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T. S. Zveryachenko

*Northern Kazakhstan State University, Petropavlovsk, Republic of Kazakhstan line, timurzv@mail.ru*

A. S. Zveryachenko

*Military Medical Academy named after S.M. Kirov, St. Petersburg, Russian Federation, zveryachenko\_andrei@mail.ru*

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# Antibacterial Remedies

## Based on the Flora of Northern Kazakhstan

T. S. Zveryachenko & A. S. Zveryachenko

Northern Kazakhstan State University, Petropavlovsk, Republic of Kazakhstan line  
Military Medical Academy named after S.M. Kirov, St. Petersburg, Russian Federation  
e-mail: timurzv@mail.ru & zveryachenko\_andrei@mail.ru

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**Abstract** - It was developed by us a medicinal products line that includes components of *Populus balsamifera* L., *Populus tremula* L., *Chelidonium majus* L., *Glycyrrhiza uralensis* Fisch. The basis for the creation of the line was vegetative parts of balsam poplar. It was developed and patented a new method of obtaining biologically active substances from balsam poplar. It was determined the dynamics of changes in various indices of herbal raw material. The antiseptic tincture and suppositories on the base of substance were developed. Selected fraction of terpenoids uses as remedy and effectively fights all forms of lung tuberculosis pathogens, removes bacterial and fungal infection, helps to improve immunity, and forces the bronchus cure. An aerosol form of medication on the basis fraction of terpenoids is being developed. It is developed composition of the dosage form (syrup), which includes wartwort alkaloids, phenolic compounds of aspen, an extract of licorice, for the treatment of concomitant assident fungus and bacterial flora.

**Keywords**- *balsam poplar, aspen, wartwort, licorice, essential oil, terpenoids, flavonoids, tuberculosis, bacterial and fungal infection, multidrug resistance, antioxidant, immunity, tincture, suppository, aerosol, dosage form.*

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### I. INTRODUCTION

Flora of North Kazakhstan is a rich resource of biologically active substances with broad-spectrum agent. It was developed by us a medicinal products line that includes components of balsam poplar (*Populus balsamifera* L.), aspen (*Populus tremula* L.), wartwort (*Chelidonium majus* L.), licorice ural (*Glycyrrhiza uralensis* Fisch).

The basis for the creation of the medicinal products line was vegetative parts of balsam poplar. Balsam poplar is a unique composition of biologically active substances and plant vitality. Bark, buds and leaves of poplar have been used for a long time in folk medicine for inflammatory and fungus disease treatment, wound healing. Research works that were begun more than 60 years ago in Lithuania, Canada, USA, Russia, confirmed the presence of high-performance compounds. However, only Kazakhstan's developments were registered in state registration of medicines, and the medicine was put into the first production in North Kazakhstan.

According to plant taxonomy poplar belongs to the willow family. Poplar is a deciduous tree up to 40-45 m high and more than 1 m in diameter. Balsam poplar grows naturally in North America (from Labrador till 65 northern latitude). To the south it extends till the states of Colorado and Michigan. In former Soviet Union

balsamic poplar naturally grew only on Kamchatka. Compared to other poplars it has large resinous buds.

Cultivation of balsam poplar in the forest culture of Kazakhstan was begun in 1960-1965 due to its extremely high growth rate and ability to reproduce by cuttings and seeds.

There are about 1,400 ha of plantations of balsam poplar at the age of 35-50 in the North Kazakhstan region, mainly located in the Ishim River floodplain. To expand the production base it is being established new own plantations of poplars.

Aspen is widespread in the natural forests of North Kazakhstan on an area about 26,600 hectares. Industrial preparation is underway. Aspen distinguished by its columnar trunk, which is 35 m height and 1 m in diameter. It grows very fast, but has susceptible wood to diseases. The bark of young trees is smooth, light green or greenish-gray. That bark is widely used in folk medicine and has antimicrobial, anti-inflammatory, antitussive, choleric, and anthelmintic properties.

Naturally wartwort didn't grow in North Kazakhstan. About 20 years ago it was carried and cultivated for medical purposes and adapted very well to local conditions. Nowadays wartwort is widespread in forests, woodland parks and moistened lowlands.

Wartwort has been used for a long time in folk medicine due to its antibacterial, antifungal, antiviral, diuretic, antispasmodic, cholagogue, sedative, analgesic properties.

