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STUDY OF THE ESSENTIAL OIL OBTAINED FROM JUNIPER PLANT BY PHYSICAL-CHEMICAL METHODS

© ***A.B. Hasanova***

Institute of Bioresources of the Ministry of Science and Education of the Republic of Azerbaijan (Ganja), av. H. Aliyeva, 419, Ganja, AZ2000 (Azerbaijan), e-mail: ayshe_hesenova@rambler.ru

Plants are natural resource with a perfect structure that can meet many needs of people and show diversity since hundreds of years. All over the world and in our country, plants have been used for a long time in the preparation of tea, spices, perfumes, ointments and medicines used in the treatment of diseases. Essential oils arelarge group of biologically active compounds contained in plants. In the article, the process of extracting essential oil from the juniper plant (*Juniperus communis* L.) at different yields was studied. Juniper fruit 200.47 g; 200.28 g; when 200.54 g, the average amount of essential oil according to wet raw material was determined to be 1.2±1.4%. The composition of the essential oil obtained from juniper fruit, rich in biologically active substances and important for treatment, was studied by physicochemical methods (chromatographic, Ultraviolet (UV) and Infrared (IR) spectroscopic). In the chromatogram of the essential oil, it was determined that there is a peak of monoterpenoids with a tricyclic structure. Absorption bands obtained as a result of spectral analysis ensure the correctness of the composition of the synthesized essential oil. On the basis of medicinal properties of the juniper plant (*Juniperus communis* L.), the obtained essential oil can be used in medicine and cosmetology in the preparation of ointments and lotions with various ingredients.

Keywords: Juniper plant (*Juniperus communis* L.), essential oil, monoterpenoid, sesquiterpenoid, chromatography, Ultraviolet (UV) spectroscopy, Infrared (IR) spectroscopy.

Introduction

One of the most important issues facing the chemical industry in modern times is the process of industrial and domestic waste recycling in order to use natural resources efficientlyand save raw materials, protect the environment [1–3].

The properties of plants that are important for human health have been studied in laboratories since 1926. In recent years, the reasons such as the increased number of side effects and resistance of the body to synthetic ingredients used in skin diseases and skin care, especially as antimicrobial agents, have increased the importance of natural plant-based medical preparations [4–6]. Taking into account the fact that there are more counter-indications of synthetic substances used in the field of pharmacology and cosmetology, our goal is to further expand the fields of application of natural substances. It is the study of the biodiversity of the flora of our republic, the protection and efficient use of the gene pool.

Cultivated, wild and medicinal plants, rich in biologically active substances, which are important in various fields of medicine, have always been in the center of attention. Because such compounds play an important role in the obtaining of pharmaceutical forms, which is the main field of pharmacology. The purchase of therapeutically important oils, ointments, and cosmetics from natural resources and biologically active substances obtained from them and their application is one of the current problems.

The World Health Organization (WHO) reported that the number of medicinal plants used for treatment is about 20000. Since 1940 essential oils obtained from natural plants as raw materials have been used in many industries such as medicine, food, perfumery and cosmetology. In particular, antimicrobial properties have been studied and important results have been obtained [7].

Essential oils are a mixture of multicomponent volatile organic substances produced by plants, which form their characteristic aroma, and whose composition consists mainly of terpenes and terpenoids. In other words, essential oils consist of a mixture of simple

Hasanova Aisha Baba gyzy – Tech. Sci. PhD, docent "Oils and ointments" head of the laboratory, e-mail: ayshe_hesenova@rambler.ru

aliphatic and cyclic terpenoids (especially mono- and sesquiterpenes), their alcohols and ketones, accompanied by derivatives of benzoic acid and phenylpropane.

The essential oil industry is one of the most profitable industries in the world agro-industrial complex. The essential oil flora includes about 3000 plant species. In the last 40 years, the production of essential oils in the world oil production increased from 50000 tons to 250000 tons per year. It is produced by about 300 cultivated and wild species of essential plants. Most essential oils are obtained from tropical or subtropical plants, and only a few (coriander, anise, peppermint, etc.) are cultivated in more temperate latitudes [8].

Experimental Part

Common juniper – scientific name Juniperus communis is a naturally distributed plant in the northern zones of Europe. It grows in Azerbaijan in the Greater and Lesser Caucasus, in the Nakhchivan mountains, on the flat plateau, and on dry stony slopes in the lower and middle mountain belts.

