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Technology for Isolation Essential Oil from the Buds of *Populus balsamifera* L.

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Abstract

The article presents the results of the development of technology for isolating essential oil from the buds of *Populus balsamifera* L. and a medicinal substance based on it. For the first time, a technology has been developed for the quantitative isolation of essential oil from the buds of *Populus balsamifera* L. by the barothermal method at a temperature of 140 °C, under a vacuum of 1333–2000 Pa. The main components of the essential oil of *Populus balsamifera* L. buds, isolated by the barothermal method, were determined to be α-bisabolol, β-eudesmol, and 2-phenylethyl-2-methylbutanoate. A technological scheme for the production of essential oil substance from *Populus balsamifera* L. buds has been developed, including 2 stages of the main technological process and 2 stages of auxiliary work, and its pilot industrial regulations. The control points during the isolation and preparative production of the essential oil substance from the *Populus balsamifera* L. buds are the major components α-bisabolol and β-eudesmol. The main critical points in the production of a substance based on the essential oil of *Populus balsamifera* L. buds are pressure, temperature, and duration of isolation. The stages of production of the substance are controlled using a standard sample of α-bisabolol (1). A quantitative determination of 108 components of essential oil from *Populus balsamifera* L. buds was carried out, as well as standardization for organoleptic properties and major components. A comparative analysis of essential oil from *Populus balsamifera* L. buds collected in the vicinity of Karaganda and the North Kazakhstan region was carried out. A substance based on essential oil from *Populus balsamifera* L. buds has antimicrobial, wound-healing, and antitumor activities with relatively low toxicity.

1. Introduction

A promising and potential source of medicinal substances, including essential oils, are the buds of *Populus balsamifera* L., which has industrial reserves of raw materials in Kazakhstan.

The diversity of the chemical composition, including more than 70 biologically active compounds of various classes, including polyphenolic compounds, fatty and organic acids, vitamins, trace elements, tannins, lignins, and essential oils, allows us to recommend the buds of different species of *Populus L.* as a renewable raw material for the development and production original medicines.

Populus L. buds are a rich source of essential oil [1–4], which is a complex mixture of mono- and sesquiterpenes, as well as hydrocarbons, alcohols, ketones, phenolic compounds, aldehydes, and acids. The practical value of the essential oil of *Populus balsamifera* L. buds lies in the possibility of creating antimicrobial, antitumor, anti-inflammatory, and immunomodulating agents based on it [5–6]. In addition, this essential oil can be used in perfumery, soap making, and the food industry.

One of the promising biologically active compounds of *Populus L.* buds is α-bisabolol – a monocyclic sesquiterpene found in essential oils of many

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plant species. Antitumor, neuroprotective, cardioprotective, and antimicrobial activities have been determined for α-bisabolol [7]. α-Bisabolol is a part of the skin care cream and is included in many cosmetic formulations due to its soothing effect, good absorption, and the absence of irritation or photosensitivity after its use [8].

2. Experimental part

Raw materials of buds *Populus balsamifera* L. were collected in March-April 2023 (at rest) in the vicinity of the village of Pribrezhnoye, North Kazakhstan region, and in the vicinity of Karaganda (Central Kazakhstan).

Essential oils from the samples of *Populus balsamifera* L. were isolated via hydrodistillation using a Clevenger apparatus and by barothermal method, and the oil was extracted from the raw material using organic solvents.

The refractive index and boiling point of essential oil samples were determined using generally accepted methods [9].

Chromatography-mass spectrometric analysis of essential oils was carried out using an Agilent 6890 gas chromatograph equipped with an MSD 5973 mass-selective detector. The essential oil components were identified by comparing their mass spectra and linear retention indices (relative to C_8-C_{24} alkanes) with the data presented in the database [9].

2.1. Barothermal oil isolation

An installation has been developed and created for obtaining essential oil of *Populus balsamifera* L. buds using a barothermal method, combining 2 processes – high pressure and hydrodistillation (Fig. 1).