## II. CHEMICAL COMPONENTS

The study of balsam poplar was commenced by Kazakhstan scientist M.I. Goryaev in the 1950s [1]. Researches were continued in the 70s by scholars of the Institute of Botany of Academy of Sciences of Lithuanian SSR, which conducted its first study of antimicrobial activity of components of balsam poplar, and proved that this action is determined primarily by the sesquiterpene hydrocarbons and alcohols, and that the mutual influence of components increases the overall antibacterial activity [2].

In the 80s scientists of the Samara State Medical University joined the study of balsam poplar [3,4], and returning to Kazakhstan, complex researches of balsam poplar buds were initiated at the Northern Kazakhstan University (Petropavlovsk), led by Professor V.V. Polyakov [5,6,7]. Initially, both groups of researchers worked on the poplar at that time direction of separation of individual substances of natural objects, and their targeted chemical transformation. But along with the positive results there was demonstrated a strong increase in the toxicity of synthetic products [8]. The new realities after Kazakhstan's independence set new, more urgent problems: its own pharmaceutical production hardly provided 10% of the necessary health care products, which required the immediate practical research results in the form of preventive and medical products. North-Kazakhstan researches of balsam poplar were continued and deepened within the framework of cooperation with the Institute of Phytochemistry (Karaganda), initiated by the Government of Kazakhstan Republican target scientific-technical program "Development and industrial application of herbal remedies to provide medical institutions and population of the Republic with domestic medicines".

Although the experimental batches were produced at the Institute of Phytochemistry, they have not appeared in the medical institutions of the Republic yet. There was a similar situation in Russia, where research developments have received scientific recognition, but so far not taking shape into actual production and commercially produced medications.

Since year 2002, a team of scientists from Almaty and Petropavlovsk has been developing medications based on biologically active substances of balsam poplar. As a result there was developed a complex processing technology, were registered and implemented into industrial production three medicines of antimicrobial, antiseptic and anti-inflammatory action in Kazakhstan.

The chemical constitution of medications of vegetative parts of balsam poplar buds was studied in detail. Studies conducted in the Siberian State Technological University (Krasnoyarsk) found that the major components are extractives, which accounts for up to 52%, of which 12% are volatile components [9]. As part of water-extractable there was revealed the presence of substances with the reducing ability (up to 8%), tannins (up to 6%), substances of protein nature (up to 1%). The remaining mass is for starch, pectin, pigments and phenolics. Spirit-soluble substances are 80% lipid substances. The share of polysaccharides is up to 17%, of which more than 50% is cellulose. As part of the mineral components there is the presence of micro- and macro-elements (S, N, P, Na, K, Ca, Mg, Fe, Cu, Zn).

Chemical constitution of extractives of balsam poplar was studied in detail by Academician S.M. Adekenov (Institute of Phytochemistry, Karaganda) and Professor V.V. Polyakov [7].

Table 1 : Chemical constitution of extractives of balsam poplar

<i>Component</i>	<i>Content, %</i>
<b>1. Organic acids (2,5%)</b>	
Glycolic acid	38,90
Pyruvic acid	1,12
Oxalic acid	0,54
Malonic acid	1,19
Succinic acid	1,08
Glutaric acid	1,10
Fumaric acid	2,26
<b>Continuation of the table 1</b>	
Adipic acid	7,40
Salicylic acid	3,25
Oxaloacetic acid	0,17
Malic acid	28,50
Ketoglutaric acid	1,14
Aconitic acid	0,38
Tartaric acid	0,83
Citric and isocitric acid, etc. (total 21)	4,60
<b>2. Fatty acids (70%)</b>	
Lauroleic acid	5,0
Lauric acid	2,9
Stearic acid	22,7
Palmitic acid	17,5
Oleic acid	21,6
Margarine acid	9,6
Myristinic acid	4,6
Linoleic acid	4,9
Linolenic acid	3,3
Arachidonic acid	2,0
And others. (total 17)	
<b>3. Carbohydrates (19%)</b>	
Ribose	20,0
Glucose	45,0
Fructose	20,0

4. Polyphenols (6,78%) Total 14 compounds of plant polyphenols: pinostrobin, pinocembrin, chrysin, tektohrizin, apigenin, kaempferol, quercetin, myricetin, galangin, 5,5,3',4-tetrahydroxy, -7-methoxyflavone, 2,6-dihydroxy -4'-methoxychalcone and etc.	
5. Microelements, mg/kg	
Zinc	52,00
Manganese	8,25
Copper	6,17
Cobalt	0,015
Iodine	1058,00
6. Amino acids: asparagine, alanine, valine, leucine	
7. Vitamins: A, C, P	
8. Prostaglandins: E <sub>1</sub> , E <sub>2</sub>	
9. Phenolic glycoside: populin, salicin	
10. Phenol acids: gallic acid, caffeic acid, ferulic acid, cinnamic acid	
11. sterols, mono-, diglycerides	

