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ANTIMICROBIAL ACTIVITY OF WATER-ETHANOLIC EXTRACTIONS FROM *QUERCUS ROBUR* L. LEAVES AND BUDS

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The problem of finding new antimicrobial drugs based on medicinal plant raw materials in modern pharmaceutical practice, is still relevant. There are interesting plant objects that have an antimicrobial action due to the content of a complex of biologically active substances in them. *Quercus robur* L. is a promising plant object, medicinal plant raw materials of which can be used for the development of new antimicrobial drugs.

The aim of the study is screening of the antimicrobial activity of water-ethanolic extractions from *Quercus robur* L. leaves and buds.

Materials and methods. The determination of the minimum inhibitory concentration was carried out by the method of double serial dilutions in Mueller-Hinton nutrient broth (Bio-Rad, USA). As test cultures, strains of microorganisms of the American Type Culture Collection (ATCC) were used: *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), as well as *Candida albicans* (a clinical strain). The incubation was carried out at the temperature of 35°C for 24 hours. Simultaneously, an experiment was carried out to establish a "negative" control. The results were assessed visually by the presence / absence of the growth of microorganisms in test tubes with the corresponding dilutions of the test samples.

Results. In the course of the study, it was found out that-water-ethanolic extractions of *Quercus robur* L. leaves have the greatest antimicrobial effect against strains of *Staphylococcus aureus* and *Escherichia coli*. The water-ethanolic extractions of *Quercus robur* L. buds exhibit a pronounced antimicrobial activity against *Pseudomonas aeruginosa* and *Candida albicans* strains.

It was revealed that the preparation of *Quercus robur* L. leaves tincture in the raw material:extractant ratio of 1:5 has a pronounced antimicrobial effect on the strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and with a higher multiplicity of dilution – on the strains of *Escherichia coli* and *Candida albicans*. The drug tincture of *Quercus robur* L. buds in the raw material:extractant ratio of 1:5 has a pronounced antimicrobial effect on the strains of microorganisms *P. aeruginosa, S. aureus, E. coli* and *C. albicans* in an eight-fold dilution. With respect to *P. aeruginosa* strains, antimicrobial activity was observed in 16-fold dilutions. The most pronounced antimicrobial effect was recorded against the *C. albicans* strain in a 32-fold dilution.

As a result of the study, it can be concluded that to obtain the antimicrobial drugs – tincture of *Quercus robur* L. leaves and buds – is advisable to use the optimal extractant – 70% alcohol in a raw material:extractant ratio of 1:5. With these parameters of extraction, the greatest antimicrobial effect is observed in relation to the studied strains of the microorganisms. 70% alcohol has also a better penetrating ability into the deep layers of the epidermis in comparison with higher concentrations.

Conclusion. The results of the screening analysis of the antimicrobial activity will be used as a justification for the introduction of antimicrobial drugs based on the leaves and buds of the *Quercus robur* L. in a medical and pharmaceutical practice.

Key words: English oak; *Quercus robur* L.; leaves; buds; water-ethanolic extractions; tincture; minimum inhibitory concentration; antimicrobial activity

List of abbreviations: ATCC – American Type Culture Collection; MRSA – Methicillin-resistant Staphylococcus aureus; CLSI – Clinical and Laboratory Standards Institute; MRS strains – Methicillin-resistant Staphylococcus; MIC – Minimum inhibitory concentration; SP RF – State Pharmacopoeia of the Russian Federation; CFU / ml – Colony forming units / ml; SMR-1547 – Index Herbarium of the Samara State University, Department for Ecology, Botany and Nature Protection Faculty of Biology; G, MUK – "Guidelines", "Methodical instructions"; *Q.* – *Quercus* L. (eg, *Q. robur*). – English oak

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АНТИМИКРОБНАЯ АКТИВНОСТЬ ВОДНО-СПИРТОВЫХ ИЗВЛЕЧЕНИЙ ЛИСТЬЕВ И ПОЧЕК ДУБА ЧЕРЕШЧАТОГО (*QUERCUS ROBUR* L.)

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Проблема поиска новых противомикробных препаратов на основе лекарственного растительного сырья в современной фармацевтической практике является по-прежнему актуальной. Интерес представляют растительные объекты, обладающие антимикробным действием благодаря содержанию в них комплекса биологически активных веществ. Дуб черешчатый – *Quercus robur* L. является перспективным растительным объектом, лекарственное растительное сырье которого может быть использовано при разработке новых антимикробных препаратов.

Цель. Проведение скрининга антимикробной активности водно-спиртовых извлечений листьев и почек дуба черешчатого.

Материалы и методы. Определение минимальной ингибирующей концентрации проводилось методом двойных серийных разведений в питательном бульоне Мюллера-Хинтона (Bio-Rad, США). В качестве тестовых культур были использованы штаммы микроорганизмов Американской коллекции типовых культур (АТСС): *Staphylococcus aureus* (АТСС 29213), *Escherichia coli* (АТСС 25922), *Pseudomonas aeruginosa* (АТСС 27853), а также *Candida albicans* (клинический штамм). Инкубацию проводили при температуре 35°С в течение 24 часов. Параллельно проводился опыт для постановки «отрицательного» контроля. Оценку результатов проводили визуально по наличию/отсутствию роста микроорганизмов в пробирках с соответствующими разведениями исследуемых образцов.

