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PHYTOCHEMICAL AND MICROBIOLOGICAL ASPECTS OF THE *NIGELLA SATIVA* L. HERBS STUDY

A.R. Mubinov, V.A. Kurkin, E.V. Avdeeva, S.D. Kolpakova, A.V. Zhestkov

Samara State Medical University
89, Chapaevskaya Str., Samara, Russia, 443099

E-mail: v.a.kurkin@samsmu.ru

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Nigella sativa L. is a promising plant object, herbal medicinal raw materials of which can be comprehensively used for the development of drugs with an antimicrobial activity.

The aim of the study was to screen and compare the antimicrobial activity of water-ethanolic extractions from the *Nigella sativa* L. herbs with a eucalyptus tincture of as a reference preparation.

Materials and methods. Chromatograms of the extracts were obtained by thin layer chromatography in the system of chloroform – ethanol – water (26:16:3). The detection of adsorption zones was carried out in daylight, in the UV light at λ=254 nm and λ=365 nm, as well as by treatment with reagents – a 3% alcohol solution of aluminum chloride and a solution of diazobenzosulfonic acid in a 20% sodium carbonate solution. The next step was to determine the minimum inhibitory concentration by the method of double serial dilutions in Mueller-Hinton nutrient broth (Bio-Rad, USA). As test cultures, the strains of the American Type Culture Collection (ATCC) microorganisms were used: *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), as well as *Candida albicans* (a clinical strain). Simultaneously, an experiment to establish a "negative" control was carried out. For the comparative evaluation of the studied samples activity, its activity was compared with the reference preparation with a proven antimicrobial activity – a eucalyptus tincture.

Results. For all water-ethanolic extractions and the *Nigella sativa* L. herb tincture, the adsorption zones characteristic of flavonoids with Rf_1 = 0.28, Rf_2 = 0.15, Rf_3 = 0.11 were revealed, and under the action of an alcoholic solution of aluminum chloride, the fluorescence of the adsorption zones was also enhanced, which indicates the phenolic nature of these compounds. In the course of the study, it was found out that all water-ethanolic extractions from the *Nigella sativa* L. herbs have the greatest antimicrobial effect against the *Pseudomonas aeruginosa* strain. When compared with the reference preparation – a eucalyptus tincture, it was notified that the specified tincture of the *Nigella sativa* L. herbs has an advantage in the antimicrobial activity over the strain of *Pseudomonas aeruginosa* – the action at the 16-fold dilution vs the 4-fold dilution. The action on the *Escherichia coli* and *Candida albicans* strains is comparable for the both tinctures.

Conclusion. The obtained results of phytochemical and microbiological analyses will be used as a rationale for the introduction of antimicrobial preparations based on the *Nigella sativa* herbs in medical and pharmaceutical practice.

Keywords: *Nigella sativa* L.; herbs; water-ethanolic extractions; tincture; minimum inhibitory concentration; antimicrobial activity

Abbreviations: ATCC – American Type Culture Collection; CLSI – Clinical and Laboratory Standards Institute; MRS-strains – methicillin-resistant *Staphylococcus*; MRSA – methicillin-resistant *Staphylococcus aureus*; SP RF XIV ed. – State Pharmacopoeia of the Russian Federation XIV edition; CFUs/ml – Colony forming units/ml; MIC – minimum inhibitory concentration; Gs – Guidelines; *N.* – *Nigella* L. (eg. *N. sativa*).

ФИТОХИМИЧЕСКИЕ И МИКРОБИОЛОГИЧЕСКИЕ АСПЕКТЫ ИЗУЧЕНИЯ ТРАВЫ ЧЕРНУШКИ ПОСЕВНОЙ (*NIGELLA SATIVA* L.)

А.Р. Мубинов, В.А. Куркин, Е.В. Авдеева, С.Д. Колпакова, А.В. Жестков

Федеральное государственное бюджетное образовательное учреждение высшего образования
«Самарский государственный медицинский университет»
Министерства здравоохранения Российской Федерации
443099, Россия, г. Самара, ул. Чапаевская, д. 89

E-mail: v.a.kurkin@samsmu.ru

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Чернушка посевная – *Nigella sativa* L. является перспективным растительным объектом, лекарственное растительное сырье которой может быть комплексно использовано для разработки препаратов с антимикробной активностью.

Цель. Проведение фитохимического скрининга и сравнения антимикробной активности водно-спиртовых извлечений травы чернушки посевной (*Nigella sativa* L.) с препаратом сравнения – настойкой эвкалипта.

