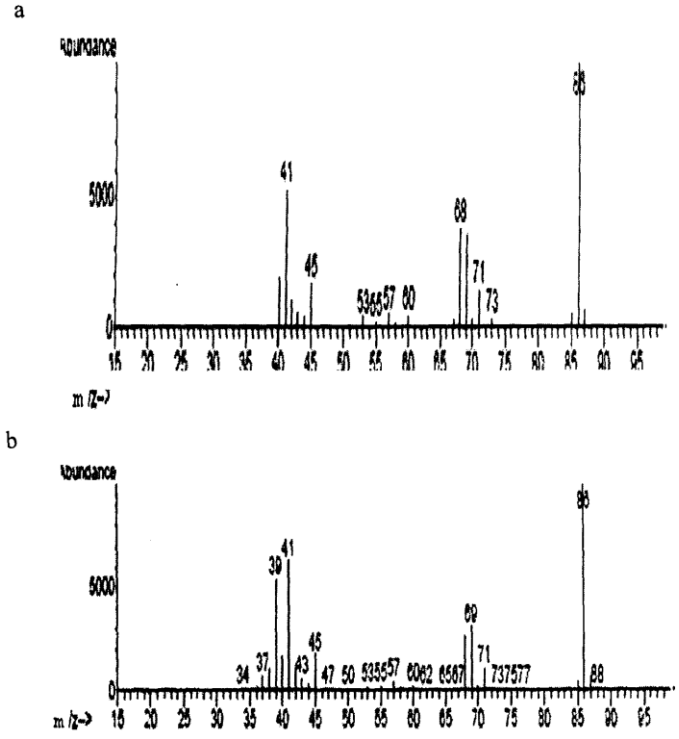


Fig. 3.4 The MS-spectrum the studied compound (a) compared with the mass spectrum of lauric acid (b).

Among unsaturated fatty acids dominant linolenic acid (43,6) (Fig. 3.5).

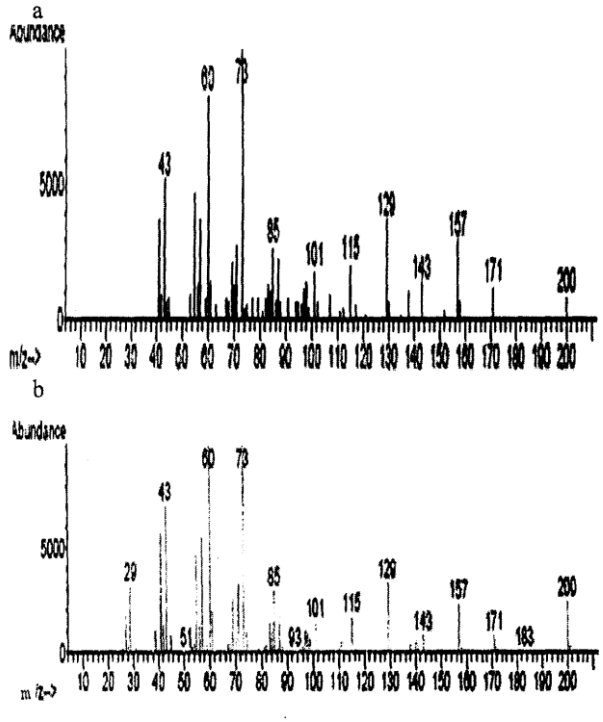


Fig. 3.5 The mass spectrum of the studied compound (a) compared with the mass spectrum of linolenic acid (b).

The content of myristic acid was 81,6 mkg/100 mg, which in terms of the sum of fatty acids amounted to 24,8% (Fig. 3.6).

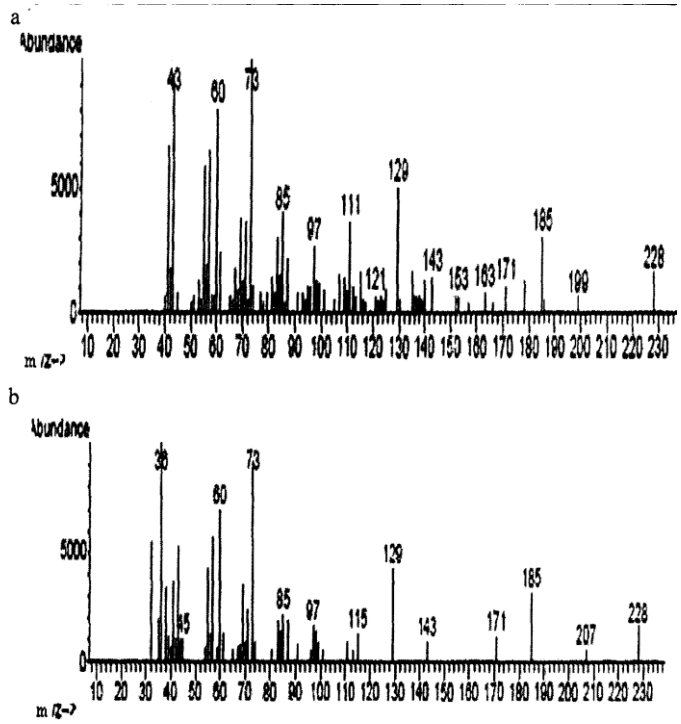


Fig. 3.6 The MS- spectrum of the studied compound (a) compared with the mass spectrum of myristic acid (b).

The content of stearinic acid in recalculated to all acids was 3,74% (Fig. 3.7).