Результаты. В ходе проведенного исследования установлено, что водно-спиртовые извлечения листьев дуба черешчатого оказывают наибольший антимикробный эффект в отношении штаммов *Staphylococcus aureus* и *Escherichia coli.* Водно-спиртовые извлечения почек дуба черешчатого проявляют выраженную антимикробную активность в отношении штаммов *Pseudomonas aeruginosa* и *Candida albicans.*

Выявлено, что препарат настойка листьев дуба черешчатого в соотношении «сырье – экстрагент» (1:5) обладает выраженным антимикробным эффектом на штаммы *Pseudomonas aeruginosa, Staphylococcus aureus,* а при большей кратности разведения на штаммы *Escherichia coli* и *Candida albicans.* Препарат настойка почек дуба черешчатого в соотношении «сырье – экстрагент» (1:5) обладает выраженным антимикробным эффектом в отношении штаммов микроорганизмов *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* и *Candida albicans* при восьмикратном разведении. В отношении штаммов *Pseudomonas aeruginosa* антимикробная активность наблюдалась при 16 кратном разведении. Максимально выраженный антимикробный эффект был зафиксирован в отношении штамма *Candida albicans при 32-кратном разведении*.

В результате проведенного исследования можно сделать вывод о том, что для получения противомикробных препаратов – настойки листьев и почек дуба черешчатого, целесообразно использовать в качестве оптимального экстрагента спирт 70% в соотношении «сырье – экстрагент» (1:5). При данных параметрах экстракции отмечается наибольший антимикробный эффект в отношении изучаемых штаммов микроорганизмов. Также спирт 70% обладает лучшей проникающей способностью в глубокие слои эпидермиса по сравнению с более высокими концентрациями.

Заключение. Полученные результаты скринингового анализа антимикробной активности будут использованы в качестве обоснования для внедрения антимикробных препаратов на основе листьев и почек дуба черешчатого в медицинскую и фармацевтическую практику.

Ключевые слова: Дуб черешчатый; *Quercus robur* L.; листья; почки; водно-спиртовые извлечения; настойка; минимальная ингибирующая концентрация; антимикробная активность

Список сокращений: АТСС – Американская коллекция типовых культур (American Type Culture Collection); MRSA – Метициллинрезистентный золотистый стафилококк (Methicillin-resistant *Staphylococcus aureus*); CLSI – Clinical and Laboratory Standards Institute; MRS-штаммы – Метициллин-резистентные стафилококки (Methicillin-resistant *Staphylococcus*); МИК – Минимальная ингибирующая концентрация; ГФ РФ – Государственная Фармакопея Российской Федерации; КОЕ/мл – Колониеобразующие единицы / мл; SMR-1547 – Индекс гербария Самарского государственного университета, кафедра экологии, ботаники и охраны природы, биологического факультета; МУК – Методические указания; *Q.* – *Quercus* L. (н-р, *Q. robur*)

INTRODUCTION

Currently, obtaining new antimicrobial drugs based on plant raw materials is an urgent task of modern pharmacy. The spread of antimicrobial resistance poses a serious danger, which reduces the effectiveness of measures for the prevention and treatment of human infectious diseases [1, 2].

In terms of the search for new antimicrobial drugs, a promising object is a representative of the genus *Quercus* L. – English oak (*Q. robur* L.). In the *Quercus* L. genus, there are more than 500 species from the temperate and subtropical regions of the Northern Hemisphere. In Russia, 19 species grow in the wild, about 50 species have been introduced [3, 4]. *Quercus* L. is one of the most

important forest-forming species in Europe and the European part of Russia [3, 4]. *Q. robur* L. is rather widely used in folk medicine as a remedy for the prevention and treatment of diseases of the gastrointestinal tract, gynecological diseases, as well as for otorhinolaryngological and dermatological diseases. An official classic medicinal product of *Q. robur* L. is a decoction of the bark as an agent with astringent and anti-inflammatory properties [5–7]. Recently, a large number of investigations have been carried out to study the antimicrobial properties of *Q. robur* L. barks, as well as the preparations based on it [8–16]. *Q. robur* L. bark is a part of many complex preparations, such as «Stomatofit», «Tonsilgon N», «Dentos», etc., and it is also used to obtain extracts for medical and cosmetic purposes[1]. In addition to tannins, *Q. robur* L. bark contains triterpenes (Fridelin, Fridelinol, 3-Fridelanol), flavonoids such as quercetin, quercitrin, leukoanthocyanidin, etc. [13, 17–19]. As a group of biologically active compounds, flavonoids have a number of valuable pharmacological properties, such as anti-inflammatory, diuretic, choleretic, antispasmodic, antiviral, antimicrobial, etc.[2] [18–20]. Earlier, in order to search for new antibacterial agents, a study of *Quercus incana* species was carried out. During it, two substances were isolated: 4-hydroxydecanoic acid and 4-hydroxy-3-(hydroxymethyl) pentanoic acid. The isolated compounds were tested for the antifungal activity against *Aspergillus niger* and *Aspergillus favus*. The antibacterial activity of the isolated compounds was determined by diffusion into agar wells. The compound 4-hydroxydecanoic acid exhibited a great antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus* (gram-positive).

The obtained compounds showed an antibacterial activity against *Staphylococcus aureus* with an inhibition zone of 16 mm and 13 mm. The compound 4-hydroxy-3-(hydroxymethyl) pentanoic acid was moderately active against *Bacillus subtilis* and *Micrococcus luteus* with the inhibition zone of 5 mm and 9 mm. Both compounds were inactive against *Escherichi coli* and *Shigella fexneri* [11].

A profile of polyphenols in extracts obtained from the bark of *Q. robur*, *Q. macrocarpa* and *Q. Acutissima* was studied. As a result of it, antioxidant, antibacterial, antifungal and antitumor activities were revealed. In comparison with extracts of other species, *Q. robur* exhibited a significant antimicrobial activity against *Pseudomonas aeruginosa* [13].