Материалы и методы. Методом тонкослойной хроматографии в системе хлороформ – этанол – вода (26:16:3) были получены хроматограммы извлечений. Детектирование зон адсорбции проводилось при дневном свете, в УФ-свете при λ=254 нм и λ=365 нм, а также обработкой реактивами – спиртовым раствором алюминия (III) хлорида 3% и раствором диазобензосульфокислоты в 20% растворе натрия карбоната. Далее проводилось определение минимальной ингибирующей концентрации 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 (ATCC 29213*), *Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), а также Candida albicans* (клинический штамм). Параллельно проводили опыт для постановки «отрицательного» контроля. Для сравнительной оценки активности изучаемых проб сопоставляли активность с препаратом сравнения – настойкой эвкалипта с доказанной антимикробной активностью.

Результаты. Для всех водно-спиртовых извлечений и настойки травы чернушки посевной выявлены характерные для флавоноидов зоны адсорбции с Rf_1 = 0,28, Rf_2 = 0,15, Rf_3 = 0,11, также под действием спиртового раствора алюминия (III) хлорида 3% происходит усиление флуоресценции зон адсорбции, что говорит о фенольной природе данных соединений.

В ходе проведенного микробиологического исследования установлено, что все водно-спиртовые извлечения травы чернушки посевной оказывают наибольший антимикробный эффект в отношении штамма *Pseudomonas aeruginosa*. Отмечено, что препарат – настойка травы чернушки посевной на 70% спирте этиловом имеет преимущество по антимикробной активности к штамму *Pseudomonas aeruginosa* – *действие* при 16-ти кратном разведении против 4-кратного у настойки эвкалипта. Действие на штаммы *Escherichia coli* и *Candida albicans* сравнимо для обеих настоек.

Заключение. Полученные результаты фитохимического и микробиологического анализа будут использованы в качестве обоснования для внедрения антимикробных препаратов на основе травы чернушки посевной в медицинскую и фармацевтическую практику.

Ключевые слова: Чернушка посевная; *Nigella sativa* L.; трава; водно-спиртовые извлечения; настойка; минимальная ингибирующая концентрация; антимикробная активность

Список сокращений: АТСС – Американская коллекция типовых культур (American Type Culture Collection); CLSI – Институт клинических и лабораторных стандартов (Clinical and Laboratory Standards Institute); MRS-штаммы – метициллин-резистентные стафилококки (Methicillin-resistant *Staphylococcus*); MRSA – метициллинрезистентный золотистый стафилококк (Methicillin-resistant *Staphylococcus aureus*); ГФ РФ XIV изд. – Государственная фармакопея Российской Федерации XIV издания; КОЕ/мл – колониеобразующие единицы; МИК – минимальная ингибирующая концентрация; МУК – методические указания; *N.* – *Nigella* L. (н-р, *N. sativa*).

INTRODUCTION

The creation of new antimicrobial drugs based on herbal raw materials, has always been and remains an urgent task of modern pharmacy. The proliferation of various new viral and microbial infections does not make possible a stable improvement in the quality of life and health of people and in many ways aggravates the course of other associated diseases in patients. Currently, the increase in antimicrobial resistance poses a serious danger, which consists in the reduction of the effectiveness of measures for the prevention and treatment of human infectious diseases [1–3].

The species of *Nigella* L genus have been used in folk medicine as promising medicinal and food plants since ancient times and are of great interest [4–6]. The genus *Nigella* L. consists of 24 species growing in the Mediterranean, southern and southeastern Europe, the Caucasus, Asia Minor and Central Asia, North Africa; about 11 species are distributed in the Commonwealth of Independent States [7, 8].

In modern medical practice, the *N. damascena* L. seeds and *N. sativa* L are mainly used. In the world publication stream, there is quite a lot of information about the pharmacological activity of various groups of biologically active compounds contained in the seeds and, respectively, in the *Nigella saliva* L. seeds oil. There is a high content of unsaturated fatty acids in the fatty oil, a high thymoquinone content and the presence of nigellone as components of the essential oil, carbohydrates, as well as lipolytic enzymes [6, 8–10].

The most studied are the *Nigella* L. seeds as well as the fatty oil obtained from them by cold pressing. It is reported that the *Nigella* L. essential oil has a strong antibacterial activity against Gram-positive (*Staphylococcus aureus)* and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria [11, 12]. It has also a synergistic effect in combination with streptomycin and gentamicin, while its additive effect is manifested in combination with spectinomycin, erythromycin, tobramycin, doxycycline, chloramphenicol, nalidixic acid, ampicillin, lincomycin and co-trimoxazole, and is similar to topical mupirocin [11]. The activity against many multiresistant resistant Gram-positive and Gram-negative bacteria has also been described [11–14]. The researchers believe that these antimicrobial properties are due to the high

content of thymoquinone, particularly, thymohydroquinone [12–15].