The results of the study of extractive substances of juniper, growing in the territory of the Western region of Azerbaijan, soluble in ethyl alcohol are presented.

Essential oils are extracted from the leaves, flowers and fruits of plants by various methods: extraction through water vapor; mechanically; extraction with organic solvents; enfleurage method.

The application of these methods depends on the morphological-anatomical structure, nature and content of the essential oil of the processed plant. The juniper fruit was extracted with 96% ethyl alcohol as a solvent. The extraction process was carried out in a Soxhlet extractor. Soxhlet extractor (Soxhlet apparatus) is a device for the continuous extraction of sparingly soluble solids from solid materials. The Soxhlet extractor is mounted on a round bottom flask containing the extracting solvent and is equipped with a reflux condenser. In the center of the apparatus is a reservoir in which is placed a sleeve made of thick cardboard or paper and filled with a solid sample from which the extraction will be performed. The solvent is heated to the boiling point, it evaporates and, passing through the side outlet, enters the reflux condenser, where it condenses and flows into the sleeve. While the sleeve is being filled with solvent, the target substance is extracted into this solvent. As soon as the liquid level in the sleeve reaches the top level of the siphon, the sleeve is emptied: the substance solution is drained into the original flask and the cycle is repeated again. Thus, the device allows multiple extractions due to the reuse of a relatively small volume of solvent, while the extractable substance accumulates in the main flask. The extraction efficiency is additionally increased due to the fact that the sleeve is located directly above the flask and is heated by the vapors of the boiling solvent.

The process was carried out in two stages: the actual separation of the components from the fruit of the juniper plant and the separation of the solvent. In order to obtain essential oils from juniper fruits by the extraction method, the crushed plant fruit was first mixed with a solvent in a device. The obtained mixture is extracted using a solvent for 5–6 hours at a temperature of 78–80 °C in an extractor. Then the filtration process is carried out. The filtrate is separated into oil and solvent fraction at a temperature of 70–78 °C in the distillation unit. The separated fraction can be used in the extraction again.

The component composition of essential oil of juniper fruit was determined by the gas-liquid chromatography method in AutoSystem XL (PerkinElmer) flame ionization detector chromatograph.

The UV/Vis 6850 spectrophotometer manufactured by JENWAY was used to study the essential oil obtained from the juniper plant by UV-spectroscopic method. The optical transmittance of the device is 0.1 nm. The spectra were recorded in the 200–500 nm wavelength range in steps of 1.0 nm.

The IR spectrum of the essential oil obtained from the juniper fruit was recorded on the "ALPHA" IR-Fourier spectrometer of the German "Bruker" company. The IR spectra of the substances were recorded in the wave number range of 600–4000 cm^{-1} at a temperature of 25 °C.

Results and discussion

The essential oil of common juniper is a light yellow liquid. The amount of essential oil in juniper fruit was 1.2±1.4%.

The obtaining processof essential oil based on extraction of juniper plant in ethyl alcohol as an extract agent (organic solvent) and the composition and purity of the obtained essential oil were studied by chromatographic, Ultraviolet (UV) and Infrared (IR) spectroscopic methods.

Based on the experiments, the process of extracting the essential oil of juniper fruit at different yield rates was studied in 3 options, and a favorable option was determined. The yield of essential oils obtained as a result of the experiments was calculated according to the wet raw material based on the formula written below [9]:

$$\text{Yield of essential oil, \%} = \frac{\text{Amount of essential oil, g}}{\text{Amount of raw materials, g}} \cdot 100\%$$

The results of the essential oil obtained at different extraction rates are given in Table 1. In the determined optimal options, juniper fruit is 200.47 g; 200.28 g; When 200.54 g was taken, the average amount of essential oil was 1.2±1.4% according to the wet raw material.

The simplest methods of determining the authenticity of essential oils are to determine their organoleptic properties (color, transparency, smell, taste) and physicochemical properties (density, refractive index, kinematic viscosity, pH). Physico-chemical properties of the obtained essential oil were studied by different methods and the obtained results are presented in Table 2.