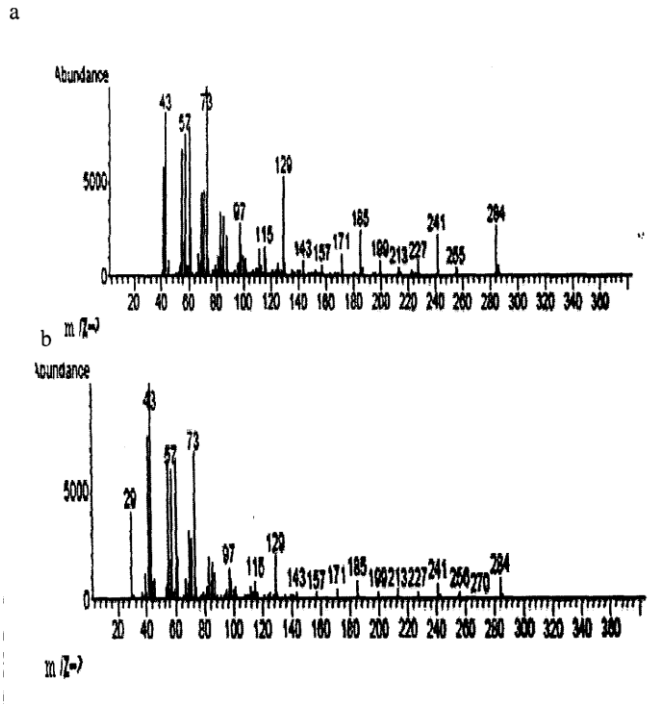


Fig. 3.7 The MS-spectrum of the studied compound (a) compared with the mass spectrum of stearinic acid (b).

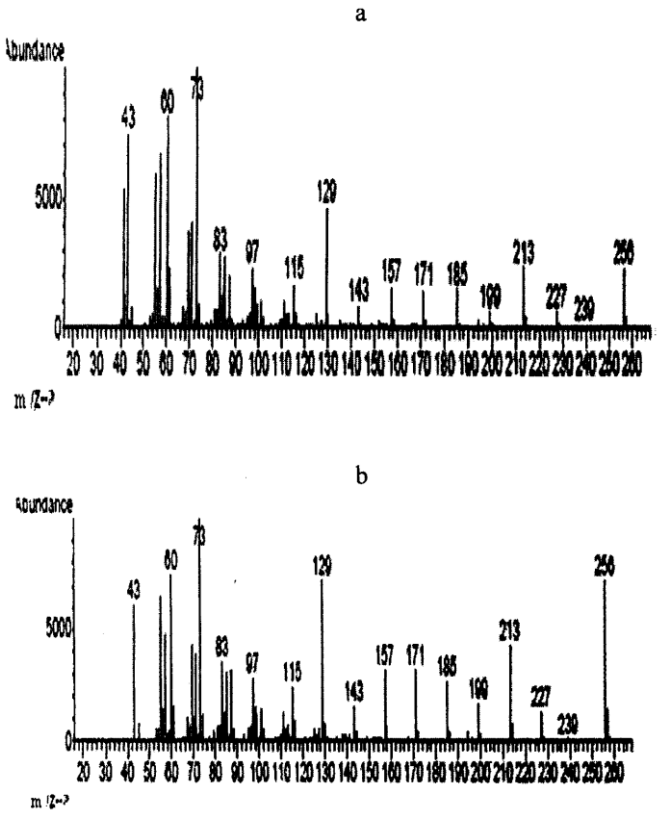


Fig. 3.7 The mass spectrum of the studied compound (a) compared with the mass spectrum of palmitinic acid (b).

3.3 Research the components content of volatile oil of *J. sabina*

3.3.1 Obtain and quantitative determination of volatile oil

Quantitative determination of essential oils in raw material conducted by used the method of water distillation, by next measuring volume of obtaining essential oils and its calculation of volume-weight percentage. For this purpose used the Ginsberg apparatus (Fig. 3.8).

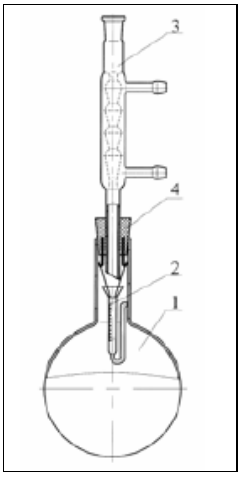


Fig. 3.8 Ginzberg apparat construction

Note: 1- round-bottomed flask; 2- graduated receiver; 3 – condenser; 4 – stopper.

Water distillation methodic. 20 g of raw material was grinded. Puted the sample to the round bottom flask (volume of flask 700-800 ml). Added 300 ml of water. The top of the flask fixed by calibrated receiver. The flask equipped together with vertical refrigerator. The flask was heated to boiling and boil slowly during 2 hours. After the distillation the layer of essential oil was separated [18].

The essential oil content calculated (in the volume-weighted percentage) using the formula:

$$X = \frac{A \times 100}{Б}$$

А – volume of essential oil, ml; Б – mass of raw materials, g.

Content of essential oil in volume-weight percentage (X) in recalculated to the dry raw material recalculated used a formula:

$$X = \frac{V \times 100 \times 100}{m \times (100 - w)}$$

V - volume of essential oil, ml; m – mass of raw materials, g; W – lost in the weight during drying, %.

Content the essential oil in volume-weight percentage is 1,63%.

J.sabina essential oil – oily liquid, yellow color, has specific, unpleasant smell.

3.3.2 Determination the physical-chemical properties of *J. sabina* essential oil

Determination of some physicochemical parameters of the obtained essential oil was carried out according to the pharmacopoeial method. The obtained results are shown in Table 3.6.