The extractions from the seeds of *Nigella* L. can be used as an antimalarial and antiviral agents [16]. The recent studies of a number of scientists showing a great promising character of *Nigella* L. in the prevention and treatment of COVID-19, are of particular interest. That confirms the uniqueness of this plant once again [17, 18].

In view of the proven spectrum of antimicrobial activity for seeds and, accordingly, for the *N. sativa* L. oil, from the complex processing perspective of raw materials, it seems appropriate to study the antimicrobial properties of water-ethanolic extractions and preparations based on the *N. sativa* L herb. These studies will expand a spectrum of representations about the *N. sativa* L. pharmacological activity and evaluate the possibility of using this object in the creation of domestic drugs used in the antibacterial therapy.

For the 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. Comparing the activity with the reference preparation – a eucalyptus tincture[1,2], for which antimicrobial properties, including the ones against the MRS strains, have been proven, should be also carried out. [19, 20].

THE AIM of the study was to screen and compare the antimicrobial activity of water-ethanolic extractions from the *Nigella sativa* L. herbs with a eucalyptus tincture as a reference preparation.

MATERIALS AND METHODS

The objects of the study were experimental *N. sativa* L. water-ethanolic extractions at various concentrations of a chemically pure ethanol grade (40%, 70%, 96%) (alcohol 96%, CJSC "Hippocrat", Russia, Samara, series: 360919) in the raw materials. For the selection of the ethyl alcohol concentration, the raw materials-to-the extractant ratio was 1:30. The *N. sativa* L. tincture was also obtained with 70% ethyl alcohol in the materials-to-the extractant ratio of 1:5 by fractional maceration with the inclusion of the final thermal stage – 0.5 hours at the temperature of 70°C.

The required alcohol concentrations of were obtained by diluting 96% alcohol according to Table No. 5 of the appendix to the State Pharmacopoeia of the Russian Federation XIV edition (SP RF XIV ed.)[3]. 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 [21]. In addition, compounds of the terpenoid nature also pass into the liquid phase, it was found in the course of the preliminary phytochemical studies.

Analyzed samples of raw materials

The *N. sativa* L. herb was harvested from July to August 2021 in the Ulyanovsk region, the Cherdakly district, Cherdakly). *N. sativa* L. commercial seeds («Nora zdorov'ya», the habitat is Egypt) were used for cultivation. From the position of the phytochemical analysis results of various samples by their origin and places of harvesting, this raw material was chosen as the most valuable relative to the content of phenolic and terpenoid compounds, and also as perspective for the industrial cultivation in the European part and the south of Russia. The species specificity of the analyzed objects was also confirmed using determinants and a plant atlas[4].

Reference preparation

The reference preparation with the established antimicrobial activity was a eucalyptus tincture in 70% ethyl alcohol of a commercial production in a bottle of 25 ml (LLC "Hippocrat", series: 010620, the date of production: 01.06.2020, the expiration date: 5 years).

Test cultures

The following 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). A clinical strain of *Candida albicans* was isolated from a patient's sputum with bronchopulmonary pathology at "Samara State Medical University Clinics", the structural subdivision of Samara State Medical University. The study was conducted in accordance with the approval of the Bioethics Committee at Samara State Medical University (protocol No.196 dated October 31, 2018).

Research methods

For the primary phytochemical analysis of the stud-

[1] State Register of Medicines. Available from: https://www.rlsnet.ru/tn_index_id_5478.htm.
[2] State Pharmacopoeia of the Russian Federation, XIV edition. Ministry of Health of the Russian Federation. Vol. 1–4, 2018. Available from: https://femb.ru/record/pharmacopea14.
[3] Ibid.
[4] *Nigella sativa* L. Plantarium. Plants and lichens of Russia and neighboring countries: open online galleries and plant identification guide. Available from: https://www.plantarium.ru/lang/en/page/view/item/25118.html

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ied extracts composition, the thin-layer chromatography (TLC) method was used, which was carried out in accordance with GM.1.2.1.2.0003.15 "Thin-layer chromatography" of the SP RF XIV ed.[5].

The TLC was carried out using Sorbfil PTSKh-AF-A-UF chromatographic plates; 0.02 ml of water-ethanolic extractions and the *Nigella sativa* herbs tincture were applied with a micropipette. Nearby, a witness solution was applied with a micropipette – a standard sample (SS) of rutin, which met the requirements of the SP RF XIV ed., and was provided for research by the Center for Collective Use of the Institute of Pharmacy of Samara State Medical University. The determination was carried out in the chloroform – ethanol – water (26:16:3) system. The resulting chromatogram was viewed in the UV light at λ=254 nm and λ=365 nm, it was also treated with a 3% alcohol solution of aluminum chloride ($AlCl_3$) and a solution of diazobenzosulfonic acid in a 20% sodium carbonate solution (DSA).