2.1.2. Effect of pressure on essential oil yield

When extracting essential oil from *Populus balsamifera* L. buds, the pressure in the apparatus was changed within the following limits – from 0.5 to 2.8 atm. The upper limit (2.8 atm) is the maximum permissible for this laboratory installation. It is not advisable to use higher pressure due to the high temperature, which negatively affects the quality of the obtained essential oil, and the increased cost of equipment, along with an increase in the danger of operation. During the studies with pressure changes, the data shown in Table 1 were obtained.

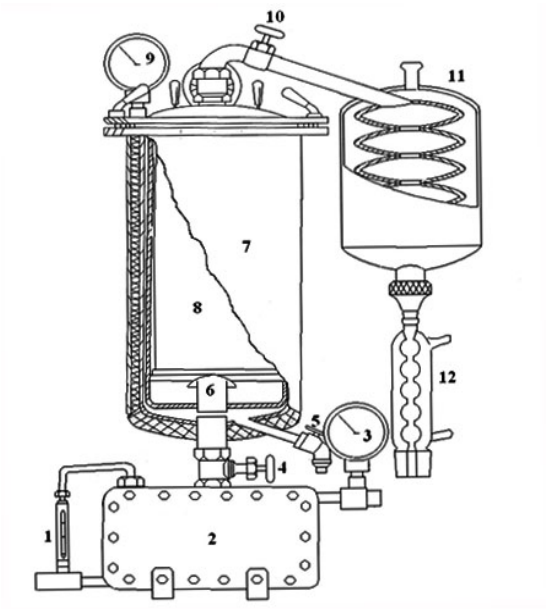


Fig. 1. Diagram of a barothermal installation: 1 – water level indicator; 2 – steam generator; 3 – steam generator pressure indicator; 4 – valve for allowing steam into the reactor; 5 – lower exhaust valve; 6 – steam distributor; 7 – reactor; 8 – container; 9 – reactor pressure indicator; 10 – upper exhaust valve; 11 – water refrigerator; 12 – reverse water refrigerator.

Table 1. Effect of pressure changes on the yield of essential oil

Pressure, atm	0.50	1.00	1.50	1.80	2.00
Yield, %	0.19±0.01	0.34±0.03	0.50±0.02	0.57±0.07	0.57±0.01

The optimal operating mode of the installation is a pressure of 1.5 up to 1.8 atm, which gives the highest yield of essential oil from 0.50 to 0.57%. Graphically, the reliance of the yield of essential oil on pressure is presented in Fig. 2.

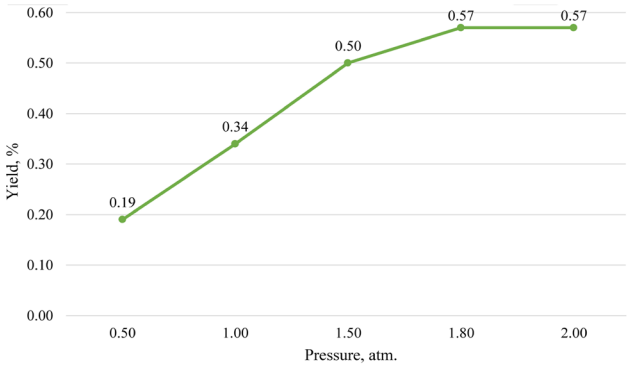


Fig. 2. Graph of essential oil yield versus pressure.

Table 2. Effect of changing the height of the raw material layer on the yield of essential oil

Height of the raw material layer, cm	1.50	3.00	4.50	6.00	7.50	9.00	10.50	12.00	13.50	15.00
Yield, %	0.21±0.03	0.36±0.01	0.52±0.03	0.58±0.05	0.60±0.07	0.50±0.02	0.43±0.02	0.37±0.04	0.37±0.03	0.30±0.01

2.1.3. The correlation of drug yield and layer height

When conducting experiments on a laboratory installation, the height of the layer of raw materials was changed (from 1.5 to 15.0 cm) and the correlation between the yield of essential oil and this indicator was studied. With increasing layer height, due to greater contact of steam with the raw material and, therefore, greater saturation, the yield increases. But when the layer height is more than 7.5 cm, a decrease in yield is observed, which is a consequence of the compaction of the raw material, which cakes in lumps and makes it difficult for steam to contact the raw material. During the experiment, data were obtained (Table 2).