Table 3.6

Some physical-chemical properties of *J. sabina* essential oil

Indicator	Methodic	Result
Refraction index	Get acquainted with the employment of a refractometer	Refraction index of essential oil of *Juniperus Sabina* herb at 20˚ is 1,12.
Solubility	The tested oil was added to different test tubes. 96% ethanol was added to one, purified water to the other	Essential oil soluble in 96% alcochol, unsoluble in water
Colour and transparency	Place 5 ml of a volatile oil into a cylinder (d= 2-3 cm)	It is light green liquid.
Odour	Place two drops of a volatile oil on a strip of a filtrate paper and compare the odour	Essential oil with characteristic, upleasure smell.

According results of study, essential oil is oily, light green, clear liquid, with a characteristic, unpleasant smell.

3.3.3 Chromatographyc study of *J. sabina* essential oil

For the identification the components of essential oils used one-dementional chromatograph in a thin layer of sorbent (TLC).

In experiment we used several types of chromatographic systems and chromogenic compounds:

а) Solvent system - chlorophorm, chromogenic solution – 0,1 n solution potassium permanganate;

б) Solvent system - chlorophorm, chromogenic solution – 20% alcohol solution of phosphoric molibdene acid [22].

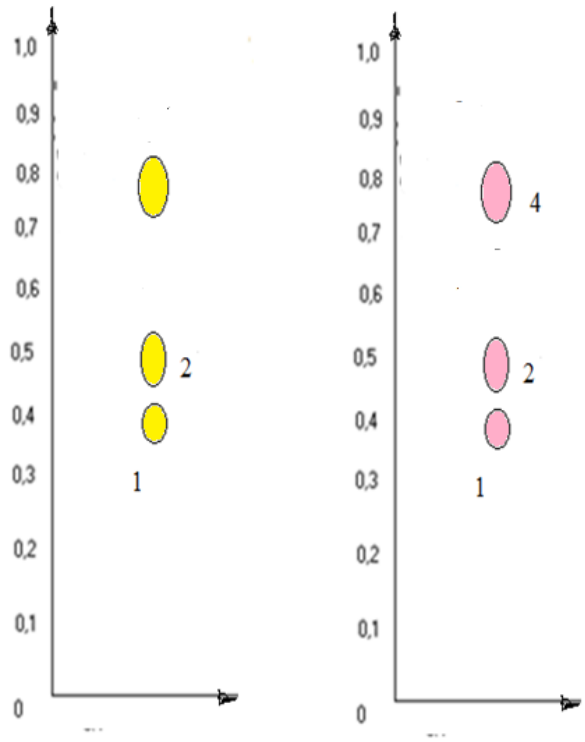


Fig. 3.9 Scheme of essential oil chromatogram

Note: solvent system – chlorophorm; chromogenic solution – 0,1 n solution of potassium permanganate.

Yellow color of spots changed on pink after the processing of chromatograms 0.1 N potassium permanganate solution indicates the presence in essential oil 3 compounds which have reducing properties (Fig 3.9, Table 3.7).

Table 3.7

Results of the chromatographic determination of *J.sabina* essential oil

№ of spots	Color of spots	Rf
1	Yellow	0,44
2	Yellow	0,52
3	Yellow	0,85

Emergence of of yellow spots on pink background in the processing of chromatograms 0.1 N potassium permanganate solution indicates the presence of the essential oil of not less than 3 compounds having reducing properties.

The appearance of blue spots on green background after the processing of chromatograms phosphoric molibden acid indicates the presence in the essential oil no less than 6 compounds of terpenoids nature (Fig. 3.10).

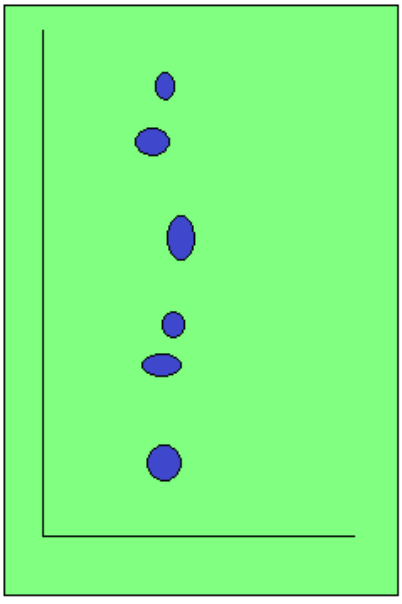


Fig. 3.10 Scheme of essential oil chromatogram

Note: solvent system – chlorophorm; chromogenic solution – 20% alcohol solution of phosphoric molibdene acid.

3.3.4 Chromatography-mass-spectrometry study of *J. sabina* volatile oil

By used chromatography-mass spectrometric method was established of *J. sabina* essential oils chemical composition [5].

The methodic. To determine the component composition of the essential oil a portion of herbal drugs (5 g) was placed in a vial of 20 ml, added an internal standard - tridecane. 10 ml of water was added to the sample and volatile compounds of the sample were distilled off with steam for 2 hours using an air-cooled reflux condenser. In the distillation process, volatile compounds are adsorbed on the inner surface of the reflux condenser [30].

