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Результаты изучения фармакологической активности основных сесквитерпеновых лактонов листьев *Лавра Благородного*

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Аннотация. Введение. *Цель. Изучение актуальных видов фармакологической активностиизвлечений и основных сесквитерпеновых лактонов из листьев лавра благородного.* ***Материалы и методы*** *В работе использованы образцы листьев лавра благородного, собранные в южных регионах Российской Федерации в основные фазы роста и развития растений. Также использовались образцы листьев лавра, приобретённые в торговой сети от различных производителей, имевшие соответствующие сертификаты качества и безопасности. Исследование токсичности и противодиабетической активности проводили по известным методикам. Исследование противотуберкулёзной активности осуществлялось нитратредуктазным методом.* ***Результаты*** *Эфирное масло, сесквитерпеновые лактоны (особенно дегидрокостуслактон) в сравнении с известными противотуберкулёзными препаратами проявляют выраженную активность, в отношении клинических штаммов туберкулёза.Сравнение эффективности курсового применения разработанного сбора с листьями лавра благородного по отношению к сбору прототипу (без листьев лавра), показалочто исследуемый антидиабетический сбор позволяет снизить уровень глюкозыв крови (до 7,6±0,15 ммоль/л в сравнении с 11,2±0,8 ммоль/л при использовании сбора прототипа и с 24,5±2,3 ммоль/л у интактных животных), а также нормализовать показатели общего холестерина, β-липопротеидов и инсулина.* ***Заключение.*** *Эфирное масло и сесквитерпеновые лактоны проявляют противотуберкулезную активность, в отношении исследованных клинических штаммов. Разработанный авторами сбор (с листьями лавра благородного) обладает гипогликемической активностью.*

Ключевые слова: листья лавра благородного, гипогликемическая активность, противотуберкулёзная активность

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Research article

Results of studying the pharmacological activity of the main sesquiterpene lactones of *Laurus Nobilis Leaves*

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Abstract. Introduction. *The aim. To study the actual types of pharmacological activity of extracts and basic sesquiterpene lactones from the LaurusnobilisL. leaves of the noble laurel.* ***Materials and methods.****The work uses samples of Laurusnobilisleaves collected in the southern regions of the Russian Federation during the main phases of plant growth and development. We also used samples of Laurusnobilisleaves purchased in the retail chain from various manufacturers, which had the appropriate quality and safety certificates. The study of toxicity and antidiabetic activity was carried out using well-known methods. The study of antitubercular activity was carried out by the nitrate reductase method.* ***Results and discussion.*** *Essential oil, sesquiterpene lactones (especially dehydrocostuslactone), in comparison with known antituberculosis drugs, show pronounced activity against clinical strains of tuberculosis. A comparison of the effectiveness of the course application of the developed collection with Laurusnobilisleaves in relation to the prototype collection (without laurel leaves) showed that the studied antidiabetic collection reduces blood glucose levels (to 7.6±0.15 mmol/L compared with 11.2 ±0.8 mmol/L when using the prototype collection and from 24.5±2.3 mmol/l in intact animals), as well as normalize the indicators of total cholesterol, β-lipoproteins and insulin.* ***Conclusion.*** *Essential oil and sesquiterpene lactones exhibit anti-tuberculosis activity against the studied clinical strains. The collection developed by the authors (with leaves of noble laurel) has hypoglycemic activity.*

Keywords: leaves of *Laurusnobilis L.,* hypoglycemic activity, antituberculosis activity

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Introduction. There is an increasing demand for environmentally friendly natural products and herbal medicines (HM) for the prevention and treatment of various diseases. The main method of treatment in traditional medicine is the use of HM. They are an indispensable means of correcting the body's condition with historically proven effectiveness and safety. Laurus nobilis leaves, as follows from scientific literature and the results of our research, contain sesquiterpene lactones. To date, more than 30 compounds of this class have been isolated and identified from this raw material.

The fruits and leaves of the noble laurel (*Laurus nobilis* L.) are used in folk medicine in different countries for rheumatism, dermatitis, viral infections, as an antiseptic, diuretic, hypoglycemic, antibacterial, anti-inflammatory and gastric secretion stimulant, for diarrhea, hysteria, as an antidote for snake bites. The leaves of the noble laurel contain sesquiterpene lactones. These compounds have shown various pharmacological activities in experiments: antibacterial, antifungal, anti-inflammatory (inhibit the production of nitric oxide), hepatoprotective, neuroprotective [1].