The optimal height of the raw material is from 6.0 to 7.5 cm. Based on the data in Table 2, a graph was constructed (Fig. 3).

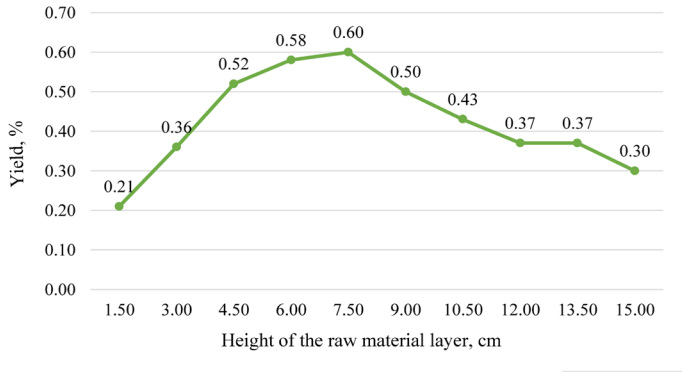


Fig. 3. Graph of the correlation of the yield of essential oil and the height of the raw material layer.

2.1.4. Extraction time duration

When extracting essential oil, observations were made to determine the dependence of the product yield on the extraction time. With increasing time of bud processing, the yield of essential oil increases. The most effective period is 1.5–2.5 h, then the product yield begins to sharply decrease, which indicates the inappropriateness of using a longer extraction time (Table 3).

The optimal time for extracting raw materials is 2 h. Based on the above, we can give an example of one installation cycle.

Installation cycle:

1) Filling the container with raw materials to a height of 6.0–7.5 cm;

2) Power on;

3) Filling the steam generator with water;

4) Heating water in the steam generator to boiling point;

5) Injection of pressure in the steam generator, due to the formation of steam in the steam generator, up to 1.5–1.8 atm;

6) Turning on the refrigerator;

7) Warming up the reactor with the generated steam (when valve 4 is opened, steam is supplied from the steam generator to the reactor, then the steam supply is stopped by it);

8) Draining the condensate formed as a result of heating through valve 5 from the reactor;

9) Injection of the reaction atmosphere in the reactor by opening valve 4;

10) When the pressure reaches 1.25 atm the collection of the aqueous emulsion of essential oil begins through valve 10 (we open the valve slightly and monitor the temperature of the water leaving the refrigerator; if it gets hot, we increase the water flow). When valve 10 opens, the rate of pressure growth in the reactor decreases. It is necessary to maintain its growth without allowing pressure to decrease;

11) When the pressure reaches 1.6–1.7 atm the collection of the thick substance begins through valve 5. The collection occurs along with condensate, which is separated after cooling;

Table 3. Effect of extraction time on essential oil yield

Extraction time, h	1	2	3	4	5	6	7	8
Yield, %	0.25±0.01	0.45±0.03	0.32±0.02	0.05±0.05	0.01±0.01	0.02±0.03	0.01±0.02	0

12) When the pressure reaches 1.8 atm and higher, gradually opening valve 10 to the end, we collect the aqueous emulsion of essential oil (while continuing to monitor the temperature of the water in the refrigerator);

13) In addition, throughout the entire operating time of the installation (for 2 h), it is necessary to monitor the water level in the steam generator; when the water level decreases to less than half, turn off the power;