As can be seen from the table, the density of essential oils (GOST ISO 279-2014 [10]) is usually less than one. The refractive index (GOST ISO 280-2014 [11]) is almost constant for all oils (Table 2). According to the value of the refractive index, it can be judged that certain components are predominant in the oil. The highest breakdown is characteristic of oils with a high content of aliphatic terpenes with three double bonds, and the lowest for tricyclic terpenes. Identification of the components included in juniper oils was carried out by various methods: introducing known pure substances, measuring the physical constants of substances isolated by preparative chromatography, IR and UV spectroscopy methods.

Chromatographic, Ultraviolet (UV) and Infrared (IR) spectroscopic methods are used to check the purity of essential oil and to analyze its composition.

To determine the naturalness and composition of essential oils, enantioselective gas-liquid chromatography is used, which allows determining the ratio of diastereomers of oil components that can serve as chemomarkers of their origin. Chromatograms allow to determine the composition of essential oils or a detailed "map" of the distribution of all its components, the authenticity and naturalness of the essential oil. A common method for the analysis of complex mixtures of terpene compounds (mono-, sesqui-, diterpenes) is gas-liquid chromatography [12–16].

To carry out chromatography, a small sample (0.001 μl) of essential oil is evaporated (at a temperature of 250 °C) into a narrow quartz capillary tube (inner diameter 0.25 mm) with a length of 30 meters using a microsyringe. Under the influence of a carrier gas (usually helium, hydrogen or nitrogen) constantly flowing through this tube, the essential oil in the form of vapor moves through the tube. Simultaneously, the column temperature rises from 50 to 220 °C at a rate of 3–4 degrees/min. The inner surface of the tube (which is called a column) is covered with a thin layer (0.25 microns) of a neutral liquid of a polymeric nature [17–19].

As a result of chromatographic analysis, a chromatogram is obtained, that is, a graphical representation of the composition of essential oils is obtained in the form of peaks.

Under these conditions, more than 70 components were found in oils. Monoterpene, sesquiterpene and oxygen-containing compounds were found in oils. According to the results of the study, two significant conclusions can be drawn: the qualitative analysis of the essential oils of the studied juniper species is the same, but there are significant differences in quantitative terms. They differ both in the amount of mono- and sesquiterpenoids, and in individual components. The amount of monoterpenoids 78.90% (α-pinene 32.90%, camphene 1.00%, p-pinene 10.50%, myrcene 8.62%, Δ^3-karene 1.83%, α-phellandrene 1.80%, α-terpinene 1.50%, dipentene 0.10%, 3-phellandrene 4.15%, cineole 2.24%, γ-terpinene 0.50%, p-cymol 1.28%, terpinolene 1.20%, α-thujone 2.28%; the amount of sesquiterpenes and oxygenated compounds: 21% (X_4 0.1%, geranial 1.0%, peral 0.80%, longycyclene 1.12%, siberene 0.82%, dicycloether 0.20%, bornyl acetate 3.48%, caryophyllene 1.41%, longifolene 0.95%, terpineol 3.80%, murolene 0.12%, α-cadinene 2.20%, γ-cadinene 2.00%, chamazulene 2.90%, α-bisabolol 0.10%, α-curcumene 0.10%). The figure 1 shows the chromatogram of essential oil of juniper fruit.

The analysis and percentage of the individual components in the essential oil were calculated according to ISO 4724:2004 and CTO 18393365-004-2010 document [11]. In the chromatogram of the essential oil, the capture of the peak of monoterpenoids with a tricyclic structure coincides with the gas-liquid chromatographic profile (reference) for the essential oil presented in the document ISO 4724:2004 and CTO 18393365-004-2010. The symmetry factor calculated for the α-pinene peak in the chromatogram is 7.4. The chromatographic conditions chosen were 5.7 with a partition coefficient to resolve the α-pinene peaks.

Table 1. Material balance of the extraction process

The name of the raw material		Amount of raw materials, g	Amount of essential oil, g	Yield of essential oil,% (%)	Average amount, %
N1	Juniper the fruit (*Juniperus communis* L.)	200.47	2.21	1.10	
N2	Juniper the fruit (*Juniperus communis* L.)	200.28	2.21	1.37	1.2±1.4
N3	Juniper the fruit (*Juniperus communis* L.)	200.54	2.29	1.14	