A study to determine the antimicrobial activity of a *Q. robur* bark methanol extract (a solution of 80% methanol in water) was carried out. It was tested by diffusion in agar for *Staphylococcus aureus, Enterobacter aerogenes,* and *Candida albicans* strains [14]. As a result of the study, the possibility of using oak bark extracts as a bactericidal agent against *Staphylococcus aureus* strains and a bacteriostatic agent against *Enterobacter aerogenes* was determined [14]. Lipophilic extracts were active against the strains of *Staphylococcus aureus, Enterobacter aerogenes*, and *Candida albicans* [14].

In addition to water-ethanolic extractions of the *Q. robur* L. barks, water-ethanolic extractions from other types of this plant raw materials such as oak leaves and buds, are of interest. At the moment, a sufficient number of studies have been carried out to investigate *Q. robur* L. leaves by both Russian and foreign scientists, whose attention was mainly focused on the study of morphological and anatomical features, a chemical composition and antimicrobial properties [21–24]. The leaves of the *Quercus* representatives are promising objects for obtaining drugs with antimicrobial properties [21–24]. In the authors' opinion, the study of *Q. robur* L. buds, along with leaves, is also an urgent direction, because the buds of some plants are capable of exhibiting an antimicrobial activity and have a valuable chemical composition [25, 26].

One of the serious factors in the successful treatment of the infectious diseases, is a decrease in the resistance of pathogenic microorganisms to antimicrobial drugs [9, 27]. Staphylococci or methicillin-resistant strains (MRS strains), which are the cause of nosocomial and community-acquired infections, are of particular interest. Among the MRS strains, *Staphylococcus aureus* (MRSA) is most often found, its strains are resistant to many members of the β-lactam antibiotics group, including penicillins, cephalosporins, monobactams, carbapenems, etc. [9, 27]. The gram-negative bacterium *E. coli,* which is present in the human intestine and can cause various infectious diseases of the gastrointestinal tract and genitourinary system, is not a less dangerous strain [27, 28]. The study of the antimicrobial properties of water-ethanolic extractions and preparations based on the leaves and buds of the *Q. robur* L. will expand the spectrum of *Q. robur* pharmacological activities, and assess the possibilities of using this object in the creation of drugs in antibacterial therapy.

For an objective assessment of the antimicrobial activity of the studied raw materials, it is necessary to conduct a screening analysis of water-ethanolic extractions and determine the minimum inhibitory concentration (MIC) in relation to the main clinically significant strains of the microorganisms.

THE AIM of the study was to screen the antimicrobial activity of water-ethanolic extractions from the leaves and buds of *Quercus robur* L.

The research tasks included.

1. Screening analysis of the antimicrobial activity of water-ethanolic extractions of *Q. robur* L. leaves and buds;

2. Determination of the optimal concentration of

[1] State Register of Medicines. Available online: https://www.rlsnet.ru/tn_index_id_6283.htm

[2] Assessment report on Quercus robur L., Quercus petraea (Matt.) Liebl., Quercus pubescens Willd., cortex EMA/HMPC/3206/2009. Available online: https://www.ema.europa.eu/en

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the extractant and the conditions for the extraction of raw materials for the creation of preparations based on *Q. robur* L. plant raw materials.

MATERIALS AND METHODS

The objects of the study were water-ethanolic extractions of *Q. robur* L. leaves and buds at various concentrations of a chemically pure ethanol grade (40%, 70%, 80%, 96%) (alcohol 96%, ZAO "Hippocrat", Russia, Samara, series: 360917). The preparations of *Q. robur* L. leaves tincture and *Q. robur* L. buds tincture 70% alcohol in the raw materials: extractant ratio of 1:5, were also obtained by the method of fractional percolation. The required alcohol concentration was obtained by diluting 96% alcohol according to Table No. 5 of the appendix to the State Pharmacopoeia of the Russian Federation of the XIV edition [7]. For most flavonoid-containing plants, the optimal extractant is 70% ethanol, since this concentration of ethyl alcohol allows the maximum amount of flavonoids in the plant to be extracted and has a better penetrating ability into the deep layers of the epidermis compared to higher concentrations [7, 18, 19].

Analyzed samples of raw materials

The leaves of *Q. robur* L. were harvested from May to July 2020 (Samara region, Pohvistnevsky district, Pervomaiskaya Str., 2020). The buds of *Q. robur* L. were harvested from March to April 2020 (Samara region, Pohvistnevsky district, Pervomaiskaya Str., 2020).

The species specificity of the analyzed objects was confirmed with the help of the identifiers of the Russia central zone [3, 4]. In addition to the identifiers, the method of comparison with reliably known samples of the herbarium fund of Samara University was used. The inventory number of the main herbarium specimen is SMR-1547 (Herbarium Department for Ecology, Botany and Nature Protection Faculty of Biology, Samara University, 2021)[3].

Test cultures

The strains of the American Type Cultures Collection (ATCC) were used as test cultures: *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), as well as *Candida albicans* (clinical strain).

Research methods

The MIC was determined by the method of double serial dilutions in broth (a tube test, a macro method) in accordance with the methods described in "Guidelines" (G) 4.2.1890-04 [27, 29]. The method of double serial dilutions in comparison with diffusion method allows a qualitative assessment of the presence of the antimicrobial effect by visual assessment in comparison with the standard, and determination of the minimum inhibitory concentration of the studied sample, which slows down the growth of the studied strains of the microorganisms [27, 29]. Mueller-Hinton nutrient broth (Bio-Rad, USA) was used as a nutrient medium [29].