The adsorbed compounds were washed after cooling the system by slowly adding 3 ml of particularly pure pentane to a 10 ml dry vial. The wash was concentrated by purging (100 mL / min) with particularly pure nitrogen to a final extract volume of 10 μl, which was completely removed by a chromatographic syringe. Further concentration of the sample was performed in the syringe to a volume of 2 μl. The introduction of the sample into the chromatographic column was performed in splitless mode (without flow separation), which allowed to enter the sample without loss on division and increase the sensitivity of the chromatographic method. The sample injection rate is 1.2 ml / min for 0.2 minutes. Chromatography conditions: Agilent Technologies 6890 chromatograph with mass spectrometric detector 5973; capillary chromatographic column DB-5 ext. diam. 0.25 mm and 30 m long; carrier gas velocity (helium) 1.2 ml / min; temperature of the sample heater - 250 degrees; the temperature of the thermostat is programmed from 50 to 320 degrees with a speed of 4 degrees / min. To identify the components the NIST05 and WILEY 2007 mass spectrum libraries with a total number of spectra of more than 470,000 in combination with the AMDIS and NIST identification programs were used. The internal standard method was used for quantitative calculations.

The calculation of the content of components was performed according to the formula:

$$C=K1 \cdot K2,$$

where K1 = P1 / P2 (P1 - peak area of the test compound, P2 - peak area of the standard); K2 = 50 / M (50 is the weight of the internal standard (μg) entered in the sample, M - is the sample weight (g)).

The results are shown in Table 3.8 and Fig. 3.7.

Table 3.8

The components of Savin essential oil

№	Rt	Compound	Content, %
1	4.93	Benzaldehyde	1.8
2	6.64	Limonene	0.3
3	7.08	Phenylacetaldehyde	11.0
5	7.97	*trans*-Linalool oxyde	1.5
6	8.45	*cis*- Linalool oxyde	5.0
7	8.81	Kedrol	12.4
8	9.97	β-Phelandrene	27.1
9	11.48	α-Eudesmole	14.6
10	12.92	α-Tujone	30.9
11	13.084	α-Pinene	120.4
12	14.21	Cinnamic aldehyde	3.0
13	14.29	Mircene	6.2
14	15.69	2,4-decadienal	9.8
15	16.98	Eugenole	1.4
16	30.27	Nonadecane	23.0
17	31.65	Eicosane	20.5
18	32.98	Cheneicosane	100.2
19	34.09	Docosane	23.0
20	34.86	Tricosene-1	12.0

21	35.27	Tricosane	150.3
22	36.23	Tetracosane	26.7
23	37.25	Pentocasane	30.1
24	39.09	Heptacosane	15.1
25	40.27	Squalen	25.9

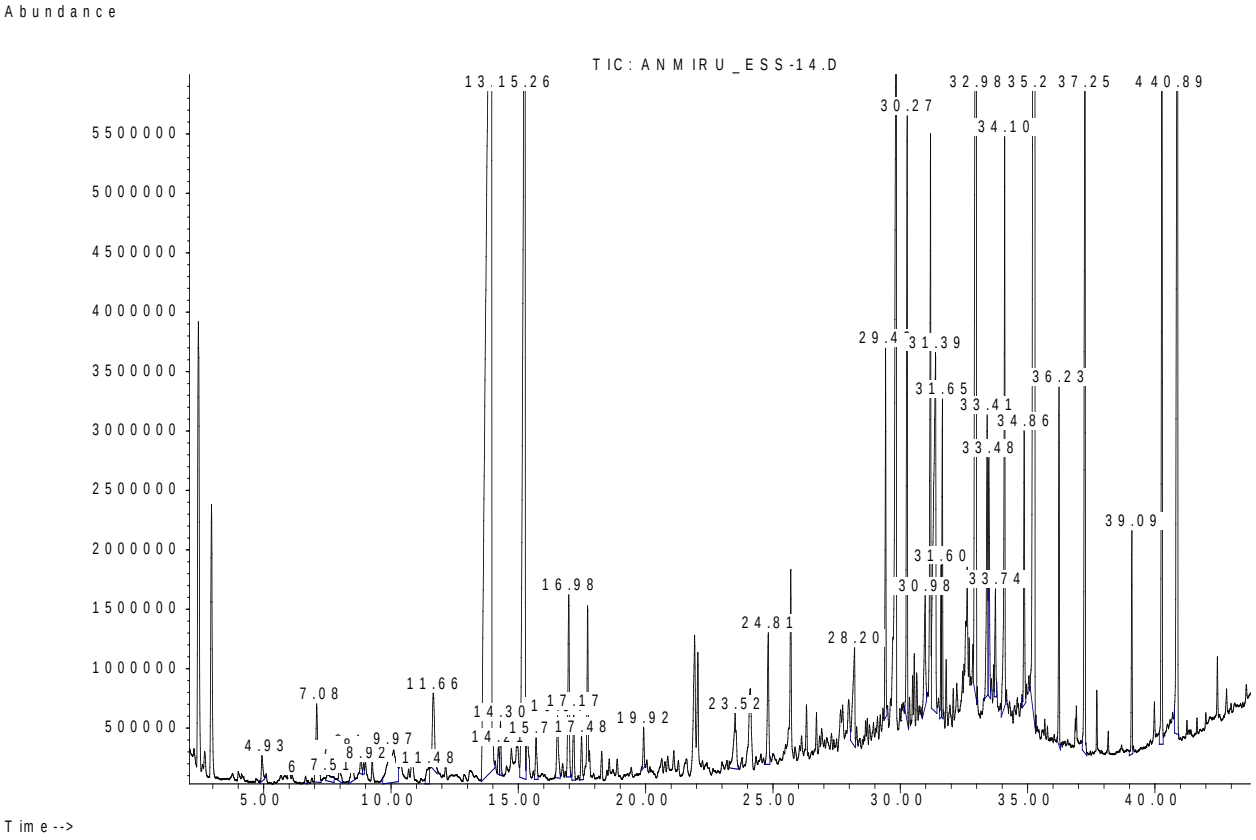


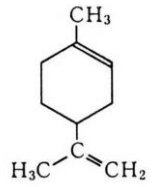
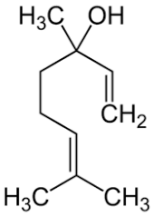
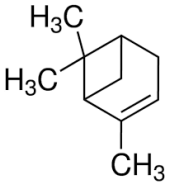
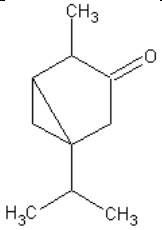
Fig. 3.11 Chromatogram of essential oil components of *J. sabina*