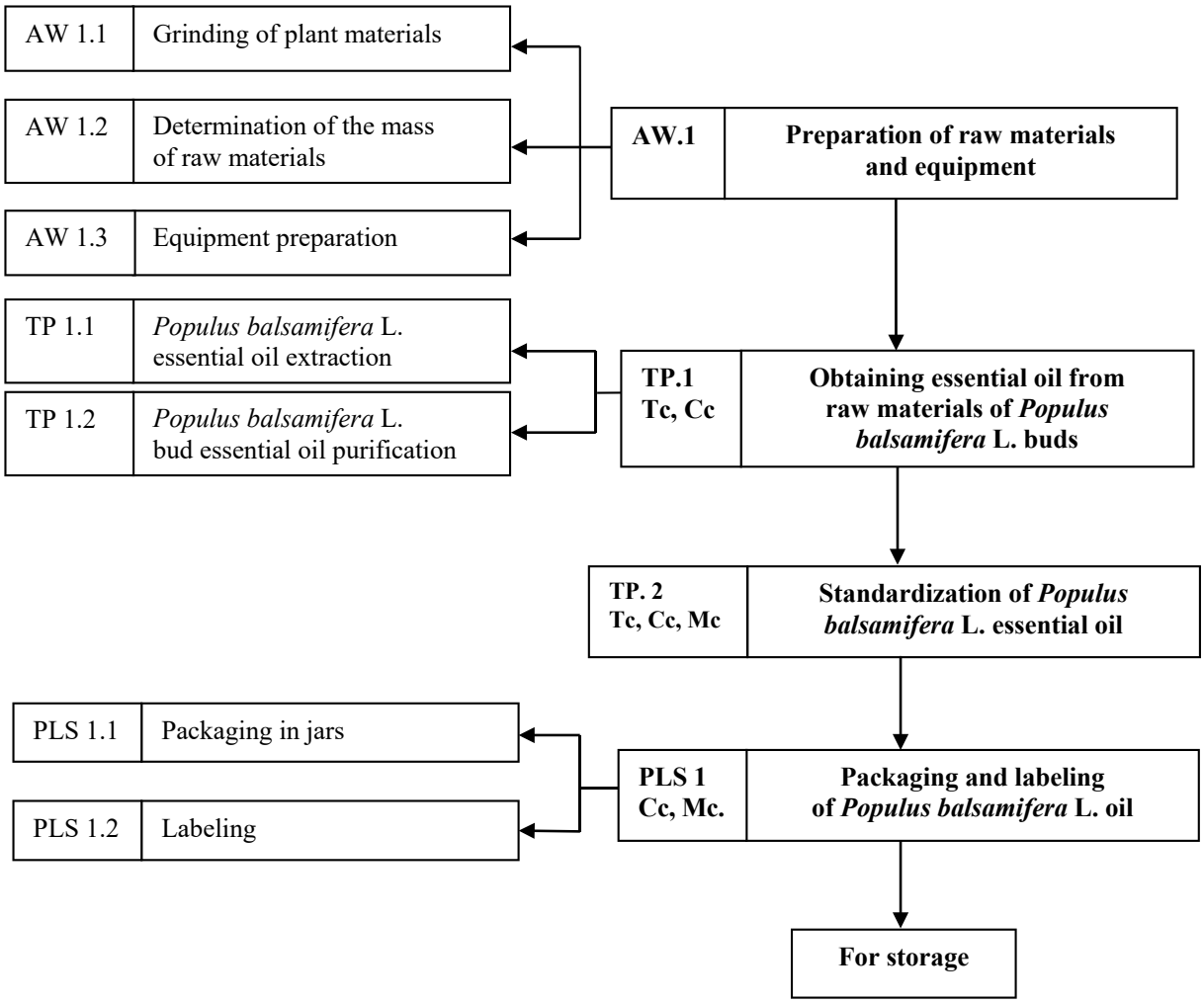
14) With a gradual decrease in pressure, we continue collecting both the aqueous emulsion and the thick substance, without ceasing to monitor the temperature of the water in the refrigerator;

15) When the pressure reaches 0 atm in the steam generator we remove the container with raw materials from the reactor; We remove waste materials from the container.

The optimal conditions for the process of extracting *Populus balsamifera* L. oil have been determined. The proposed method is economically beneficial since it eliminates the use of expensive flammable organic solvents, significantly reduces the time of release of *Populus balsamifera* L. oil, and the final product does not contain residual amounts of solvents that pollute the substance and increase its toxicity. In addition, it eliminates 4 technological operations (preparing the extractant, isolation, settling, and evaporation) and reduces the number of devices involved in the operation.