Methods

Testing of the studied samples was carried out in the volume of 1 ml of each sample dilution in water-ethanolic extractions.

Preparation of working solution

To determine the sensitivity, the nutrient broth was poured 0.5 ml into each tube. In addition to the number of tubes required to dilute the sample, one tube was used to set up a "negative" control. A working solution of a test sample was prepared from a stock solution using a liquid nutrient medium (Mueller-Hinton nutrient broth). The concentration of the working solution was calculated based on the required maximum concentration in a series of dilutions, taking into account the dilution factor of the drug during the subsequent inoculation [27].

Using a micropipette with a sterile tip, the working solution in the amount of 0.5 ml was introduced into the first tube containing 0.5 ml of broth. Then it was thoroughly mixed, and with a new sterile tip, 0.5 ml of the test solution in broth was transferred into the second tube containing initially 0.5 ml of broth. The procedure had been repeated until the entire required dilution series was prepared. 0.5 ml of broth was removed from the last test tube. Thus, a number of test tubes with the solutions of the tested samples of the water-ethanolic extractions from *Q. robur* L. leaves and buds were obtained, the concentrations of which differed in the adjacent test tubes by a factor of 2. Simultaneously, additional series of serial sample dilutions were prepared for testing control strains [27].

Inoculum preparation

For inoculation, a standard microbial suspension was used. It was equivalent to 0.5 according to McFarland's standard, diluted 100 times in nutrient broth. After that, the concentration of the microorganism in it would be approximately 10^6 CFU/ml. 0.5 ml of inoculum was introduced into each tube containing 0.5 ml of the corresponding dilution of the test sample, and into one tube with 0.5 ml of nutrient broth without a sample ("negative" control). The final concentration of the microorganism in each tube reached the required concentration of about 5×10^5 CFU/ml. The inoculum was introduced into test tubes with sample dilutions not later than 15–30 min from the moment of its preparation [27].

[3] Herbarium Department for Ecology, Botany and Nature Protection Faculty of Biology, Samara University. SMR-1547. Available from: http://sweetgum.nybg.org/science/vh/collection-index/collection-index-details/?irn=124749.

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Table 1 – Results of testing extracts from Q. robur L. leaves and buds

Object / Microorganism	Dilution ratio *						
	1	2	3	4	5	6	7
	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Pseudomonas aeruginosa							
Q. robur L. leaves 40%	–	–	+	+	+	+	+
Q. robur L. leaves 70%	–	–	–	+	+	+	+
Q. robur L. leaves 80%	–	–	–	–	+	+	+
Q. robur L. leaves 96%	–	–	–	+	+	+	+
Q. robur L. buds 40%	–	–	–	+	+	+	+
Q. robur L. buds 70%	–	–	–	–	+	+	+
Q. robur L. buds 80%	–	–	–	–	–	+	+
Q. robur L. buds 96%	–	–	–	–	–	+	+
Tincture of *Q. robur* L. leaves 70%	–	–	–	–	+	+	+
Tincture of *Q. robur* L. buds 70%	–	–	–	–	–	–	+
Staphylococcus aureus							
Q. robur L. leaves 40%	–	–	–	+	+	+	+
Q. robur L. leaves 70%	–	–	–	–	–	+	+
Q. robur L. leaves 80%	–	–	–	–	+	+	+
Q. robur L. leaves 96%	–	–	–	–	+	+	+
Q. robur L. buds 40%	–	–	–	+	+	+	+
Q. robur L. buds 70%	–	–	–	+	+	+	+
Q. robur L. buds 80%	–	–	–	–	+	+	+
Q. robur L. buds 96%	–	–	–	–	+	+	+
Tincture of *Q. robur* L. leaves 70%	–	–	–	–	+	+	+
Tincture of *Q. robur* L. buds 70%	–	–	–	–	+	+	+
Escherichia coli							
Q. robur L. leaves 40%	–	–	–	+	+	+	+
Q. robur L. leaves 70%	–	–	+	+	+	+	+
Q. robur L. leaves 80%	–	–	–	–	–	–	+
Q. robur L. leaves 96%	–	–	–	–	–	+	+
Q. robur L. buds 40%	–	–	–	+	+	+	+
Q. robur L. buds 70%	–	–	–	+	+	+	+
Q. robur L. buds 80%	–	–	–	–	–	+	+
Q. robur L. buds 96%	–	–	–	–	+	+	+
Tincture of *Q. robur* L. leaves 70%	–	–	–	–	–	+	+
Tincture of *Q. robur* L. buds 70%	–	–	–	–	–	+	+
Candida albicans							
Q. robur L. leaves 40%	–	–	–	+	+	+	+
Q. robur L. leaves 70%	–	–	–	+	+	+	+
Q. robur L. leaves 80%	–	–	–	–	+	+	+
Q. robur L. leaves 96%	–	–	–	–	+	+	+
Q. robur L. buds 40%	–	–	–	–	–	+	+
Q. robur L. buds 70%	–	–	–	–	–	+	+
Q. robur L. buds 80%	–	–	–	–	+	+	+
Q. robur L. buds 96%	–	–	–	–	–	+	+
Tincture of *Q. robur* L. leaves 70%	–	–	–	–	–	–	+
Tincture of *Q. robur* L. buds 70%	–	–	–	–	–	+	+

Note: + presence of microorganism growth; absence of microorganism growth

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Table 2 – Minimum inhibitory concentrations of ethanol ("negative" control)