As seen from Tabl. 3.8 in essential oil identified 25 compounds of different chemical structure: monoterpenoids (acyclic, monocyclic), aromatic compounds [15].

Amongs terpenoids are established limonene, trans-linalool oxide, cis-linalool oxide, linaloole, squalen (Table 3.9).

Table 3.9

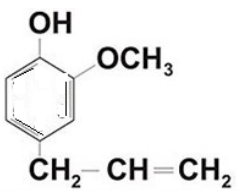
Most tipical terpenoids for *J. sabina* essential oil

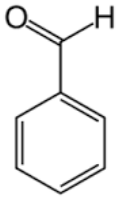
Compound	Chemical formula	Chemical structure
Limonene	$C_{10}H_{16}$	
Linalool	$C_{10}H_{18}O$	
Squalen	$C_{30}H_{50}$	
α-Pinene	$C_{10}H_{16}$	
α-Tujone	$C_{10}H_{16}O$	

Aromatic compounds are represented by eugenole, benzaldehyde (Table 3.10).

Table 3.10

Most typical aromatic compounds for *J. sabina* essential oil

Eugenol	$C_{10}H_{12}O_2$	

Benzaldehyde	C_7H_6O	

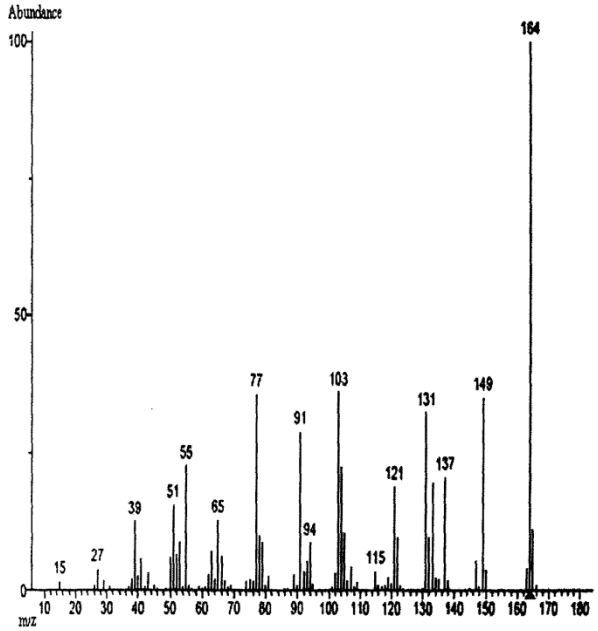


Fig. 3.12 MS-spectrum of eugenol identified in the essential oil *J. sabina*

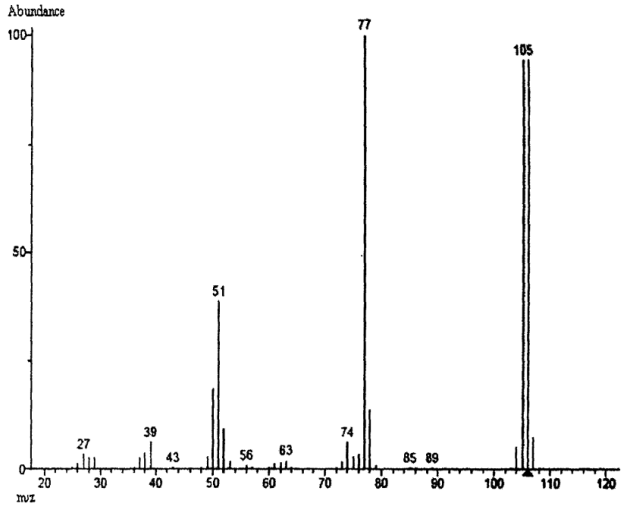


Fig. 3.13 MS-spectrum of benzaldehyde identified in the essential oil *J. sabina*

In quantitative content among terpenoids are dominated squalen (25,9 %), α-pinene (120,4 %), α-tujone (30,9 %).

Other compounds identified in the essential oil are represented by higher

alcohols, aldehydes, ketones.

The most pharmacologically valuable compounds identified in the essential oil are linalool, α-pinene, α-tujone, squalene, eugenole.

It has been scientifically proven that bicyclic monoterpenoid α-pinene, identified in the essential oil, have antimicrobial, fungicidal, antistaphylococcal action. Due to the content of geraniol and eugenol, the essential oil can have anti-inflammatory and antiseptic effects; linalool oxides – bactericidal.

Squalene is readily easily absorbed by the skin due to the fact that it is directly a protective component of human skin, thus can accelerate the penetration of drugs through the skin, has a regenerating, anti-inflammatory effect.

Taking into account the obtained results, it can be assumed that the obtained and studied biologically active substances (LF and essential oil) can be used in the future to create medicines for the treatment of skin diseases caused, for example, *S. aureus*, since investigated in this theses compounds according to the literature have bactericidal activity against this microorganism.