A technological scheme for the production of essential oil substances from *Populus balsamifera* L. buds has been developed, which is presented below (Fig. 4).

As can be seen from Fig. 4, the technological scheme for extracting essential oil from *Populus balsamifera* L. buds consists of three auxiliary works



AW – Auxiliary works; TP – Technological process; Tc – Technical control; Cc – Chemical control; Mc – Microbiological control; PLS – Packaging, labeling and shipping

Fig. 4. Process flow diagram for the production of essential oil from *Populus balsamifera* L. buds.

and two technological processes. The use of a barothermal method for the production of essential oil from *Populus balsamifera* L. buds significantly reduces the extraction time, eliminates the use of organic solvents, and the quality of the essential oil by increasing the amount of component composition and increasing the quantitative content of biologically active compounds.

2.2. Quantitative determination of essential oil

During the experiments, we used a method for the quantitative determination of *Populus balsamifera* L. essential oil according to the State Pharmacopoeia of the Republic of Kazakhstan [10].

A sample of the raw material was placed in a wide-necked round-bottomed or flat-bottomed flask with a capacity of 700–800 mL, 300 mL of water was added and closed with a rubber stopper with a reflux condenser. Metal hooks were fastened into the cork from below, which, using a thin wire, suspended the graduated receiver so that the end of the condenser was exactly above the funnel-shaped extension of the receiver, without touching it. The division value of the graduated part of the receiver is 0.025 mL. The receiver should fit freely in the neck of the flask, without touching the wall of the neck, and be at least 50 mL from the water level. The flask with the contents was heated to boil gently for 1–1.5 h. Vapors of water and essential oil are condensed in the condenser, and the liquid form is collected in the receiver. The oil was allowed to settle in the graduated elbow of the receiver, and the water flowed back into the flask through the smaller elbow of the receiver. After distillation and cooling were completed, the volume of the settled layer of essential oil was counted and its percentage amount *X* was calculated using the formula:

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

where *V* is the volume of essential oil, mL; *m* is the mass of the raw material sample, g; *w* – loss in weight of raw materials during drying, %.

The control points for the isolation and preparative production of a substance based on essential oil from *Populus balsamifera* L. buds are: the content of the major components α-bisabolol, β-eudesmol, and 2-phenylethyl 2-methylbutanoate.

The main critical points in the production and production of *Populus balsamifera* L. bud essential oil substance are pressure, temperature, and isolation time.

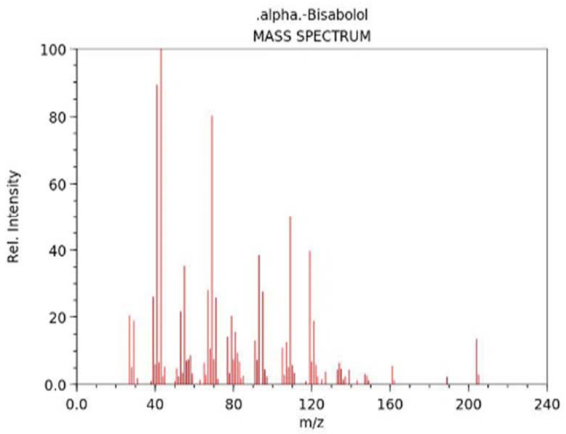


Fig. 5. Mass spectrum of α-bisabolol reference sample.

2.3. Standardization

Control of the stages of production of a substance based on the essential oil of *Populus balsamifera* L. buds is carried out using a standard sample of α-bisabolol (1).

α-Bisabolol – $C_{15}H_{26}O$ (2-(4-methyl-3-cyclohexenyl)-6-methyl-5-hepten-2-ol) also known as levomenol, is a natural monocyclic sesquiterpene alcohol, a viscous liquid with a faint, distinctive odor. Molecular weight: 222.38 g/mol; boiling point 153 °C/16 hPa; 129–130 °C/3.4 hPa. The refractive index is 1.491–1.500 [7, 9].

The authenticity of the tested oil is determined by determining the color, smell, taste, density, and refractive index.