Table 2. Physicochemical characteristics of essential oil

№	Color	Transparency	Smell and taste	Density at 20°C, g/sm^3	N_D, 20 °C	Kin. visco. 20 °C, mm^2/s	pH	Brix 25 °C, °Bx	ABS (absorbance measurement)
N1	yellow	clear	specific	0.9769	1.3645	1.741	4.25	0.5	0.959
N2	yellow	clear	specific	0.8162	1.2356	1.631	5.12	0.1	1.155
N3	yellow	clear	specific	0.8445	1.2564	1.625	6.31	1.5	1.211

According to the UF spectroscopy method, we can note that the highest wavelength of 230 nm is equal to the absorption spectrum of 2.524%. This behavior means that the absorbance around this wavelength can take maximum amount. The absorbance at the wavelength range (280–360 nm) represents minimum values and the radiation reached to the skin is a maximum. In the wavelength greater than 480 nm the absorbance takes approximately equal amounts, and this means that the quantity of sunlight reached to human skin is about equal [20, 21].

Infrared spectroscopy substantially complements UV spectroscopy. The IQ-spectra of essential oil obtained from juniper fruit and its components were recorded on a zinc-selenide crystal in the "Alpha" Fourier spectrophotometer manufactured by the German company "BRUKER" in the wavenumber interval of 600–4000 cm^{-1}, based on the principle of reflection of the beam from the sample and KBr crystal plates. by creating a thin sample layer between it and drawing it at room temperature based on the principle of radiation transmission.

The main component of Fourier IR spectrophotometers is the Michelson interferometer. Its main elements are three mirrors. The light-splitting mirror (plate) divides the beam of light into two parts: one of them is stationary, and the second is reflected from a moving (scanning) mirror. Both reflected beams fall back on the beam-splitting mirror, where they are combined and directed to the detector (photoreceiver).

A moving mirror creates an optical path difference for the two light beams. When this difference is $(n+½)\times\lambda$, the transmitted rays cancel each other, and the reflected ones, on the contrary, are strengthened. As a result, an interferogram, that is, the dependence of the recorded beam intensity on the difference in optical paths, is obtained. For monochromatic light, this dependence is cosinusoidal. In the case of polychromatic light, this dependence is complex, it provides all the spectral information about the light beam falling on the detector. Then the interferogram is transformed into an IR spectrum by Fourier transformation.

For the chemistry of sesquiterpenoids, two parts of the spectrum are most important. Absorption bands in the region of approximately 3650–2650 cm^{-1} (excluding C-H vibrations) in the case of terpenoids are almost always characteristic of O-H bond vibrations. When properly interpreted, absorption bands in this region can serve as evidence for the presence of hydroxy or related groups. Absorption in the second and more important part of the spectrum (approximately 1820–1640 cm^{-1}), if it is sufficiently intense, usually corresponds to C=O vibrations. By the position of the absorption maximum in this region, it can be determined whether the compound is a saturated or conjugated ester, aldehyde, ketone, acid, lactone, or anhydride. Important, but less general, are data on absorption in other parts of the spectrum. Thus, a weak absorption band in the region of 3050 cm^{-1} indicates the presence of a methylene group in the cyclopropane ring [22, 23].

IR analysis indicated presence of myrcene (ester functional group identified with three bands positioned at 1743 cm^{-1}, 1238 cm^{-1}, 1021 cm^{-1}), possible presence of apigenin-7-glucoside (ketone functional group identified with with a band positioned at 1743 cm^{-1}), α-bisabolol (C-O vibration identified with a band at 1050 cm^{-1}, C-C-C vibration of tertiary alcohol identified with a band at 919 cm^{-1} and isopropyl group tentatively identified with two bands at 1452 cm^{-1} and 1374 cm^{-1}) and dicycloether identified by the C-O-C functional group with two bands at 1080 cm^{-1} and at 1167 cm^{-1} (Fig. 2).