The minimum inhibitory concentration (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[6]. The method of double serial dilutions in comparison with a diffusion method allows a qualitative assessment of the antimicrobial effect presence by a visual assessment in comparison with the standard, and the determination of the minimum inhibitory concentration of the studied sample, which slows down the growth of the microorganisms strains under study. Mueller-Hinton nutrient broth (Bio-Rad, USA) was used as a nutrient medium [22].

Methods

Testing of the samples under study 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, 0.5 ml of the nutrient broth was poured 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.

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 up, and with a new sterile tip, 0.5 ml of the test solution in the 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 the 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 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.

Inoculum preparation

For the inoculation, a standard microbial suspension was used. According to McFarland's standard, it was equivalent to 0.5 units diluted 100 times in the nutrient broth. After that, the concentration of the microorganism in it would be approximately 10^6 CFUs/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 (a "negative" control). The final concentration of the microorganism in each tube reached the required concentration of about 5×10^5 CFUs/ml. The inoculum was introduced into the test tubes with the sample dilutions not later than 15–30 min from the moment of its preparation.

The tubes were closed with sterile gauze and cotton stoppers, and all the tubes with the tested strains, except the tube with the "negative" control, were incubated at 35°C 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.

Assessment of microorganisms growth

To determine the presence of the microorganisms growth, the test tubes with inoculations were viewed in the transmitted light. The growth of the culture in the presence of the test sample was carried out by the comparison with the tube of the "negative" control containing the original inoculum and stored in the refrigerator. The MIC was determined by the lowest concentration of the test sample, which suppresses the visible growth of the microorganisms.

[5] State Pharmacopoeia of the Russian Federation. Ministry of Health of the Russian Federation. XIV edition. Vol. 1–4, 2018.

[6] Determination of the sensitivity of microorganisms to antibacterial drugs. Guidelines. 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.)

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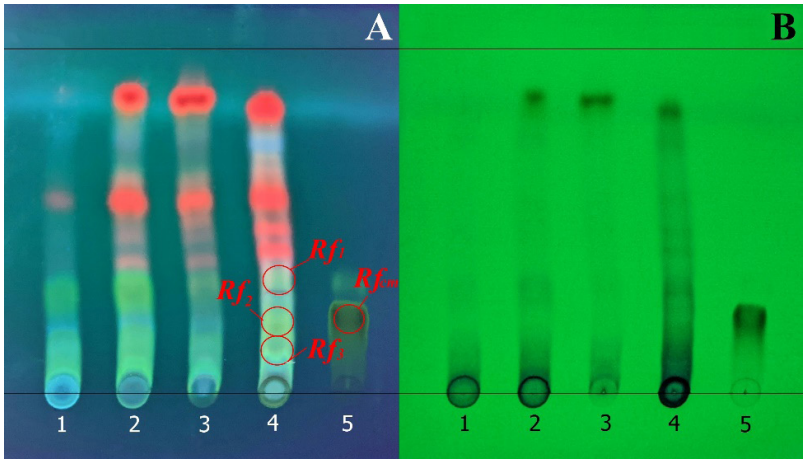

Figure 1 – Chromatogram of the analysis of water-ethanolic extractions and *N. sativa* L. herb tincture

Note: A – detection in UV light at 365 nm; B – detection in UV light at 254 nm. 1 – 40% water-ethanolic extraction; 2 – 70% water-ethanolic extraction; 3 – 96% water-ethanolic extraction; 4 – *N. sativa* L. herb tincture; 5 – SS rutin.

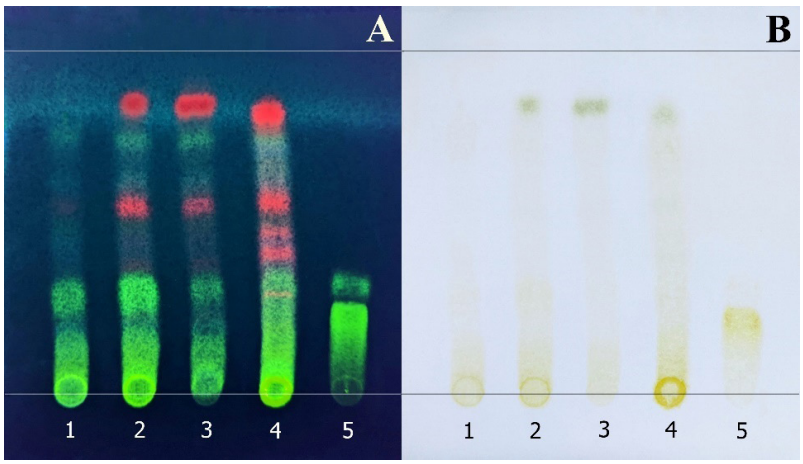

Figure 2 – Chromatogram of the analysis of water-ethanolic extractions and *N. sativa* L. herb tincture

Note: A – detection in UV light at 365 nm after $AlCl_3$ treatment; B – detection after DS**A** treatment. 1 – 40% water-ethanolic extraction; 2 – 70% water-ethanolic extraction; 3 – 96% water-ethanolic extraction; 4 – *N. sativa* L. herb tincture; 5 – SS rutin.