3.3.5 Determination of the residual content of chloroform in the lipophilic fraction

In accordance with the requirements for extracts obtained with chloroform, the residual content of this solvent in the lipophilic fraction was determined. The method of gas chromatography (GC) was used.

Preparation of the tested solution. 1.0 g of lipophilic fraction (LF) was placed in a container with a capacity of 20.0 ml, which is tightly closed (3 samples), 0.5 ml of water P, 0.5 g of sodium chloride and 3.0 ml of an internal standard solution (n-butanol) were added .

The container was sealed with a rubber gasket with a fluoroplastic coating. Containers with samples were alternately placed in a thermostat and held at a temperature of 120 0 C for 30 min.

Preparation of internal standard solution. 100 mg of n-butanol was placed in a volumetric flask with a capacity of 10.0 ml. The volume of the solution was brought up to the mark with dimethylacetamide (DMAC) and mixed. 5.0 ml of the resulting

solution was placed in a volumetric flask with a capacity of 500.0 ml. The volume of the DMAC R solution was brought up to the mark and mixed.

Preparation of the solution of the standard sample (StSp). 5 ml of the internal standard solution was placed in a volumetric flask with a capacity of 100 ml, 12.7 μl of hexane was added, the volume was brought up to the mark with the internal standard solution and mixed (Solution 1).

3.0 ml of Solution 1 was placed in a container with a capacity of 20.0 ml, which is tightly closed (3 samples), 0.5 ml of water , 0.5 g of sodium chloride and 1.0 g of the substance were added. The container was sealed with a rubber gasket with a fluoroplastic coating. The samples were placed one by one in a thermostat and kept at a temperature of 120 °C for 30 minutes.

Preparation of a solution for checking the suitability of a chromatographic system. 5 ml of DMAC solution was placed in a volumetric flask with a capacity of 200 ml, 1.5 g (exact measure) of methanol, 1.5 g (exact measure) acetone, 0.3 g (exact measure) methylene chloride, 1.5 g (precise measure) of ethyl ether, 0.445 g (precise measure) of toluene, 0.1 g (precise measure) of pyridine. The volume of the DMAC solution was brought up to the mark and mixed.

Then 3.0 ml of the resulting solution was placed in a container with a capacity of 20.0 ml, which is tightly closed, 0.5 ml of water P, 0.5 g of sodium chloride were added and the container was sealed with a rubber gasket with a fluoroplastic coating. The samples were placed one by one in a thermostat for the analysis of the equilibrium vapor phase and kept at a temperature of 120 0 C for 30 min.

Checking the suitability of StSp. The chromatographic system was considered suitable if the following conditions were met: the degree of separation of the peaks, calculated from the peaks of the solvents from the chromatograms of the solution for checking the suitability of the StSp, had to be at least 1.5; the relative standard deviation calculated for the solvent peak areas from the chromatograms of the StSp solution should not be more than 6.0%; the symmetry factor of the peaks, calculated from the solvent peaks from the chromatograms of the solution to check the suitability of the StSp, should have been between 0.8 and 1.5.

1.0 ml of the gas phase was chromatographed on the tested, control and StSp in turn on a gas chromatograph with a flame ionization detector, under the following conditions: column: capillary quartz, size 60 x 0.32 mm, 1.8 μm DB - 624 or similar, for which the requirements of the test "Check of suitability of the chromatographic system"; the temperature of the column thermostat was programmed from 40 °C (5 min. delay) to 200 °C (20 min. delay), temperature increase - 5 °C/min; temperature of the evaporator block - 230 °C, flow separation - 1:5; detector temperature − 290 °C; the speed of the carrier gas (helium) is 2 ml/min.

The gas phase was chromatographed over the solution of the standard sample, obtaining from 2 to 6 chromatograms. Relative standard deviation was calculated for solvent peak areas from the obtained chromatograms. Obtaining parallel chromatograms (n_0) was stopped when the requirements for the suitability of the chromatographic system were reached (Fig. 3.14, 3.15).

The content of chloroform (X), in ppm, in LF was calculated according to the formula:

$$X = \frac{B_i \cdot 3 \cdot 10^6 \cdot \rho \cdot V_o}{(B_0 - B_i) \cdot m \cdot 100 \cdot 1000}$$

where B_i – the average value of the chloroform peak area from the chromatograms of the tested solution; B_0 – the average value of the chloroform peak area from the StSp chromatograms; V_0 – volume of chloroform, μl; ρ – density of chloroform; m – weight of the sample, g.

Results were considered valid if the requirements of the «Chromatography System Suitability» test were met.

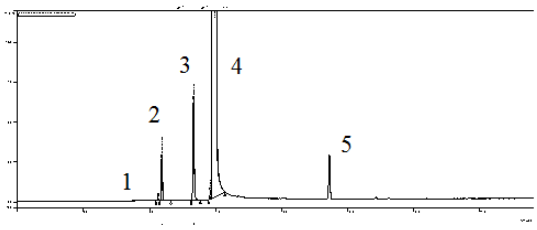


Fig. 3.14 Chromatogram of StSp solution: 1 – system peak; 2 - chloroform; 3 - internal standard; 4 – DMAC R; 5 - system peak.