According to scientific literature, the sesquiterpene lactones costunolide and zaluzanin D are responsible for the observed in experiments *in in vitro* and *in vivo* antitumor activity. They have a strong growth inhibitory effect on human myeloid leukemia cells (HL -60) and cause apoptosis. It is known that the α-methylene-γ-butyrolactone part of sesquiterpene lactones is important for the manifestation of cytotoxic (antitumor) activity. Costunolide also exhibited a gastroprotective effect. The ability of the main sesquiterpene lactones from laurel leaves to inhibit the increase in ethanol content in the blood was discovered [2]. It is also known that laurel leaf extract significantly reduced blood glucose levels and improved the regeneration of pancreatic β-cell islets in animals. It also had a positive effect on liver enzymes (aspartate aminotransferase, γ-glutamyl transferase and alanine aminotransferase), urea, creatine kinase, total protein, calcium and iron levels, restoring them to almost normal values [1, 2].

It has been established that the populations of Mycobacterium tuberculosis (MBT) existing during the infection are physiologically heterogeneous and therapeutic drugs used to slow down the glycolytic phase cannot affect the slow phase and the growth-dead phase, which prevails in lung granulomas, causing the need for long-term therapy. In this regard, the search for new substances with anti-tuberculosis activity that can reduce the rate of development of drug resistance and are capable of affecting different stages of the disease is relevant. In recent years, researchers have again turned to products of natural origin. This is due not only to the problem of multiple drug resistance, but also to the search for new leading substances in a number of poorly studied classes of natural compounds. Among such promising classes, sesquiterpene lactones [3–5], terpene, phenolic and polyacetylene compounds [6–9] have been widely studied in the scientific literature in recent years.

As follows from the results of the study of the leaves of the noble laurel, sesquiterpene lactones exhibit a diverse spectrum of pharmacological activity. However, it can already be stated that the antibacterial (in a broad sense), anti-inflammatory, antidiabetic and cytotoxic activities found in extracts and individual compounds from the leaves of the noble laurel are promising areas of research for the development of new drugs.

The aim of the study was to investigate the current types of pharmacological activity (anti-tuberculosis, anti-diabetic) of extracts and the main sesquiterpene lactones from the leaves of noble laurel of Russian origin.

Materials and research methods. The work used samples of leaves of the noble laurel, collected in the Krasnodar Territory (the environs of the city of Gelendzhik, the city of Khosta), the Republic of Crimea (the environs of the city of Alushta, the city of Yalta, the urban-type settlement of Nikita, the Nikitsky Botanical Garden) in the main phases of growth and development of plants . In addition, the study used samples of laurel leaves purchased in the retail network from various domestic manufacturers that had the appropriate quality and safety certificates. The objects of our study were: aqueous extract, essential oil and sesquiterpene lactones (costunolide, dehydrocostuslactone), previously isolated from the leaves of the noble laurel.

Determination of sensitivity of mycobacterium tuberculosis (MBT) to official anti-tuberculosis drugs and to samples of essential oil, sesquiterpene lactones (costunolide, dehydrocostuslactone) of noble laurel by the nitrate reductase method developed in the Central Research Institute of Tuberculosis of the Russian Academy of Sciences. The method is based on the use of nitrate reductase reaction for early detection of MBT. For this purpose, sodium nitrate is introduced at the rate of 1 g/l together with drugs before coagulation during preparation of a dense nutrient medium. The method is used for *M. tuberculosis strains* with nitrate reductase activity. Detection of nitrate reductase reaction was carried out using standard Griess reagent (7.5% aqueous solution). Nitrate reductase activity was determined by the amount of sodium reduced from nitrate, giving a color reaction with the Griess reagent, which is a criterion for MBT growth. The study was conducted for 12 days [10]. For the determination, the Lowenstein-Jensen medium was used, to which anti-tuberculosis drugs and essential oil from laurel leaves were added

before coagulation in the threshold concentrations described below, inhibiting the growth of MBT strains [10]. The comparison drugs and the studied compounds were used in the following concentrations: streptomycin - 10 mg / ml (1); isoniazid - 2 mg / ml (2); kanamycin - 45 mg / ml (3); rifampicin - 20 mg / ml (4); ethambutol - 7.5 mg / ml (5); prothionamide - 30 mg / ml (6); essential oil of laurel - 20 mg / ml (7); costunolide - 5 mg / ml (8); dehydrocostus lactone - 5 mg / ml (9).