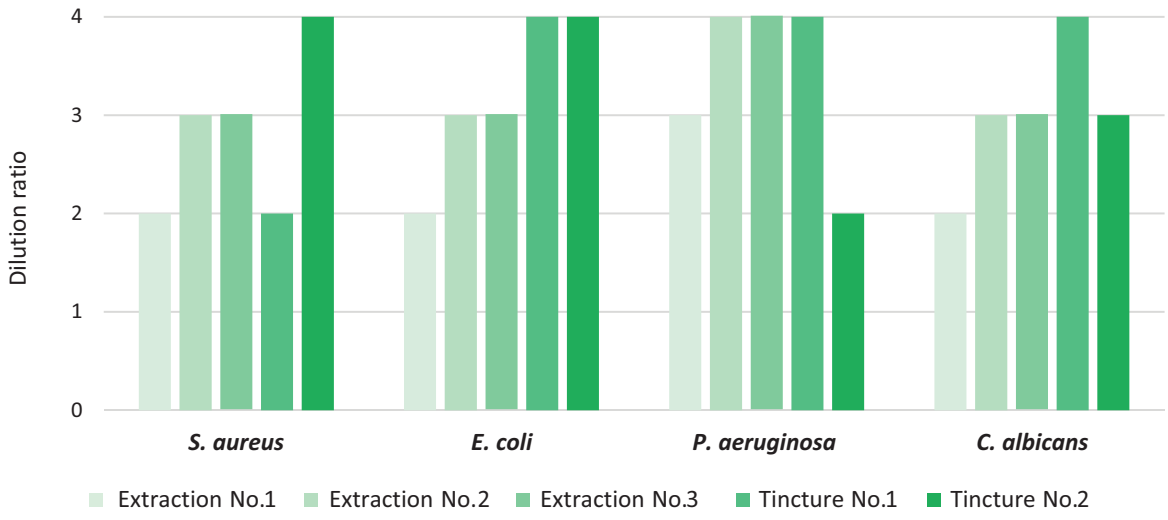

Figure 3 – Comparative diagram of the antibacterial activity of water-ethanolic extractions of *N. sativa* L. herbs and the reference preparation

Note: extraction 1 – 40% water-ethanolic extraction; extraction 2 – 70% water-ethanolic extraction; extraction 3 – 96% water-ethanolic extraction; tincture 1 – *N. sativa* L. herbs tincture; tincture 2 – eucalyptus tincture.

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Table 1 – Testing results of extractions from *N. sativa* L. herbs and reference preparation

Object	Dilution ratio						
	1	2	3	4	5	6	7
	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Staphylococcus aureus							
40% *N. sativa* L. herbs	–	–	+	+	+	+	+
70% *N. sativa* L. herbs	–	–	–	+	+	+	+
96% *N. sativa* L. herbs	–	–	–	+	+	+	+
70% *N. sativa* L. herbs tincture	–	–	+	+	+	+	+
70% eucalyptus tincture (reference preparation)	–	–	–	–	+	+	+
Escherichia coli							
40% *N. sativa* L. herbs	–	–	+	+	+	+	+
70% *N. sativa* L. herbs	–	–	–	+	+	+	+
96% *N. sativa* L. herbs	–	–	–	+	+	+	+
70% *N. sativa* L. herbs tincture	–	–	–	–	+	+	+
70% eucalyptus tincture (reference preparation)	–	–	–	–	+	+	+
Pseudomonas aeruginosa							
40% *N. sativa* L. herbs	–	–	–	+	+	+	+
70% *N. sativa* L. herbs	–	–	–	–	+	+	+
96% *N. sativa* L. herbs	–	–	–	–	+	+	+
70% *N. sativa* L. herbs tincture	–	–	–	–	+	+	+
70% eucalyptus tincture (reference preparation)	–	–	+	+	+	+	+
Candida albicans							
40% *N. sativa* L. herbs	–	–	+	+	+	+	+
70% *N. sativa* L. herbs	–	–	–	+	+	+	+
96% *N. sativa* L. herbs	–	–	–	+	+	+	+
70% *N. sativa* L. herbs tincture	–	–	–	–	+	+	+
70% eucalyptus tincture (reference preparation)	–	–	–	+	+	+	+

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

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

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

Note: "+" presence of microorganism growth; "**–**" – absence of microorganism growth.