Object / Microorganism	Dilution ratio *						
	1	2	3	4	5	6	7
	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Pseudomonas aeruginosa							
Ethanol 40%	–	–	+	+	+	+	+
Ethanol 70%	–	–	+	+	+	+	+
Ethanol 80%	–	–	–	+	+	+	+
Ethanol 96%	–	–	+	+	+	+	+
Staphylococcus aureus							
Ethanol 40%	–	–	–	+	+	+	+
Ethanol 70%	–	–	–	+	+	+	+
Ethanol 80%	–	–	–	+	+	+	+
Ethanol 96%	–	–	+	+	+	+	+
Escherichia coli							
Ethanol 40%	–	–	–	+	+	+	+
Ethanol 70%	–	–	–	–	+	+	+
Ethanol 80%	–	–	–	+	+	+	+
Ethanol 96%	–	–	+	+	+	+	+
Candida albicans							
Ethanol 40%	–	–	–	+	+	+	+
Ethanol 70%	–	–	–	+	+	+	+
Ethanol 80%	–	–	–	+	+	+	+
Ethanol 96%	–	–	–	+	+	+	+

Note: + presence of microorganism growth; – absence of microorganism growth

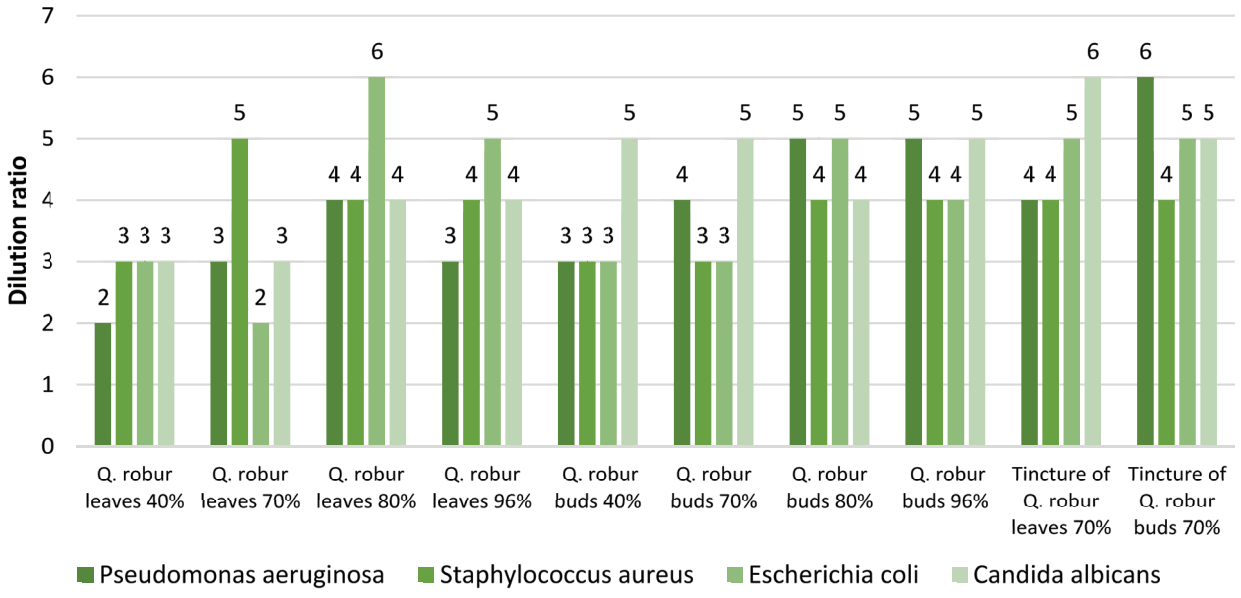

Figure 1 – Comparative diagram of antibacterial activity of water-ethanolic extractions of *Q. robur* L. leaves and buds (the abscissa is the serial number of the dilution ratio)

The tubes were closed with sterile gauze and cotton stoppers, and all tubes with the tested strains, except for the tube with the "negative" control, were incubated at 35℃ for 20–24 hours. The tube with the "negative" control was placed in a refrigerator at 4°C, and stored until the results were taken into account [27].

Assessment of microorganisms growth

To determine the presence of the microorganism growth, the test tubes with crops were viewed in transmission. The growth of the culture in the presence of the test sample was carried out by comparison with the tube of the "negative" control containing the original inoculum and stored in the refrigerator. MIC was determined by the lowest concentration of the test sample, which suppresses the visible growth of the microorganism [27].

Assessment of experimental results

The results were assessed visually by the presence/absence of the microorganisms growth of in test tubes with the appropriate dilutions of the test samples [27]. The minimum inhibitory concentration was the lowest concentration of the studied sample, which completely suppressed the growth of the microorganisms strain. At the same time, according to the requirements of the "Guidelines" (G 4.2.1890-04)[4] for determining the sensitivity of microorganisms to the antibacterial drugs, as well as the recommendations of the Performance Standard for Antimicrobial Susceptibility Tests (Clinical And Laboratory Standards Institute (CLSI))[5], the presence of turbidity, and the detection of a small number of the microorganisms (one colony) were not taken into account when registering the experimental result. The experiment was repeated three times [27, 29].

RESULTS AND DISCUSSIONS

During the screening of the antimicrobial activity of the extracts from *Q. robur* L. leaves and buds, the following results were obtained.