The hypoglycemic activity was studied using a previously developed collection containing the herb of common galega (goat's rue) - 11.8%, roots of medicinal dandelion - 11.8%, flowers of small-leaved linden - 5.6%, leaves of white mulberry - 11.8%, roots of high elecampane - 11.8%, leaves of common lingonberry - 11.8%, valves of fruits of common bean - 11.8%, leaves of noble laurel - 11.8%, herb of drooping bidens - 11.8%. A patent of the Russian Federation was received for this collection [11].

Pharmacological studies of this collection were conducted on the model of experimental diabetes mellitus, which was carried out by intraperitoneal administration of alloxan solution at a dose of 25 mg/100 g of animal weight to 60 male Wistar rats weighing 180-200 g. Then the animals were divided into 3 groups of 20 rats each. The first group consisted of control animals that received only alloxan; 20 rats of the second group, with alloxan diabetes, were treated with the studied collection for 6 days. In the 3rd comparison group, rats with alloxan diabetes were treated with the prototype collection for 6 days. The 4th group included 10 intact animals.

The prototype collection (antidiabetic collection) was used in the studies, including the valves of the common bean fruit, the leaves of the white mulberry, the walnut, the common lingonberry, the lesser periwinkle, the herb of the common goat's rue (galega), the knotweed (knotweed), the common agrimony, the roots of the common dandelion, the high elecampane, the inflorescences of the scepter-shaped mullein, the flowers of the small-leaved linden (A.s. No. 2430735 dated 30.12.2009). The collection is recommended as a hypoglycemic agent for the prevention and treatment of mild to moderate type 2 diabetes.

The infusion (water extract) was administered to the animals of the 2nd group orally through a tube in a volume of 0.5 ml per 100 g of body weight of the experimental animals for 6 days, 2 times a day before the administration of alloxan, and in the next 4 days after the administration of alloxan. The composition of the prototype, which was administered to the animals of the third group according to a similar scheme, was used as a comparison drug. On the 11th day, the animals were decapitated, followed by laboratory tests. Metabolic changes after the action of the proposed collection on animals with the alloxan diabetes model were studied by the content of total cholesterol (C) in (mmol / l), low-density lipoprotein cholesterol (LDL) and very low-density lipoprotein cholesterol (VLDL) in (mmol / l), triglycerides (TG) in (mmol / l) and glucose (GL) in (mmol / l) in the blood serum. The glucose level in the blood serum of all groups of animals was analyzed on the 4th day after the administration of alloxan.

Research results and their discussion.

Study of anti-tuberculosis activity of essential oil and individual sesquiterpene lactones of bay laurel leaves

Table 1 – Results of tests of essential oil and sesquiterpene lactones of bay laurel in comparison with standard preparations (1-6) in relation to MBT strains

MBT strain code	1	2	3	4	5	6	7	8	9
2425	+	–	+++	++	–	++	–	–	–
2314	+	+++	+++	++	+++	+++	+++	+	+
2280	+	++	–	+	–	–	+	–	–
1830	+	+++	++	+++	+++	+++	++	++	+
2354	+	–	+++	++	+++	+	+	+	–

2324	+	+++	+++	++	++	+++	+++	—	+
1974	+	—	—	—	—	—	++	+	—
3049	+	+++	—	—	+	+++	++	+	++
1929	+	—	—	—	++	—	++	+	—
2153	+	—	+	+++	+++	+	+	+	—

Note: " **—" –** no growth; " +" – poor growth; "++" – moderate growth; "+++" – massive growth.

Culture growth on media with medicinal preparations and the test substance means resistance to this preparation. Decrease (absence) of growth – sensitivity is preserved, i.e. the preparation is effective. Thus, essential oil and sesquiterpene lactones (costunolide, dehydrocostuslactone) exhibit anti-tuberculosis activity with respect to the studied strains. The highest activity on the studied strains of *M. tuberculosis was* demonstrated by the sesquiterpene lactone dehydrocostuslactone.

Study of hypoglycemic activity of a collection using bay laurel leaves.