The density of the oil at 20 °C is 0.76 g/cm³, which can be useful information to determine its type. The refractive index of oil at 20 °C is 1.3715. Below is the mass spectrum of a reference sample of α-bisabolol (Fig. 5).

2.4. Pharmacological studies

The acute toxicity of *Populus balsamifera* L. oil in a solution of dimethyl sulfoxide (in a ratio of 1:1 was studied using a method generally accepted in toxicology [11] on intact white outbred mice (weighing 19–20 g), rats (weighing 100–130 g) with a single subcutaneous injection. LD_{50} for mice is 5–6 mL/20 g body weight, for rats 10–15 mL/100 g body weight.

Chronic toxicity was determined using generally accepted methods in toxicology [11]. *Populus balsamifera* L. oil in a solution of dimethyl sulfoxide (1:1 ratio) in doses of 1–1.5 ml/100 g body weight of rats and 0.4–0.6 mL/20 g body weight of mice was administered subcutaneously daily for 10 days.

The antitumor activity of *Populus balsamifera* L. oil was studied on white outbred rats and mice with transplanted strains of sarcoma 45, Walker carcinosarcoma, Pliss lymphosarcoma and sarcoma 37. The antitumor effect was determined by daily administration of *Populus balsamifera* L. oil in a solution of dimethyl sulfoxide (1:1 ratio) for 10 days in maximum tolerated doses. Treatment of animals after transplantation of the tumor homogenate began from the moment of the appearance of measurable tumor nodes. To assess antitumor activity, we used the percentage of tumor growth inhibition.

The antibacterial properties of *Populus balsamifera* L. bud oil were studied by direct exposure of the oil to the cultures of *Staphylococcus aureus* and *Staphylococcus epidermidis*. The nutrient medium for growth was standard meat peptone agar (MPA).

3. Results and discussion

3.1. Study on the essential oil composition

The information available in the literature [1–4] on the essential oil composition of *Populus balsamifera* L. buds contains incomplete, sometimes contradictory information. Therefore, the study of the composition and biological activity of essential oil from the buds of *Populus balsamifera* L. is an urgent task.

According to the literature [1], the main components in the essential oil from *Populus balsamifera* L. buds are (E)-nerolidol (64%), 1,8-cineole (10.8%) and δ-amorphene (2.8%). In the oil isolated in this experiment with the barothermal method and hydodistillation using a Clevenger apparatus, (E)-nerolidol was absent, and 1,8-cineole was found in trace amounts. Although, the content of δ-amorphene in the essential oil isolated using a Clevenger apparatus with a barothermal method is higher than 3.39% and 4.79%, respectively.

According to the literature [2], the main components in the essential oil from *Populus balsamifera* L. buds are guaiol (13.2%), n-pentacosane (9.0%), n-heptacosane (18.9%), α-eudesmol (5%) and γ-curcumene (2.1%). However, in the essential oil isolated with the barothermal method using a Clevenger apparatus, guaiol, n-pentacosane, n-heptacosane are absent. At the same time, the content of α-eudesmol in essential oils isolated using a Clevenger apparatus and the barothermal method was found to be 4.10% and 8.51%, respectively. Also in the essential oil isolated on the Clevenger apparatus, γ-curcumen was found – 11.85%.

The essential oil from *Populus balsamifera* L. buds according to the literature [3] contains: trans-α-bergamotene (3.5%), γ-curcumene (4.1%), ar-curcumene (1.5%), α-bisabolol (31.4%), and in essential oils isolated using the Clevenger apparatus and the barothermal method, the content of trans-α-bergamotene is 3.16% and 3.18%, respectively, the content of ar-curcumene is 2.76% and 7.68%, respectively, and the content of α-bisabolol is 14.14% (with the barothermal method).

In the essential oils isolated by the barothermal method and on the Clevenger apparatus, 2-phenylethyl-2-methylbutanoate is present with a content of 15.10% and 10.22%, respectively [4]. However, this compound is absent in the literature [2–3]. Moreover, in the literature [1], this compound is present in trace amounts (<0.1%) in the essential oil of *Populus balsamifera* L. buds.