When testing 40% water-ethanolic extractions of *Q. robur* L. leaves, the antimicrobial activity against the *P. aeruginosa* strain in a four-fold dilution, as well as against microorganisms *S. aureus, E. coli,* and *C. albicans* in an eight-fold dilution, was observed (Table 1). When comparing 40% water-ethanolic extractions with the "negative" standard (a minimum inhibitory concentration for 40% water-ethanol), no differences in the antimicrobial activity between the test sample and the reference sample were observed (Table 2). This indicates that there is no contribution of the complex of biologically active compounds available in the extract, to the pharmacological effect at the given extraction concentration.

For 70% water-ethanolic extractions from *Q. robur* L. leaves, the antimicrobial activity was expressed against *P. aeruginosa* in an eight-fold dilution; against *S. aureus* – when diluted 32 times; for the *C. albicans* strain – in an eight-fold dilution. Growth inhibition of the *E. coli* strain was observed in a two-fold dilution (Table 1).

An antimicrobial activity of 80% water-ethanolic extractions of *Q. robur* L. leaves was expressed against *E. coli* when diluted 64 times; against *S. aureus, C. albicans* and *P. aeruginosa* – in a 16-fold dilution (Table 1). For water-ethanolic extractions from leaves with 80% ethanol, the maximum growth retardation of microorganisms is observed. When compared with the "negative" standard of ethanol at the concentration of 80%, a significant growth inhibition of microorganisms is observed (Table 1; Table 2).

An antimicrobial activity of 96% water-ethanolic extractions of *Q. robur* L. leaves was expressed against all strains in an eight-fold dilution; for *S. aureus*, *C. albicans* and *E. coli* in a 16-fold dilution; with respect to *E. coli*, the highest activity was observed in a 32- fold dilution (Table 1). The indices of the "negative" control for 96% ethanol concentration, were significantly lower compared to the test sample (Table 2).

The analysis of 40% water-ethanolic extractions from *Q. robur* L. buds gives less antimicrobial effect in comparison with 40% water-ethanolic extractions from *Quercus robur* L. leaves (Table 1; Table 2). In particular, the extraction from the buds at the given concentration of ethanol is expressed in relation to strains of *P. aeruginosa, S. aureus, E. coli* and *C. albicans* in an eight-fold dilution; along with other strains, in 32-fold dilution, a more pronounced activity was observed against the *C. albicans* strain (Table 1).

During testing of 70% water-ethanolic extractions from *Q. robur* L. buds, a pronounced antimicrobial activity was noted against the strains of such microorganisms as *P. aeruginosa, S. aureus, E. coli* and *C. albicans* in eight-fold dilution. With respect to *P. aeruginosa* strains, an antimicrobial activity was observed in 16-fold dilutions. The most pronounced antimicrobial effect was recorded against the *C. albicans* strain in 32 fold dilutions (Table 1).

An antimicrobial activity of 80% water-ethanolic extractions of *Q. robur* L. buds was observed against the *E. coli* strain when diluted 32 times, as well as against the *S.aureus, C. albicans* and *P. aeruginosa* strains when diluted 16, 16, and 32 times, respectively (Table 1). When compared with the "negative" standard of ethyl alcohol at the concentration of 80%, a significant growth inhibition of microorganisms is observed (Table 1; Table 2).

The study of the antimicrobial activity of 96% wa-

[4] Determination of the sensitivity of microorganisms to antibacterial drugs. Guidelines. 4.2.1890-04. Clinical microbiology and antimicrobial chemotherapy. 2004; 6 (4): 306–359. The guidelines were approved and put into effect by the Chief State Sanitary Doctor of the Russian Federation – First Deputy Minister of Health of the Russian Federation G.G. Onishchenko, March 4, 2004.

[5] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2018.

ОРИГИНАЛЬНАЯ СТАТЬЯ
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ter-ethanolic extractions from *Q. robur* L. buds gave the following results: a distinct antimicrobial activity against all strains in a 16-fold dilution; against *P. aeruginosa* and *C. albicans*, the activity was also observed in 32 – fold dilutions (Table 1).

During the screening analysis of water-ethanolic extractions of *Q. robur* L. leaves and buds, the conditions for obtaining the dosage form of the tincture were determined. 70% ethanol was chosen as an extractant for the manufacture of tincture from *Q. robur* L. leaves and tincture from *Q. robur* L. buds, since this concentration is the optimal extractant for raw materials containing a complex of biologically active substances of the flavonoid group that provide an antimicrobial effect of the drug [15, 20].

The tested tincture from *Q. robur* L. leaves in 70% ethanol showed the following results. The antimicrobial effect was observed for all of these strains. In particular, in a sixteen-fold dilution, the antimicrobial activity is observed against the *P. aeruginosa* strain and the *S. aureus* strain; when diluted 32 times – against *E. coli,* and when diluted 64 times – against *C. albicans* (Table 1).

In the course of testing the preparation of *Q. robur* L. bud tincture in 70% ethanol, the following results were obtained: the antimicrobial effect was observed against the *P. aeruginosa* strain when diluted 64 times; at sixteen-fold dilution, is observed against the *S. aureus* strain in a sixteen-fold dilution; the antimicrobial activity against *E. coli* and *C. albicans* took place when diluted 32 times (Table 1).

Thus, a screening study was carried out to research the antimicrobial activity of water-ethanolic extractions from *Q. robur* L. leaves and buds, as a result of which an antimicrobial effect on a number of pathogenic microorganisms strains (*P. aeruginosa; S. aureus; E. coli; C. albicans)* was found out *in vitro*:.

According to the results of the work performed, it established that all the studied samples of water-ethanolic extractions from *Q. robur* L. leaves and buds, give a stable antimicrobial effect against *S. aureus* and *C. albicans* strains.