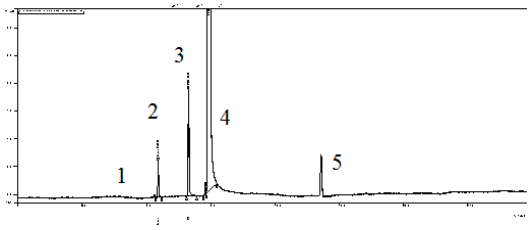


Fig. 3.15 Chromatogram of the solution of the tested sample of *J. sabina* LF: 1 - system peak; 2 - chloroform; 3 - internal standard; 4 – DMAC R; 5 - system peak

Table 3.11

Data of the peaks shown on the chromatograms of standard sample solution and the sample of lipophilic fraction of *J. sabina*

Object of study	Rt., min.	Peak aria
Chloroform	9,51	98635
LF of *J. sabina*	9,52	93129

It was established that the concentration limit of chloroform in LF of *J. sabina* does not exceed 50 ppm, which meets the requirements of Pharmacopoeia.

Conclusions

1. For the needles of J. sabina, such indicators as the loss in mass during drying and the yield of raw materials have been established.

2. The lipophilic fraction was obtained and its chemical composition was established.

3. *J. sabina* essential oil was obtained and its quantitative content and component composition were determined.

4. The residual content of chloroform in *J. sabina* lipophilic fraction was established.

GENERAL CONCLUSIONS

1. *J. sabina* needles were harvested and the moisture content after drying was determined, which was (in %) 5,80±0,38.

2. The yield of dry raw materials, taking into account its moisture content, was 53,47%.

3. A lipophilic fraction of *J. sabina* needles was obtained.

4. It was established that the yield of the lipophilic fraction from the was in terms of completely dry raw materials was 28,08 %, in the case of chloroform extraction.

5. Selection of a chromatographic system for the determination of chlorophylls and carotenoids in the lipophilic fraction was carried out. Most optimale hexane-acetone (6:3 and 6:4) turned out to be the most optimal ratio of solvents in the chromatographic system.

6. By used TLC methods in the lipophilic fraction of J. sabina after processed the chromogen reagents was detected chlorophylls and carotenoids.

7. The chromato-mass spectrometric method revealed the presence of 8 fatty acids in the lipophilic fraction, including 3 unsaturated and 5 saturated acids. By the the content saturated acids are dominated.

8. The quantitative content of carotenoids in the lipophilic fraction was determined by the spectrophotometric method, which was 1,15%.

9. The quantitative content of chlorophylls, which was 0.14% in the lipophilic fraction, was determined by the photocolorimetric method.

10. The essential oil was obtained by the method of distillation with water and its content was determined, which was 1,63% by volume and weight. The presence of reducing and terpenoid compounds was determined by the TLC method. The appearance of blue spots in green background after the processing of chromatograms phosphoric molibden acid indicates the presence in the essential oil of terpenoids nature. By used the chromatography-mass spectrometry method 25 components of essential oil were identified. The main components are terpenoids and aromatic compounds.

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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 NUTRICIOLOGY

CERTIFICATE

№ 39

This is to certify that

Lakbaibi A.

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. — **Alla KOTVITSKA**

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

Head of the department of chemistry of natural compounds and nutriciology of the NUPh, prof. — **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

IDENTIFICATION OF VOLATILE COMPOUNDS OF *JUNIPERUS SABINA* L. NEEDLES

*Lakbaibi A., Mashtaler V.V., Sydora N.V.**
National university of Pharmacy, Kharkiv, Ukraine
*University of Turku, Turku, Finland

Introduction. Under the influence of environmental factors, new, mutating microorganisms appear that are causative agents of dermatological diseases and are not sensitive to traditional drugs. Therefore, the search for natural sources of biologically active substances (BAS) for the creation of effective antimicrobial and bactericidal agents is urgent. Juniperus sabina L. is a representative of the cypress family *Cupressaceae* of the genus *Juniperus*. It is known from literary sources that lipophilic substances, in particular, volatile compounds that are part of essential oils, have a wide spectrum of antimicrobial activity [1, 4]. Therefore, the study of volatile compounds of the needles of *Juniperus sabina* L. is relevant in order to expand information on the chemical composition of the raw material and predict its further use.

Materials and methods. Volatile components were determined in the lipophilic fraction of needles obtained by chloroform. An Agilent Technology HP6890 GC chromatograph with a 5973N mass spectrometric detector was used. Analysis conditions: quartz chromatographic column, capillary HP-5MS, column length 30 m, inner diameter 0.25 mm; carrier gas – helium; speed of movement of carrier gas 1 ml/1 min.; sample volume – 2 μl.; sample introduction in splitless mode; sample injection rate 1.2 ml/1 min for 0.2 min.; thermostat temperature 50∘C with programming 4∘/min up to 220 0C; the temperature of the detector and evaporator is 250∘C [2, 5].

Results and discussion. In the lipophilic fraction 25 essential compounds of different chemical structures were found. Were identified α-pinene, sabinol, sabinene, α-pinene, α-thujene, terpinene, geraniol, cadinene, α-terpinene. According to the literature, the identified volatile compounds have an antimicrobial, bactericidal, antifungal effect, in particular, against *Staphylococcus aureus*, which can be used in the future to create medicines for the treatment of skin diseases (eczema, dermatitis) caused by this pathogen [2].