Composition and physicochemical characteristics of essential oils strongly depends on the method of its production. According of [82] essential oil was received from dry buds of spring collection with 0,5% output, transparent, yellowish color, with blurred, sweetish smell, rather bitter taste, density 0,76 g/sm<sup>3</sup>, n<sub>D</sub><sup>20</sup> =1,3715. Component composition of essential oils has more than 75 compounds. Among 30 identified terpenoid compounds prevail: nerolidol (21,4%), germacrene D (11,4%), farnesol EE (5,8%), sabinol (5,4%), β-bisabolol (4,7%), β-santalol (3,4%), trans-isoeugenol (4,7%).

A study [9] of essential oils showed that the main group is a group of sesquiterpene hydrocarbons that is 88% of buds, monoterpenes - up to 11%, diterpenes - up to 1%. Prevalent components are α-murolen, caryophyllene and gumulen, the total content of which is not less than 70% in average during the year.

According data [10] of chromatography-mass spectrometric research, the basic components are γ-turmeric, α-amorphous, α-bisabolol, α-eudesmol, β-eudesmol, γ-eudesmol, 2-phenylethyl-2-methylbutanoate.

Chemical structure of aspen crust is more studied in research [11, 12].

The main components of ethanolic extracts of aspen crust are hydroxyl - and carboxy containing compounds: about 100 aromatic compounds, relating to different classes of organic substances (acids, aldehydes, ketones, ester and ether), including 9 flavonoids, phenolic glycosides (salicin, populin), glycosides of cinnamon

and hydroxy-methoxy-phenyl spirits. Phenol compounds have intense pharmacological effect.

Chemical structure of celandine is showed in research [13, 14]. It contains alkaloids: coptisine, homochelidonine, chelerythrine, sanguinarine, protopine (over 20 alkaloids), a lot of ascorbic acids (up to 1000 mg%), carotin, flavonoids, saponins, bitter stuff, organic acids: chelidonic acid, malic acid, malonic acid, citric and succinic acids, resinous substance.

Licorice root– officinal preparation, chemical structure is close studied.

### III. TECHNOLOGICAL RESEARCH

It was determined the dynamics of changes in various indices of herbal raw material - the vegetative part of the balsam poplar - and the dependence of the terpenoid and flavonoid fraction from storage conditions.

The first batch of raw materials was kept in natural conditions at temperature from (-12)°C to (-5)°C. The second - at 0°C in a refrigerator. The third - at 10°C. Fourth - at room temperature 18-20°C. The data is presented in Figure 1.

Previously developed [7, 8, 15, 16] technology of obtaining biologically active substances from vegetative parts of balsam poplar are shown schematically in Figure 2

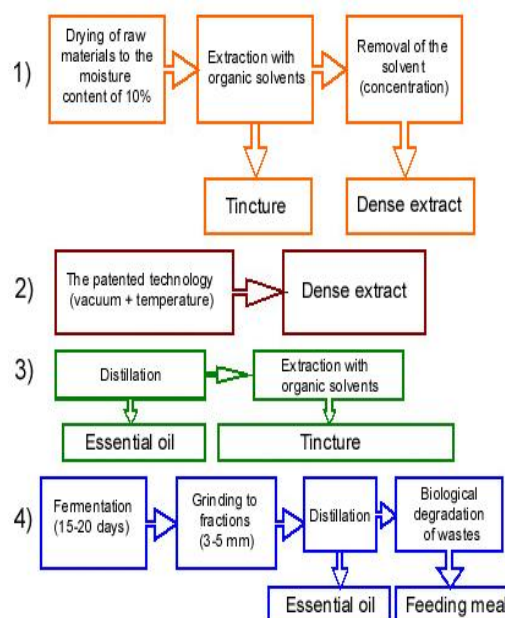


Figure 2. Four existing schemes of separation of biologically active substances from vegetative parts of balsam poplar