Assessment of experimental results

The results were assessed visually by the presence/absence of the microorganisms growth in the test tubes with the appropriate dilutions of the test samples. The minimum inhibitory concentration was the lowest concentration of the studied sample, which completely suppressed the growth of the microorganisms strain. Herewith, according to the requirements of the "Guidelines" (Gs. 4.2.1890-04)[7] 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)[8]. The presence of turbidity and the detection of a small number of microorganisms (one colony) were not taken into account when registering the experimental result. The experiment was repeated three times.

RESULTS

The results of the qualitative chromatographic study revealed a number of features of the chromatographic profiles of the studied objects. For all the water-ethanolic extractions and the *N. sativa* herbs tincture, the adsorption zones typical for flavonoids of dark yellow and green color with Rf_1 = 0.28; Rf_2 = 0.15; Rf_3 = 0.11, were revealed (Fig. 1). It is notified that the most informative are the chromatograms viewed at the wavelength of 365 nm before and after the treatment with an alcohol solution of $AlCl_3$ and DSA (Fig. 2). Under the action of the $AlCl_3$ alcohol solution, the fluorescence of the adsorption zones increases, indicating the phenolic nature of these compounds. The DSA solution oxidizes organic compounds from yellow-orange to brick red in the visible light (Fig. 2).

The rutin adsorption zone had *Rf* = 0.20, which is close to the corresponding *Rf* values in the studied objects, especially in the *N. sativa* herbs tincture. Herewith it is noted that more adsorption zones of various kinds of nature are observed in the *N. sativa* herbs tincture. And the intensity of these zones glow is higher in 70% of the water-alcohol extract and the *N. sativa* herbs tincture, respectively.

The antimicrobial activity screening of water-ethanolic extractions from the *N. sativa* L. herb, and comparing them with the eucalyptus tincture (a reference preparation), made it possible to obtain the following data.

When testing 40% of the water-ethanolic extraction (1:30) from the *N. sativa* L herbs, the antimicrobial activity against the *S. aureus, E. coli* and *C. albicans* strains in a four-fold dilution, as well as against the *P. aeruginosa* microorganism in an eight-fold dilution was observed (Table. 1). When comparing 40% of the water-ethanolic extraction with the "negative" standard (a minimum inhibitory concentration for 40% water-ethanol), there was a slight difference in the antimicrobial activity between the test sample and the "negative" standard, which showed a slightly higher activity against *E. coli* and *C. albicans* (Table 2). This fact 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 a 70% water-ethanolic extraction (1:30) from the *N. sativa* L. herbs, the antimicrobial activity was expressed against the *S. aureus, E. coli* and *C. albicans* strains in the eight-fold dilution; against *P. aeruginosa* – in the 16-fold dilution (Table 1). When compared with the "negative" standard of ethanol at the 70% concentration, there was an increase in antimicrobial properties and a growth suppression of the *P. aeruginosa* microorganisms.

The 96% water-ethanolic extraction from the *N. sativa* L. herbs (1:30) showed a similar antimicrobial activity in the 70% water-ethanolic extraction: against *S. aureus*, *E. coli* and *C. albicans* strains – in an eight-fold dilution, against *P. aeruginosa* – in a 16-fold dilution.

When compared with the "negative" standard of ethyl alcohol at the concentration of 96%, a significant growth inhibition of the *P. aeruginosa* microorganism is notified. Respectively, for the concentrations of 70% and 96% water-ethanolic extraction from the *N. sativa* L. herbs (1:30), a significant suppression of the growth of the *P. aeruginosa* microorganisms is observed.

The antimicrobial activity of the reference preparation – the 70% water-alcohol eucalyptus tincture, shows its high activity against *S. aureus* and *E. coli* – reducing the growth of microorganisms in a 16-fold dilution. A similar activity in 70% and 96% extractions from the *N. sativa* L. herbs against the *C. albicans* strain in an eight-fold dilution and a weak activity against the *P. aeruginosa* strain – suppression of a microbial growth only in a 4-fold dilution (Table 1). This fact favourably allocates an action direction of the biologically active substances based on the *N. sativa* L. herbs.

The tested 70% *N. sativa* L. herbs tincture (the tincture from the *N. sativa* L. herbs -to-70% ethanol ratio of 1:5) showed the following results. Similar to the extractions in 70% and 96% ethyl alcohol, the antimicrobial effect was observed against the *P. aeruginosa* strain in a 16-fold dilution, however, besides this prevailing effect, the antimicrobial effect against the *E. coli* and *C. albicans* strains increased stopping the growth up to the 16-fold dilution. The effect against the *S. au-*

[7] Ibid.
[8] 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.

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reus strain, on the contrary, slightly decreased to the 4-fold dilution (Fig. 3).