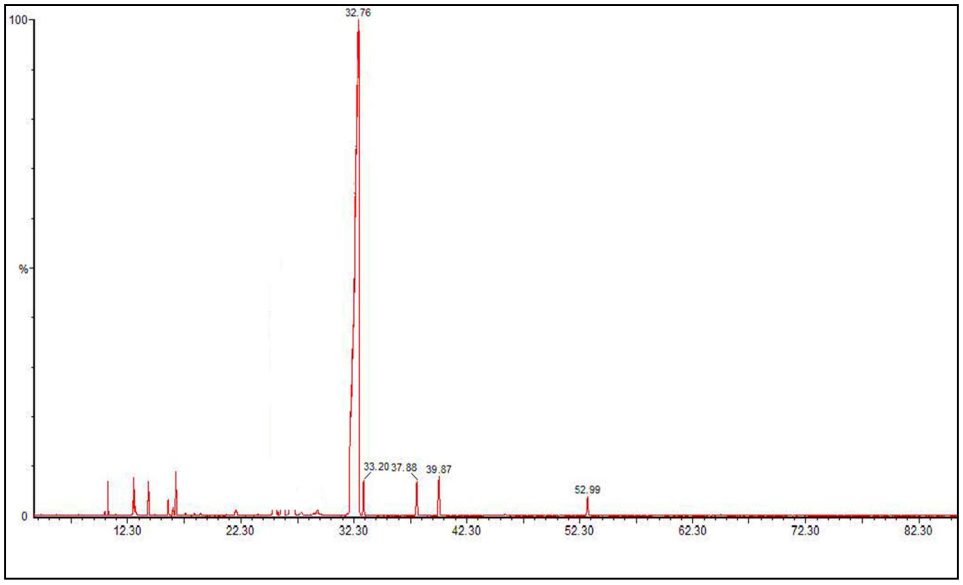

Fig. 1. Chromatogram of essential oil from juniper plant

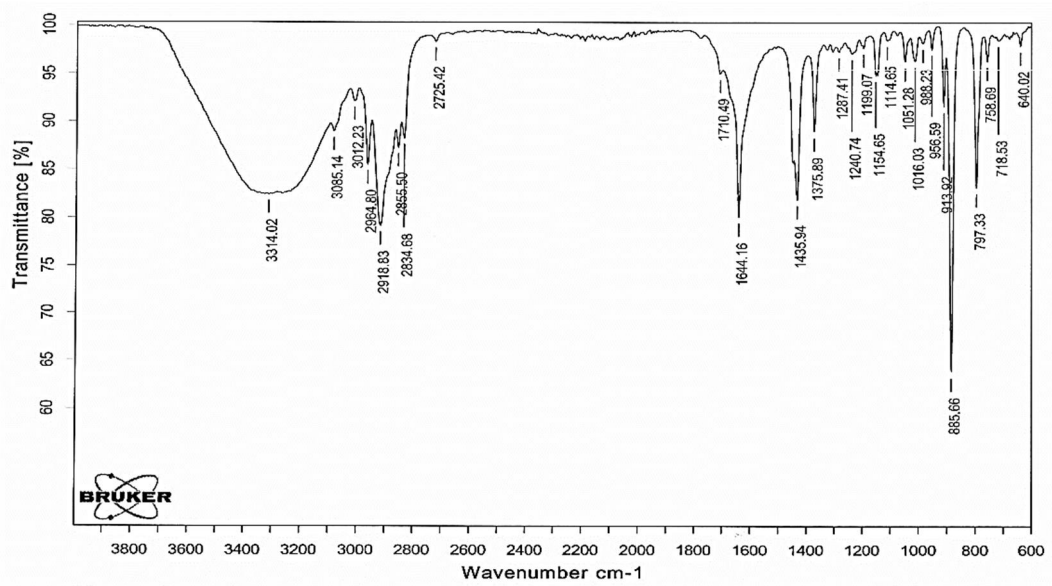

Fig. 2. IR spectrum of essential oil from juniper plant

Conclusion

The quantitative and qualitative composition of the extractive substances of Common juniper growing in the Western region of Azerbaijan was studied. It is useful in terms of studying the component composition of common juniper (Juniperus communis L.) fruit and obtaining knowledge about the synthesis and collection of individual chemical compounds. Juniper fruit 200.47 g; 200.28 g; when 200.54 g, the average amount of essential oil according to wet raw material was determined to be 1.2±1.4%. In this work, three main points can be deduced as a result of UV spectroscopic analysis, the first point shows that the wavelength absorption of the selected natural oil can be limited in the range of 230 nm. The second point is that the absorption range is at the edge of the ultraviolet region and close to the visible region, which gives us substantial evidence for the safety of human use and not being affected by visible radiation, as well as the preservation of protection in places. exposed to visible radiation. The last point is to store these oils in containers that prevent UV rays from reaching them. Absorption bands obtained as a result of IR-spectral analysis ensure the correctness of the composition of the synthesized essential oil.

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