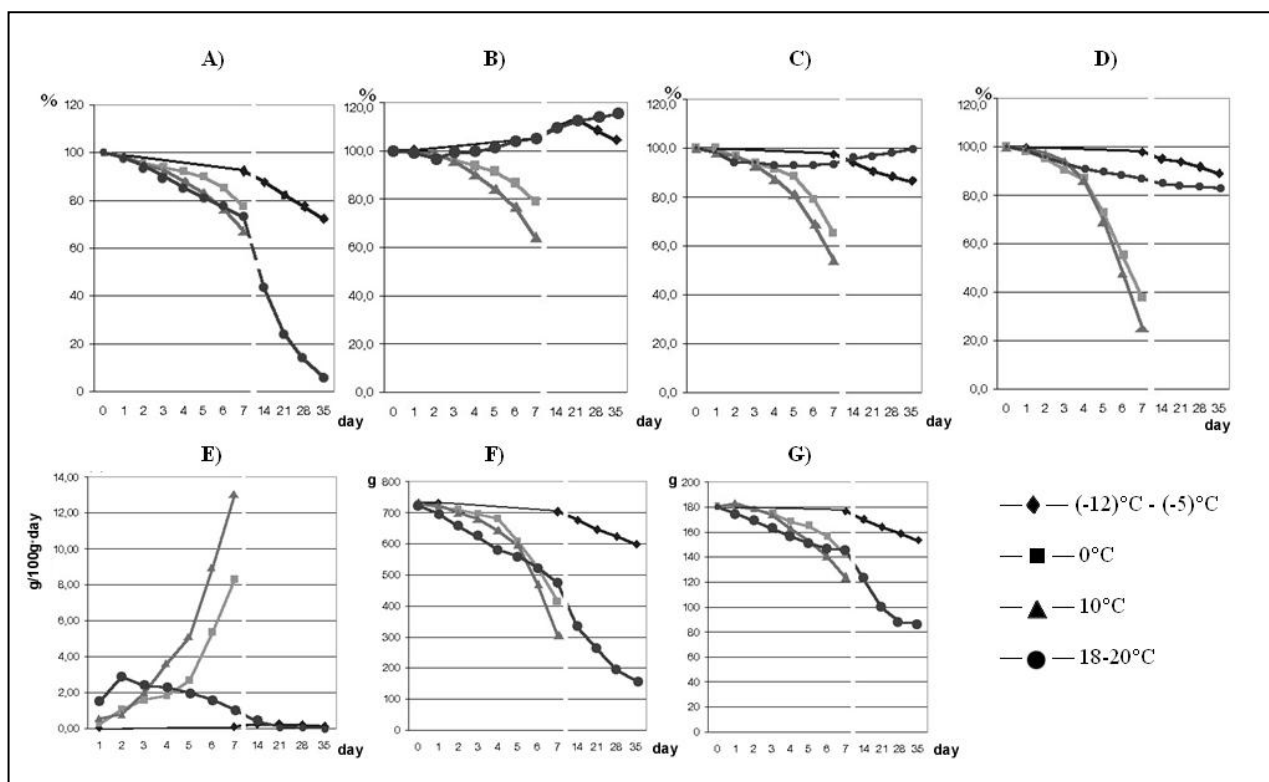


Figure 1 : Dynamics of the content in the vegetative parts of poplar: a) water, B) essential oils, C) extractives, D) of flavonoids (% of control) and changes E) intensity of respiration, F) output of adipose flavonoid substance, G) output of terpenoid fractions depending on the storage of raw materials

It was developed and patented a new method of processing. Owing to this method it was suggested the technology of obtaining the maximum range of products with the best performance and minimal production cost

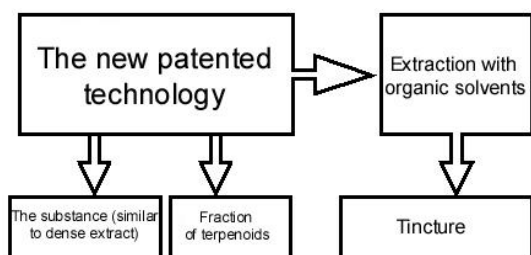


Figure 3. The scheme of the proposed technology of selection of biologically active substances from vegetative parts of balsam poplar

The derived substance in comparison with the dense extract contains 1.5 times more active substances - flavonoids (more than 7% flavonoids in recalculation on quercetin), and doesn't contain ballast materials (resin, wax). The antiseptic tincture and suppositories on the base of this substance were developed.

Qualitative composition and physico-chemical properties of the selected fraction of terpenoids differ from the essential oil obtained by conventional methods due to the peculiarities of the process.

It contains hydrocarbons (make up about 70%) and consists mainly of acyclic (Farnese) and monocyclic (type of bisabolana and type of gumulana) sesquiterpenes. In significant amounts there are also present sesquiterpene alcohols – farnesol (up to 12%) and bisabolol (up to 10%).