The following results were obtained in the course of the comparison analysis of the preparation based on the *N. sativa* L. herbs tincture and the reference preparation – the 70% eucalyptus tincture. A higher antimicrobial effect was observed against the *P. aeruginosa* and *C. albicans* strains in the 16-fold dilution in the *N. sativa* L. herbs tincture. A similar antimicrobial effect in the studied tinctures was observed against the *E. coli* strain; the eucalyptus tincture showed a higher antimicrobial activity against the *S. aureus* strain (Table 1).

DISCUSSION

Currently, one of the serious factors affecting the drug therapy success of infectious diseases is the increased resistance of pathogenic microorganisms to antimicrobial agents[9]. Staphylococci or methicillin-resistant strains (MRSs) deserve special attention and are the cause of nosocomial and hospital-acquired infections.

Among the methicillin-resistant strains, *Staphylococcus aureus* (MRSA) is particularly common; its strains are resistant to many members of the β-lactam antibiotic group, including penicillins, cephalosporins, monobactams, carbapenems, etc. [21, 23, 24]. An equally dangerous strain is the Gram-negative bacterium *E. coli*, which is present in the human intestine and can cause various infectious diseases of the gastrointestinal tract and urogenital system [25].

The potential of the vegetative plant parts (leaves, branches, and stem) of *N. sativa* L. to reduce the load on the reproductive part (seeds) was also studied by researchers from Pakistan, who performed, in addition to phytochemical screening, antibacterial and antioxidant analyses, as well as the GC-MS analysis of superpotent extracts [26]. The dried plant was subjected to the extraction by the separation method in a series of concentrations ranging from 1.562 to 200 mg/ml in different solvents. The antibacterial analysis was performed on the pathogenic strains – *Clostridium difficile*, *Pasteurella sp.*, *Pseudomonas aeruginosa*, and *Xanthomonas sp*. The isolates were subcultured on the plates with nutrient agar and incubated at 37°C for 24 hrs. The analysis was performed by diffusion into the agar disk [23, 26]. The inhibition zones were measured in the agar wells containing a plant extract. The analysis was performed for methanol, chloroform, n-hexane, n-butanol, aqueous and ethyl acetate extracts at the concentrations of 1.56-200 mg/ml. The inhibition zones were measured after 24–48 hrs. The results showed that the maximum inhibition zone (40±1.73 mm) was observed for *Xanthomonas stutzeri* among all extracts, and a 100 mg/ml chloroform extract caused the maximum inhibition zone for the growth of *Xanthomonas stutzeri* bacteria (36±1.26 mm) among all the strains. The chloroform extract at the concentration of 50 mg/ml also showed a maximum inhibition of the bacterial growth, and exactly the same pattern was observed for all the concentrations, extracts and bacterial strains [26]. In addition to the above spectrum of the pharmacological activity, other authors often mention antidiabetic and antioxidant activities of the *Nigella sativa* L. herbs [27, 28].

Given the fact of previously described studies on the antimicrobial activity of the extracts from the *N. sativa* L. herb, its component composition and the dominant group of biologically active compounds (BAC) should be also discussed and clarified. Thus, many researchers refer to the work by foreign scientists of the Biotechnology Center (Tunisia), which describes the study of the *N. sativa* L. herbs by HPLC from absolute methanol extracts. These extracts identified 14 phenolic compounds with a total content of 215 mg/100 g in the shoots, among which the dominant group is phenolcarboxylic acids, represented mainly by gallic and vinyl acids [29]. In addition, the authors established the antimutagenic activity of the studied extracts from the *Nigella sativa* L. shoots using Ames test [8, 29].

In the *Nigella sativa* L. herbs, as described above, the phenolic compounds are mainly represented by phenolcarboxylic acids and a sum of flavonoids. Probably due to the free phenolic hydroxyl groups as in the gallic acid molecule, they have a potential for the antimicrobial activity [30]. The antimicrobial activity of these compounds was also discussed by the researchers from Pyatigorsk in the study on the chemical composition and antimicrobial activity of the dry extract from the flowers of (*Tagetes patula* L. The authors explain the bactericidal effect of the studied extract on the coccus and spore-forming flora, *Salmonella gallinarum* and *Pseudomonas aeruginosa*, and the bacteriostatic effect against *Escherichia coli* with a high content of gallic acid [31]. The authors' previous phytochemical study of the *Nigella sativa* herbs composition by differential and direct spectrophotometry at the wavelengths of 254 and 365 nm, confirms the dominance of phenolic substances [32]. In addition, the TLC analysis of water-ethanolic extractions and the *N. sativa* L. herbs tincture after the treatment with a specific reagent for flavonoids – an alcohol solution of $AlCl_3$ clearly demonstrates the qualitative composition of the studied samples (Fig. 1, Fig. 2). The specificity mechanism of the reagent interaction is due to the formation of bathochromic complexes with free 3- and 5-hydroxy groups of flavonoids. This fact enhances the fluorescence of the corresponding adsorption zones at the wavelength of 365 nm [33].