The water-ethanolic 80% extract from *Q. robur* L. leaves and buds in the raw material:extractant ratio of 1:50 and preparations of tincture ~~of~~ from *Q. robur* L. leaves and buds in 70% alcohol in the raw material:extractant ratio of 1: 5, are the most effective objects, and give the maximum growth retardation of the microorganisms when diluted 32 and 64 times.

A sophisticated action with the maximum antimicrobial effect on *C. albicans* is provided by the preparation of a tincture from *Q. robur* L. leaves in the raw material:extractant ratio of 1: 5; for *S. aureus* strains – 70% water-ethanolic extractions in the raw material:extractant ratio of 1: 50 (Fig. 1). Water-ethanolic extractions from *Q. robur* L. buds have a similar stable maximum antimicrobial effect against *C. albicans*. For *P. aeruginosa, S. aureus* and *E. coli* strains, the maximum antimicrobial effect is noted in the extractant concentration of 96% ethyl alcohol.

The minimum antimicrobial activity is noted for 70% water-ethanolic extractions from the leaves of *Q. robur* L. in the raw material:extractant ratio of 1: 50 for *E. coli* strains, as well as for 40% water-ethanolic extractions from the *Q. robur* L buds in the raw material:extractant ratio of 1: 50 for *P. aeruginosa strains* (Fig. 1).

The choice in favor of 70% alcohol concentration as an extractant for obtaining tincture from the oak leaves and buds was made on the basis that for dosage forms with these extraction parameters, the greatest antimicrobial effect is observed against the studied strains of microorganisms (Fig. 1). Also, extracts at a given alcohol concentration have a better penetrating ability into the deep layers of the epidermis in comparison with higher and lower alcohol concentrations[6]. It should be noted that the leaves have a large phytomass in comparison with the buds, their collection can be carried out in a longer period of time than for the buds, the collection time of which, as a rule, falls in the winter-spring period [7].

CONCLUSION

All the results obtained in the course of the study, can serve for the creation of new antibacterial drugs based on *Q. robur* L. leaves and buds, as well as for the introduction of tincture preparations from *Q. robur* L. leaves and buds in 70% alcohol into medical and pharmaceutical practice.

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CONFLICT OF INTEREST

The authors declare no conflict of interest related to the publication of this article.

[6] State Register of Medicines. Available from: https://www.rlsnet.ru/tn_index_id_6283.htm.

[7] State Register of Medicines. Available from: https://www.rlsnet.ru/tn_index_id_6283.htm.

AUTHORS' CONTRIBUTION

Nikolay A. Ryabov – data collecting, experiment conducting, analyzing and interpreting the data obtained, preparing a draft manuscript, analyzing the literature, writing a manuscript and finally approving of it for publication; Vitaly M. Ryzhov – planning of the study, participation in the development of the concept and design of the study, collection of plant material for analysis; Vladimir A. Kurkin – final approval of the manuscript for publication, processing the results obtained, verification of critical intellectual content; Svetlana D. Kolpakova – participation in research, literature analysis; Alexander V. Zhestkov – participation in the description and analysis of the results obtained, participation in manuscript writing and its final approval for publication; Artem V. Lyamin – participation in the writing of the manuscript, critical analysis of the research.