The composition of *Populus balsamifera* L. essential oils was studied using the method of chromatography-mass spectrometry, isolated by the barothermal method from poplar buds collected in the North Kazakhstan region and by hydrodistillation using a Clevenger apparatus from *Populus balsamifera* L. buds collected in the vicinity of Karaganda.

The major components in the essential oil from *Populus balsamifera* L. buds collected in the North Kazakhstan region, isolated by barothermal method, are: α-bisabolol (1) 14.14%, β-eudesmol (2) 10.70%, 2-phenylethyl-2-methylbutanoate (3) 10.22%, ar-curcumene (4) 7.68%, γ-eudesmol (5) 6.77%, α-amorphene (6) 4.79% (Fig. 6).

The major components in the essential oil isolated using a Clevenger apparatus from *Populus balsamifera* L. buds collected in the outskirts of Karaganda are: β-eudesmol (2) – 4.35%, 2-phenylethyl 2-methylbutanoate (3) – 15.10%, γ-eudesmol (5) – 3.13%, δ-amorphene (6) – 3.39%, γ-curcumene (7) – 11.85%, epi-α-bisabolol (8) – 7.34%, α-eudesmol (9) – 4.10%, trans-α-bergamotene (10) – 3.16% (Fig. 6).

Based on the above chromatography-mass spectrometry data, it was revealed that the main component of the essential oil from *Populus balsamifera* L. buds is α-bisabolol (1), the quantitative content of which is 14.14%, and the essential oil from *Populus balsamifera* L. buds collected in the vicinity of Karaganda contains epi-α-bisabolol (8) in an amount of 7.34%. γ-curcumen (7) – 11.85% is contained only in the essential oil from *Populus balsamifera* L. buds collected in the North Kazakhstan region. β-eudesmol is isolated from the raw materials of both collections, with a content of 4.35% to 10.70% in essential oil.

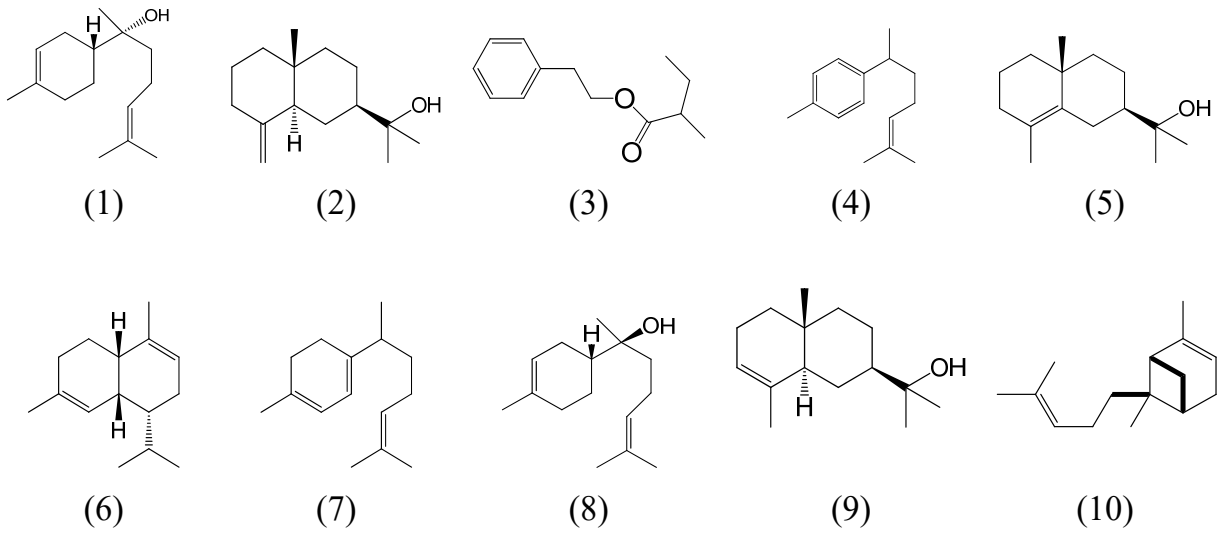


Fig. 6. Structural formulas of the molecules of the components of the essential oil of the buds of *Populus balsamifera* L.