#### IV. PHARMACOLOGICAL RESEARCH

Assessment of the microbiological activity of remedies and clinical tests was carried out on the basis of Institute of Traumatology and Orthopedics (Astana) and the National Center for Tuberculosis Problems (Almaty) as for Mycobacterium tuberculosis, Staphylococcus aureus, haemoliticus, epidermis, Streptococcus faecalis, pneumoniae, pyogenes, Enterobacteriaceae faecalis, hafniae, aerogenes, Pseudomonas aeruginosa, Peptococcus spp., mirabilis, vulgaris, Haemophilus influenzae, Branchicus

catarrhalis, Klebsigella pneumoniae, and also Candida fungus albicans [17-23].

The investigated culture were highly resistant to traditionally used antibiotics: benzylpenicillin (100%), ampicillin (77.4%), carbenicillin (96.9%), streptomycin (50.7%), kanamycin (69.4%), neomycin (93.1%), monomicin (79.3%), gentamicin (42.5%), tetracycline (53.0%), erythromycin (71.7%), lincomycin (87.8%), polymyxin (57, 5%), novobiocin (54.5%), cefixime (65.4%), cephalixin (57.8%), cephalothin (54.8%), Tsefamandol (59.9%), cefuroxime (51.4%), cefotaxime (55.9%), ceftazidime (57.3%), izoniazidum (63,9%), etambutolum (58,7%), rifadin (42,4%).

The use of remedies caused a more marked reduction of bacterial flora, particularly against gram-positive bacteria, and reduced pathogenic potential, as of gram-positive and gram-negative bacteria, which was consisted in loss of hemolytic, phospholipase, plazma-coagulase activities. In addition, treatment with remedies increased efficacy of antibiotic-resistant microorganisms by 20-40%.

Study of toxicity (acute and chronic), antitumor activity was carried out on intact animals, using different strains of mice and rats. There was shown that substances are slightly toxic. By single subcutaneous injection of LD50 for mice 4.8-6ml / 20g of body weight, for rats 8-15ml / 100 g of body weight. Chronic toxicity by daily intraperitoneal injection during 10 days 0.6-1.5 ml / 100 g of body weight of rats and 0.4-0.6 g of body weight of mice was observed. Allergenicity, teratogenicity, mutagenicity, reproductive and genotoxicity haven't been identified.

The study of pharmacodynamics has also shown that remedies cut short inflammatory processes, significantly reduce the exudative and hemorrhagic manifestations, enhance regenerative processes by stimulating proliferative activity of epithelial cells, causes marked fibroplastic reaction of communicating structures. Through the action of antioxidant components there is reduced the number of products of peroxidation of lipids in blood and there is increased own antioxidant potential of its tissues. It is established that the high antioxidant activity is not inferior to the synthetic antioxidant ionol and is based on the presence of polyphenolic compounds. Along with this, statistically significant positive changes in immunological status had been noted. The study on experimental animals of morphological changes in the nasal mucosa in experimental rhinitis showed anti-inflammatory and regenerative properties of the remedies, expressed in reduction of swelling, congestion, as well as accelerated epithelialization, which is close to the primary intention.

## V. RESULTS OF CLINICAL TESTS

Antiseptic tincture is used in the treatment of septic wounds, in the topical treatment of burns and frostbite of extremities, and inflammatory skin diseases [24, 25].

Local treatment of inflammations and I-II degree burns is implemented by the adjustment of sterile wet-drying, dressing soaked in antiseptic saline infusions (1:20) once per day. In the treatment of festering wounds, II-III degree burns, postoperative sutures it is applied sterile gauze wet-drying towels soaked in freshly prepared 2% antiseptic solution in saline infusions (1:5) until complete epithelialization.

In addition to direct local effects on the skin surface antiseptic tincture forms a protective film that prevents re-infection.

It is noted the decrease of the intensity of pain at the end of the first day, the normalization of the index of intoxication for 4-5 days, a decrease in the first three days of bacterial contamination of examined medium from  $10^8$  to  $10^3$ . Treatment time is reduced by 20-30%, the wounds heal by first intention - the scar tissue is soft, flexible, mobile. With the glance of observed results it is examined the effectiveness of infusion in plastic surgery.

Suppositories are used for the topical treatment of inflammatory gynecological and urological diseases of different etiologies, adenoid tumor, hemorrhoids. The drug is safe and can be used for the treatment and sanitization of the birth canals up to the III trimester of pregnancy inclusive.