[9] Determination of the sensitivity of microorganisms to antibacterial drugs. Guidelines. MUK 4.2.1890-04. Russian

In the course of the screening analysis of the antimicrobial activity of water-ethanolic extractions from the *N. sativa* L. herbs, the conditions for obtaining the dosage form of the tincture were determined. As an extractant for the manufacture of the *N. sativa* L. herbs tincture from, a 70% concentration of ethyl alcohol was chosen, since this concentration is the optimal extractant for this raw material containing a complex of biologically active substances of the flavonoid group. It also contains the extractions of a valuable terpenoid gamma – together providing the observed antimicrobial effect [23, 26].

The choice in favor of the 70% alcohol concentration as an extractant for obtaining the *N. sativa* L. herbs tincture was made based on the fact that in the dosage forms with these extraction parameters, the greatest antimicrobial effect is observed against the studied strains of microorganisms, especially against the *P. aeruginosa* strain. In addition to the main directed action against the *P. aeruginosa* strain, the action against the other strains – *E. coli* and *C. albicans* – is enhanced. Also, the extracts at the given alcohol concentration have a better penetrating ability into the deep layers of the epidermis in comparison with higher and lower alcohol concentrations [21, 32].

Taking into consideration the fact that the *N. sativa* L. herb has a large phytomass in comparison with seeds and is subjected to utilization at the seed collection, its collection will help to carry out a complex and versatile use of all the raw materials of the plant and will promote new medicinal herbal preparations.

CONCLUSION

Therefore, a phytochemical screening and a comparative study were carried out to research the antimicrobial activity of the experimental water-ethanolic extractions from the *N. sativa* L. herbs *in vitro*, as a result of which flavonoids were found as the main group of BAC. An antimicrobial effect was also established on the pathogenic strains of such microorganisms as *P. aeruginosa*, *E.* coli, *C. albicans*, *S. aureus*. It was found out that all the studied samples of the water-alcoholic extractions from the *N. sativa* L. herbs give a stable prevailing antimicrobial effect against the *P. aeruginosa* strain.

The *N. sativa* L. herbs tincture in the 70% ethyl alcohol (1:5) has a specific effect on the-*Pseudomonas aeruginosa* strain. In the proposed dosage form, the activity against the *Escherichia coli* and *Candida albicans* strains also increases, which is comparable to the eucalyptus tincture–used in medical and pharmaceutical practice. The results obtained in the study can serve as a basis for the creation of new antibacterial drugs based on the *N. sativa* L. herb, as well as for a further implementation of the preparations from the *N. sativa* L. tincture in the 70% ethyl alcohol in medical and pharmaceutical practice.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

ARM – collecting plant materials for analysis, experiment conducting, analyzing and interpreting the data obtained, analyzing the literature, manuscript writing and finally approving of it for publication; VAK – final approval of the manuscript for publication, processing the results obtained, verification of critical intellectual content; EVA – participation in the development of the research concept and design, critical analysis of the research results; SDK – planning the study, participation in the experiment; AVZ – participation in the description and analysis of the results obtained, writing the manuscript.

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AUTHORS

Artur R. Mubinov – postgraduate student of the Department of Pharmacognosy with Botany and the basics of Phytotherapy, Senior Specialist of «Centre for Research and Education in Pharmacy», Samara State Medical University. ORCID ID: 0000-0002-4791-4606. E-mail: a.r.mubinov@samsmu.ru

Vladimir A. Kurkin – Doctor of Sciences (Pharmacy), Professor, Head of the Department of Pharmacognosy with Botany and the basics of Phytotherapy, Samara State Medical University ORCID ID: 0000-0002-7513-9352. E-mail: v.a.kurkin@samsmu.ru

Elena V. Avdeeva – Doctor of Sciences (Pharmacy), Professor of the Department of Pharmacognosy with Botany and the basics of Phytotherapy, Samara State Medical University. ORCID ID: 0000-0002-8399-9328. E-mail: e.v.avdeeva@samsmu.ru

Svetlana D. Kolpakova – Doctor of Sciences (Medicine), Professor of the Department of General and Clinical Microbiology, Immunology and Allergology, Samara State Medical University. ORCID ID: 0000-0001-9358-4436. E-mail: Sdkolpakova@mail.ru

Alexander V. Zhestkov – Doctor of Sciences (Medicine), Professor, Head of the Department of General and Clinical Microbiology, Immunology and Allergology, Samara State Medical University. ORCID ID: 0000-0002-3960-830X. E-mail: avzhestkov2015@yandex.ru