A comparative analysis of essential oils from raw materials of *Populus balsamifera* L. buds, collected near Karaganda, with raw materials growing in the North Kazakhstan region, indicates that the quantitative yield of essential oils from both collections of raw materials is comparable, but the qualitative composition of essential oils differs from the place of collection of raw materials.

3.2. Pharmacological activity

When studying the acute toxicity of *Populus balsamifera* L. oil in doses of 5–6 mL/20 g body weight in mice and 10–15 mL/100 g body weight in rats, shortness of breath, agitation, then depression were observed: the animals died in depression on days 1–4. At necropsy of euthanized rats, the blood vessels of the peritoneum were macroscopically marked (in comparison with the control).

When studying chronic toxicity in doses of 1–1.5 mL/100 g body weight in rats and 0.4–0.6 mL/20 g body weight in mice for 10 days, no pronounced toxic effect on animals and their death were observed. Necropsy of experimental animals (rats and mice) did not reveal any pathological changes in the internal organs.

The obtained results show that *Populus balsamifera* L. bud oil is low toxic when administered subcutaneously in a solution of dimethyl sulfoxide (LD_{50} in experiments on rats 10–15 mL/100 g and mice 5–6 mL/20 g body weight).

Essential oil of *Populus balsamifera* L. buds at the maximum tolerated dose (1–1.5 mL/100 g body weight in rats and 0.4–0.6 mL/20 g body weight in mice) significantly inhibited the growth of Pliss lymphosarcoma (by 62–84%, $p < 0.05$), sarcoma 37 (up to 77%, $p < 0.01$) and Walker carcinosarcoma (up to 60%, $p < 0.05$). *Populus balsamifera* L. oil showed a weak antitumor effect on sarcoma 45 (up to 25–30%, $p < 0.05$).

Based on the results of determining antibacterial activity, it was established that *Populus balsamifera* L. bud oil suppresses *Staphylococcus aureus*, *Staphylococcus epidermidis*, as well as *Bacillus subtilis*, *Candida albicans*, i.e. has a pronounced antimicrobial effect, superior to the properties of some antibiotics. It has been established that storing oil for three years does not affect its antimicrobial activity.

4. Conclusion

Based on the results of the experiments, a method was developed for the quantitative isolation of essential oil from the raw materials of *Populus balsamifera* L. buds using a barothermal method. At the same time, the component composition of the oil is varied and the quantitative content of major components is relatively higher than that of the essential oil isolated on the Clevenger apparatus.

Based on the substance of essential oil from *Populus balsamifera* L. buds, a technological scheme for oil production has been developed, containing 2 stages of the main technological process and 2 stages of auxiliary work, and its pilot industrial regulations.

The control points for the isolation and preparative production of the essential oil substance from the *Populus balsamifera* L. buds are the major components α-bisabolol and β-eudesmol.

The main critical points in the production of essential oil substances from *Populus balsamifera* L. buds are temperature, duration, and isolation pressure. The stages of production of the substance are monitored using a standard sample of α-bisabolol.

Based on the results of a comparative analysis of the quantitative content of essential oil components from *Populus balsamifera* L. buds collected in the vicinity of Karaganda, and from raw materials growing in the North Kazakhstan region, it was revealed that the main component α-bisabolol contained at least 14.14% in the essential oil from buds collected on the territory of the North Kazakhstan region, when the essential oil from *Populus balsamifera* L. buds collected in the vicinity of Karaganda is characterized by the content of γ-curcumene 11.85%, epi-α-bisabolol 7.34%, β-Eudesmol is contained in essential bud oils from 4.35% to 10.70%.

Pharmacological and preclinical studies were carried out, and toxicity, antitumor, and wound-healing activities were determined.

Based on the above, the essential oil of *Populus balsamifera* L. buds is recommended for inclusion in transdermal patches intended for the treatment of skin diseases. The use of the essential oil of *Populus balsamifera* L. buds will have a synergistic effect on the wound healing and antiseptic activity of the medicine.

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