In intact animals, the blood glucose level was 5.6±0.09 mmol/l, in control animals with the alloxan diabetes model, on the 4th day after modeling, the glucose level increased to 24.5±2.3 mmol/l. In the experimental group of animals that received the infusion of the proposed collection according to the specified scheme, the glucose level was 7.6±0.15 mmol/l. In the group that received the prototype collection composition, the glucose level was determined as 11.2±0.8 mmol/l (Table 2).

Table 2 – Effect of the studied antidiabetic collection and the prototype collection on the blood glucose level of rats with experimental alloxan diabetes

Groups	Serum glucose level (mmol/l)	The rate of increase in glucose levels in blood serum relative to the initial level (in× once)
Intact	5.6±0.02	-
Control (alloxan)	24.5±0.27	4.4
Experience (alloxan + test collection)	7.6±0.15 P <0.001 *p* * <0.001	1.4
Comparison (alloxan+collection prototype)	11.2±0.8	2.0

Note: *p* – significance of differences relative to control; *p* * – significance of differences relative to prototype collection.

Thus, the studied collection had the ability to significantly reduce the level of glucose in the blood of rats with experimental alloxan diabetes, and this effect statistically significantly exceeded the hypoglycemic effect of the comparison drug - the prototype collection.

Effect of the studied collection and the prototype collection on the concentration of cholesterol in the blood of rats with experimental alloxan diabetes

The experiments showed that in the control animals on the 4th day after the introduction of alloxan, the cholesterol level increased to 5.12±0.05 mmol/l. In the experimental group of animals that received the infusion of the studied collection according to the specified scheme, the cholesterol level was 2.005±0.074 mmol/l. In the group that received the prototype collection, the cholesterol level was 2.961±0.082 mmol/l. The experimental data are presented in Table 3.

Table 3 – Effect of the studied collection and the prototype collection on the concentration of cholesterol in the blood of rats with experimental alloxan diabetes

Groups	Cholesterol (mmol/l)	% reduction
Control (alloxan)	5.12±0.05	-

Experience (alloxan + test collection)	2.005±0.074 p <0.001 p* <0.001	2.55 times
Comparison (alloxan+collection prototype)	2.961 ±0.082 p <0.001	1.73 times

Note: p – significance of differences relative to control; p * – significance of differences relative to prototype collection.

The experiments showed that in the control animals on the 4th day after the administration of alloxan, the level of β-lipoproteins LDL and VLDL increased to 5.97±0.011 mmol/l. In the experimental group of animals that received the infusion of the studied collection according to the specified scheme, the level of LDL and VLDL was 1.05±0.009 mmol/l. In the group that received the infusion of the prototype collection, the level of LDL and VLDL was 1.35±0.01 mmol/l (Table 4).

Table 4 – Effect of the studied collection and the prototype collection on the concentration of β- and pre-β-lipoproteins in the blood of rats with experimental alloxan diabetes

Groups	β -lipoproteins	% reduction
Control (alloxan)	5.97±0.025	-
Experience (alloxan + test collection)	1.05±0.009 p <0.001 p* <0.001	5.7 times
Comparisons (alloxan + prototype collection)	1.35±0.01 p <0.001	4.4 times

Note: p – significance of differences relative to control; p * – significance of differences relative to prototype collection.

A study of the comparative effectiveness of the course application of this collection in relation to a prototype collection of a similar composition, which has a hypoglycemic effect in the treatment and prevention of mild and moderate diabetes mellitus, showed that the studied antidiabetic collection allows to reduce the level of glucose in the blood, and also has the effect of normalizing the indicators of total cholesterol, β-lipoproteins and insulin, and may be promising from the point of view of studying the possibility of its use in the treatment of diabetes mellitus.

Conclusion. The results of the study of acute toxicity of the aqueous extract and the sum of sesquiterpene lactones of the leaves of laurel allow us to characterize them as "practically non-toxic", and the essential oil as "low-toxic" substances. Essential oil and sesquiterpene lactones (especially dehydrocostus lactone) of the leaves of laurel exhibit anti-tuberculosis activity in relation to the studied clinical strains. The collection developed by us (with leaves of laurel) has the ability to significantly reduce the level of glucose in the blood of rats with experimental alloxan diabetes, and this effect statistically significantly exceeds the hypoglycemic effect of the comparison drug - the prototype collection.

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