REFERENCES

1. Dasgupta A, Krasowski MD. Chapter 10 – Therapeutic drug monitoring of antimicrobial, antifungal and antiviral agents, Therapeutic Drug Monitoring Data (Fourth Edition), Academic Press. 2020;159–197. DOI: 10.1016/B978-0-12-815849-4.00010-4.
2. Andrade HB, Shinotsuka CR, da Silva IRF, Donini CS, Yeh Li H, de Carvalho FB, Americano do Brasil PEA, Bozza FA, Miguel Japiassu A. Highly active antiretroviral therapy for critically ill HIV patients: A systematic review and meta-analysis. PLoS One. 2017 Oct 24;12(10):e0186968. DOI: 10.1371/journal.pone.0186968.
3. Grozdova N.B., Nekrasov V.I., Globa-Mikhailenko D.A. Trees, shrubs and vines. M.: Lesn. industry. 1986: 176–178.
4. Maevsky P.F. Flora of the middle zone of the European part of Russia. 11th ed. M.: Partnership of scientific publications KMK. 2014:200–201
5. British Pharmacopoeia 2009. British Pharmacopoeia Herbal Drugs and Herbal Drug Preparations. Oak Bark. 2009; Vol. III. 7203 p.
6. European Pharmacopoeia – 8th. «01/2008:1887 corrected 6.0». 2013. Available from: http://pharmeuropa.edqm.eu
7. State Pharmacopoeia of Russian Federation. XIV ed; Vol. I–IV. *M*. 2018. Available from: http:// http://femb.ru/femb/pharmacopea.php.
8. Fatehi S, Mohammadi Sichani M, Tavakoli M. Evaluation of antimicrobial and Anti-quorum sensing activity of mazouj and ghalghaf galls extracts of oak against Pseudomonas aeruginosa. Qom Univ Med Sci J. 2018;12(10):36–45. DOI: 10.29252/qums.12.10.36
9. Pailhoriès H, Munir MT, Aviat F, Federighi M, Belloncle C, Eveillard M. Oak in Hospitals, the Worst Enemy of Staphylococcus aureus? Infection Control & Hospital Epidemiology. 2017;38(3):382–384. DOI: 10.1017/ice.2016.304.
10. Smailagić A, Ristivojević P, Dimkić I, Pavlović T, Dabić Zagorac D, Veljović S, Fotirić Akšić M, Meland M, Natić M. Radical Scavenging and Antimicrobial Properties of Polyphenol Rich Waste Wood Extracts. Foods. 2020 Mar 10;9(3):319. DOI: 10.3390/foods9030319.
11. Sarwar R, Farooq U, Naz S, Riaz N, Majid Bukhari S, Rauf A, et al. Isolation and Characterization of Two New Antimicrobial Acids from *Quercus incana (Bluejack Oak).* Biomed Res Int. 2018;2018:3798105. DOI: 10.1155/2018/3798105
12. Smailagić A., Zagorac D.D., Veljović S., Sredojević M., Relić D., Fotirić M. A., Roglić G., Natić M. Release of wood extractable elements in experimental spirit model: Health risk assessment of the wood species generated in Balkan cooperage. Food Chemistry. 2021; 338: 127804. DOI: 10.1016/j.foodchem.2020.127804.
13. Elansary O. H, Szopa A, Kubica P, Ekiert H, A. Mattar M, Al-Yafrasi MA, El-Ansary DO, Zin El-Abedin TK, Yessoufou K. Polyphenol Profile and Pharmaceutical Potential of Quercus spp. Bark Extracts. Plants. 2019; 8(11):486. DOI: 10.3390/plants8110486
14. Rao N. *In vitro* phytochemical screening, antioxidant & antimicrobial activity of the methanolic extract of *Quercus infectoria* L. 2013;5:273–277.
15. Dróżdż, Paulina and K. Pyrzyńska. Assessment of polyphenol content and antioxidant activity of oak bark extracts. European Journal of Wood and Wood Products. 2017; 76: 793–795. DOI: 10.1007/s00107-017-1280-x.
16. Sánchez-Burgosa J.A., Ramírez-Maresb M.V., Larrosac M.M., Gallegos-Infantea J.A., González-Laredoa R.F., Medina-Torresd L., Rocha-Guzmána N.E. Antioxidant, antimicrobial, antitopoisomerase and gastroprotective effect of herbal infusions from four *Quercus* species. Industrial Crops and Products. 2013;42: 57–62. DOI:10.1016/j.indcrop.2012.05.017
17. Budantsev AL. Vegetables of Russia: Wild flowering plants, their component composition and biological activity. 2nd ed. Families *Actinidiaceae-Malvaceae, Euphorbiaceae-Haloragaceae*. M., Partnership of scientific publications KMK, 2009. 774 p. Russian
18. Grotewold E. The Science of Flavonoids. 8th ed. New York: Springer. 2006:274. DOI: 10.1007/978-0-387-28822-2.
19. Bedi MK, Shenefelt PD. Herbal Therapy in Dermatology. Archives of Dermatology. 2002; 138(2): 237–238. DOI:10.1001/archderm.138.2.232.
20. Cushnie TP, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. Int. J. Antimimicrob. Agents. 2011;38: 99–107. DOI: 10.1016/j.ijantimicag.2011.02.014.
21. Scalbert A, Haslam E. Polyphenols and chemical defence of the leaves of *Quercus robur.* Phytochem-

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2021-9-2-104-113

istry. 1987;26:3191–3195. DOI: 10.1016/S0031-9422(00)82468-1.
22. Benyagoub E, Nabbou N, Dine A. Antimicrobial Effect of *Quercus robur* L. Leaves Selective Extracts from the Mezi Mountain of Djenane Bourezg (West of Algeria). Current Bioactive Compounds. 2020;16(8):1181–1190. DOI: 10.2174/1573407216666191226141609.
23. Benyagoub E, Nabbou N, Boukhalkhel S, Dehini I. The *In vitro* Evaluation of the Antimicrobial Activity of *Quercus robur* L. Methanolic and Aqueous Leaves' Extracts, from the Algerian High Plateaus Against some Uropathogenic Microbial Strains. Phytopathology. 2019:12. DOI: 10.29252/qums.12.10.36.
24. Sanchez-Burgos J, Ramírez-Mares M, Larrosa M, Gallegos-Infante J, González-Laredo R, Medina-Torres L, et al. Antioxidant, antimicrobial, antitopoisomerase and gastroprotective effect of herbal infusions from four *Quercus species*. Industrial Crops and Products. 2013;42:57–62. DOI: 10.1016/j.indcrop.2012.05.017.
25. Nassima B, Behidj-Benyounes N, Ksouri R. Antimicrobial and antibiofilm activities of phenolic compounds extracted from *Populus nigra* and *Populus alba* buds (Algeria). Brazilian Journal of Pharmaceutical Sciences. 2019; 55: e18114. DOI: 10.1590/s2175-97902019000218114.
26. Isidorov VA, Bagan R, Szczepaniak L, Swiecicka I. Chemical profile and antimicrobial activity of extractable compounds of *Betula litwinowii (Betulaceae)* buds. Open Chemistry. 2015;13(1):123–127. DOI: 10.1515/chem-2015-0019
27. The definition of the sensitivity of microorganisms to antibacterial drugs. Guidelines. MUK 4.2.1890-04. Clinical Microbiology and Antimicrobial Chemotherapy. 2004;6(4):306–359.
28. Kozlova I.V., Lekareva L.I., Bykova A.P., Myalina Yu.N., Ostrovskaja L.Yu. Candidiasis gastrointestinal tract. Experimental and Clinical Gastroenterology. 2016;(3):40–46. Russian
29. Golus J, Sawicki R, Widelski J, Ginalska G. The agar microdilution method – a new method for antimicrobial susceptibility testing for essential oils and plant extracts. J. Appl. Microbiol. 2016;121:1291–1299. DOI: 10.1111/jam.13253

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