During the studies it was dedicated 1862 strains belonging to 23 species of microorganisms dominated by Staphylococcus, Echerichia, Enterococcus, Streptococcus. Inflammatory processes were stopped by the end of the first day. On the fifth day of 90% of cases there was complete clearance of pathogens. It was noticed a positive vascular response, the acceleration of regenerative processes [26].

The terpenoid's substance uses as remedy and effectively fights all forms of lung tuberculosis pathogens, removes bacterial and fungal infection, helps to improve immunity, and forces the bronchus cure. The usage of the remedy in complex with traditional antibiotic tuberculosis therapy forces the treatment, shortens the time of being in hospitals, the quantity of relapse. Thus, it helps to decrease the wastes on treatment and rehabilitation [27].

In Kazakhstan: ill tuberculosis – 175 thousands, get ill every year – 15 thousands, die every year – 2-2,5 thousands. The main problem in lung tuberculosis treatment is appearance of multidrug resistant tuberculosis forms which are difficult to cure or cannot

be cured by traditional therapy. 20% ill people in Kazakhstan are infected with multidrug resistant tuberculosis.

In clinical trials the drug used by inhalation (with a help of nebulizer) in combination with chemotherapy in a category.

As a result of treatment, 90% of patients had an accelerated healing of specific and nonspecific changes in the larynx and bronchi.

Analysis of the period of cessation of bacterioexcretion showed that by the end of intensive phase therapy 93.3% of patients had a negative reaction of the smear.

After inhalation patients hadn't in their sputum previously determined the secondary flora in the form of *Streptococcus faecalis* and *pneumoniae*, *Staphylococcus aureus* and *haemoliticus*, *Haemoph. Influenzae*, *Haemophilus influenzae*, *Branchicus catarrhalis*, *Klebsigella pneumoniae*.

As a result of biochemical and immunological research there was found a decline in serum of patients with lipid peroxidation products (LPO): of malondialdehyde (MDA) to  $1,15 \pm 0,1$  mcmol / ml to  $0,85 \pm 0,08$  mcmol / ml and diene conjugates (DC) from  $3,14 \pm 0,2$  mcmol / ml to  $2,5 \pm 0,2$  mcmol / ml. Along with this, positive changes in immunological status had been noted in the dynamics: reduction of antituberculous somas from  $0,815 \pm 0,040$  units to  $0,653 \pm 0,02$  units and the index ratio of subpopulations of Th / Ts, with  $2,8 \pm 0,02$  to  $2,2 \pm 0,01$ , other indicators had positive unreliable dynamics.

As a result of tests achieved decrease time of hospital stay in 5-10% and quantity of backset in 3 times

## VI. PROSPECTIVE RESEARCH

Department of Education and Science of Kazakhstan allocated grant for development on its base aerosol form for prevention of tuberculosis bacterioexcretion; for development medicine to treat accompany to tuberculosis pathogenic flora.

In most cases there is necessity in a long expensive clinical treatment of pulmonary tuberculosis. In the period of active bacterioexcretion the staff of clinical units, family members and other citizens, one way or another coming into contact with the patient are be in danger of being infected. Development of an aerosol form of medication, sanitating oral cavity and bronchus, and having a prolonged effect, would reduce these risks to a minimum, and due to the security of ethereal oils it will be used as a prophylactic medication for healthy people falling under risk. Thus, the medicine is being

developed on the basis of an aerosol terpenoid substance.

Pulmonary tuberculosis in most cases is complicated by assident mycetology and bacterial flora. This factor has a large impact on the effectiveness of antibiotic therapy. Often assident flora reduces the effective dosage of antibiotics in the body of the patient, as a result of this the process of medical treatment becomes more difficult, resistance of Koch's bacillus to the used antibiotics can be developed. Development of effective medication, concomitant or prior to the use of antituberculous antibiotics will significantly enhance the strength of treatment.

To date, it is developed composition of the dosage form (syrup), which includes wartwort alkaloids, phenolic compounds of aspen, an extract of licorice. The usage of syrup with a glance to dosages is safe for children and adults, except pregnant and lactating women and patients with ulcer disease of the gastrointestinal tract.

Matched structure allows someone quickly to stop the inflammation, to kill pathogenic flora, to clear the airways from mucus that along with a mild analgesic effect, with its usability and well-chosen organoleptic properties opens broad prospects for its use. Currently it is made clinical tests.

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