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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

Aimad LAKBAIBI

1. Topic of qualification work: «Chemical study the lipophilic compounds of *Juniperus sabina*», 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 the study of lipophilic compounds of *Juniperus sabina*

4. Contents of the settlement and explanatory note (list of questions that need to be developed): obtaining a lipophilic fraction; study of the content of fatty acids and chlorophylls in the lipophilic fraction; obtaining essential oil; establishment of the component composition and physical and chemical properties of the essential oil

5. List of graphic material (with exact indication of the required drawings):
Tables – 17, figures – 16

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	Aimad LAKBAIBI 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	Aimad LAKBAIBI 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	Aimad LAKBAIBI 1.2023 Aimad LAKBAIBI 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	Preparation of lipophilic fraction of *J. sabina*	January 2023	**done**
3	Study of the content of fatty acids in the lipophilic fraction	February 2023	**done**
4	Study of chlorophylls and carotenoids	March 2023	**done**
5	Obtaining essential oil and establishing its chemical and physical properties. Study of the component content of essential oil.	April 2023	**done**
6	Preparation of a master's thesis for official defense	May 2023	**done**

An applicant of higher education Aimad LAKBAIBI

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**				
Лакбаібі Аімад	Хімічне дослідження ліпофільних сполук хвої *Juniperus Sabina.*	Chemical study the lipophilic compounds of *Juniperus Sabina.*	доцент Машталер В.В.	доцент Абу Шарк Амжад Ібрагім

Підстава: подання декана, згода ректора

Ректор

Вірно. Секретар

Ф А2.8-47-110

ВИСНОВОК

Комісії з академічної доброчесності про проведену експертизу

щодо академічного плагіату у кваліфікаційній роботі

здобувача вищої освіти

№ 112771 від « 30 » квітня 2023 р.

Проаналізувавши випускну кваліфікаційну роботу за магістерським рівнем здобувача вищої освіти денної форми навчання Лакбаібі Аімад, 5 курсу, _____ групи, спеціальності 226 Фармація, промислова фармація, на тему: «Хімічне дослідження ліпофільних сполук хвої *Juniperus Sabina.*/ Chemical study the lipophilic compounds of *Juniperus Sabina*», 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**

0%

32%

REVIEW

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

Aimad LAKBAIBI

on the topic: «Chemical study the lipophilic compounds of *Juniperus sabina*»

Relevance of the topic. The study of plants for identification and isolation of differents classes of biological active compounds it is one of the important task of modern Pharmacognosy. This is especially actual for the plants used in folk medicine and homeopathy for the treatment of widespread diseases. Among the plants that have significant pharmacological effect special place is occupied plants that belong to the poisonous. Such a plant is *Juniperus sabina*.

Practical value of conclusions, recommendations and their validity. The practical value of the qualification work is due to the fact that, as a result of the conducted research, the acquirer expanded the information on the chemical composition of the needles of *J. sabina*.

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 Aimad Lakbaibi «Chemical study the lipophilic compounds of *Juniperus sabina*» can be presented for official defense in the State Examination Commission of National University of Pharmacy.

Scientific supervisor _____Victoria MASHTALER

«7th» of April 2023

REVIEW

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

Aimad LAKBAIBI

on the topic: «Chemical study the lipophilic compounds of *Juniperus sabina*»

Relevance of the topic. Under the influence of ecological factors, new microorganisms are insensitive to traditional drugs are appear differents causative agents dermatological diseases like dermatitis, dermatitis, eczema. So, it is necessary to search for natural substances to solve this problem.

Theoretical level of work. The qualification work was performed at a high scientific level, modern methods and techniques of phytochemical research (chromato-mass spectrometry) 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. Determined the quantitative content of lipophilic fraction and essential oil from *J. sabina*. For further use in medical practice, the author suggests a lipophilic fraction and essential oil like possible components of drugs.

Practical value of conclusions, recommendations and their validity. The practical value of the qualification work is due to the fact that, as a result of the conducted research, the acquirer expanded the information on the chemical composition of the needles of *J. sabina*. Taking into account the spectrum of biological activity of the identified lipophilic compounds, it can be concluded that the obtained lipophilic fraction and essential oil can be used in the future for the development of external medicinal products for the treatment of skin diseases caused by *S. aureus.*

Disadvantages of work. In the work there are occasional unsuccessful phrases.

General conclusion and assessment of the work. The material of the qualification work of Aimad LAKBAIBI is laid out 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

«11th» of April 2023

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'ЯУКРАЇНИ
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-02 групи Аімада ЛАКБАІБІ на тему «Chemical study the lipophilic compounds of *Juniperus sabina*».

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 Аімада ЛАКБАІБІ на тему «Chemical study the lipophilic compounds of *Juniperus sabina*», науковий керівник: к.фарм.н., доц. Вікторія МАШТАЛЕР.

Голова
Завідувачка кафедри фармакогнозії **Ольга МАЛА**
Секретар **Микола КОМІСАРЕНКО**

Ф А2.2.1-32-042

НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

ПОДАННЯ
ГОЛОВІ ЕКЗАМЕНАЦІЙНОЇ КОМІСІЇ
ЩОДО ЗАХИСТУ КВАЛІФІКАЦІЙНОЇ РОБОТИ

Направляється здобувач вищої освіти Аімад Лакбаібі до захисту кваліфікаційної роботи
за галуззю знань 22 Охорона здоров'я
спеціальністю 226 Фармація, промислова фармація
освітньою програмою Фармація
на тему: «Chemical study the lipophilic compounds of *Juniperus sabina*».

Кваліфікаційна робота і рецензія додаються.

Декан факультету _____ / Світлана КАЛАЙЧЕВА /

Висновок керівника кваліфікаційної роботи

Здобувач вищої освіти Аімад Лакбаібі успішно виконав поставлені завдання, засвоїв роботу з науковими першоджерелами, методами та методиками фармакогностичного аналізу рослинної сировини, які він застосовував 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 Аімад Лакбаібі 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 /