Turkish Online Journal of Qualitative Inquiry (TOJQI)
Volume 12, Issue 10, October 2021: 820-827

DETERMINATION OF ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL FROM LAVENDER ANGUSTIFOLIUS INFLORESCENCES

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Resume. The aim of this work was to study the antimicrobial activity of essential oil from the inflorescences of lavender angustifolia. Antimicrobial activity was determined by the sensitivity of test cultures by diffusion in a solid nutrient medium. Diameter of the zone of inhibition of bacterial growth in mm - St. aureus - 28.0, St. epidermidis 26.0, E. coli 30.0, C. albicans 22.0, Bacillus subtilis 33.0. Based on the above, we can conclude that the essential oil from the inflorescences of narrow-leaved lavender has a pronounced antimicrobial effect against gram-positive cocci, bacilli and yeast-like fungi of the genus Candida, it can be recommended for the manufacture of wound healing medicines.

Keywords. angustifolia, Candida, microbes, viruses, fungi, herbal medicines.

Human life is closely connected with the plant world. In the course of evolutionary development, the human body has adapted to proteins, carbohydrates, fats and the most diverse biologically active substances (vitamins, macro and microelements, organic acids, etc.) of plant origin, without which the normal course of life processes and the development of the body as a whole are impossible. As a result of this symbiosis, some plants eventually began to serve humans as food, others as medicine.

Despite the great success in the creation of synthetic medicines, the use of herbal medicines in world medical practice not only remains stable, but also tends to increase. About half of all drugs produced in industrialized countries are obtained from natural raw materials.

Under these conditions, scientifically grounded resource management and resource conservation are becoming one of the main and decisive factors in meeting the growing needs for plant raw materials.

Due to the diverse geographical and climatic conditions, Uzbekistan is the richest region for the concentration of medicinal plants, among which there are many species that are of interest for scientific medicine.

Plants are a source for obtaining medicinal substances. Plants are an inexhaustible storehouse of natural medicinal raw materials. Throughout the history of mankind, plants have been used by people for medicinal purposes. Each plant is a kind of factory in which the synthesis of a wide variety of rare and useful substances for humans takes place. Many medicinal plants have passed through the centuries, gave health to tens of generations. The world of plants is the greatest miracle of nature, our healing wealth and the kingdom of beauty.

Nature has created numerous factors that are pathogenic for humans. However, against each disease-causing factor - from the slightest, invisible to the naked eye (microbes, viruses, fungi, protozoa, etc.), to more (injuries, wounds, etc.) - she armed a person with numerous protective and healing mechanisms. These include more than 1000 biologically active substances of the most diverse nature.

In our body, it is difficult to find such structures, organs or cells, which would not be actively influenced by dozens of biologically active substances of natural origin. Apparently, there is no disease in nature, against which healing substances would not be formed in the plant world.

Every year, a person becomes more and more convinced that in nature itself - in the great cycle of life processes, there is a solution to many problems associated not only with the occurrence of individual diseases, but also with the problem of their drug therapy. Every year people penetrate deeper and deeper into the secrets of the plant world. Thanks to tireless and purposeful scientific research, the plant world is gradually revealing its treasures to humans. Hundreds of herbal medicines developed and widely used in medical practice are a convincing example of the solution to many problems associated with the treatment and prevention of intractable diseases must be sought in nature itself. Only the painstaking work of botanists, microbiologists, chemists, pharmacologists and specialists in other branches of medicine, armed with modern scientific technology, can more fully reveal the healing properties of dozens of plants that are not yet included in a number of medicines.

However, the medicinal flora has not been fully investigated, even the studied plants are rarely used in clinical practice. This is primarily due to insufficient knowledge of the properties of many drugs, leading to a decrease in interest in herbal medicine.

According to modern concepts, a herbal medicine is a whole biogenetically developed complex that includes active biological acting substances and other secondary metabolites, proteins, essential oils, chlorophyll, trace elements, inorganic salts, vitamins, etc.

There is an opinion that such a complex, formed in a living cell, has a greater resemblance to the human body than an isolated, chemically pure active substance, is easier to assimilate and gives fewer side effects. At the same time, in modern pharmacology, some biologically active substances of plants are often used: alkaloids, essential oils, organic acids, vitamins, tannins, resins, mucus, phytoncides, etc. At the same time, the study of the therapeutic activity of medicinal plants has shown the feasibility of their use in medical practice without chemical treatment in the form of infusions, decoctions, tinctures, etc.

Lavender essential oil has the ability to eliminate nervous tension, relieve pain, disinfect the skin, improve blood circulation and treat respiratory diseases.The available raw materials and the results of preliminary pharmacological studies that open the prospects for using lavender as an effective anti-inflammatory agent indicate the relevance and advisability of a comprehensive study of this plant.

The purpose of this work was to determine additional pharmacological efficacy - the antimicrobial activity of essential oil from the inflorescences of angustifolia lavender. As you know, drugs that are not sterilized during the production process can be contaminated with microorganisms and therefore

are subject to testing for microbiological purity. Taking into account the noted circumstance, we investigated the indicator of microbiological purity of narrow-leaved lavender inflorescences in order to characterize the quality of domestic raw materials.

Materials and methods: the object of the study was the inflorescences of narrow-leaved lavender. Testing for microbiological purity in accordance with the requirements of the SP XI [2] included the quantitative determination of viable bacteria and fungi, as well as the identification of certain types of microorganisms, the presence of which is unacceptable in non-sterile medicines. It was carried out by the official two-layer agar method in Petri dishes with a diameter of 90-100 mm. A 10 g sample of the raw material was suspended in phosphate buffered saline (pH 7.0) so that the final volume of the suspension was 100 ml.

Determination of the total number of bacteria. The prepared suspension of the sample was introduced into each of two test tubes with 4.0 ml of molten thioglycol medium cooled to a temperature of 45^0 to 50^0C. The contents of the test tube were quickly mixed and transferred into a Petri dish containing 15-20 ml of the appropriate nutrient medium. The top layer of agar was evenly distributed by rapidly rocking the Petri dish. After solidification of the medium, the dishes were turned over and incubated for 5 days at a temperature of 35^0C. The crops were examined daily. After 48 hours and finally after 5 days, the number of bacterial colonies on two plates was counted, the average value was found, and, multiplying by the dilution index, the number of microorganisms in 1 g of the sample was calculated. To obtain reliable results, only those dishes were taken into account, on which from 30-300 colonies grew.

Determination of the total number of fungi. The test was carried out by the agar method described above using Sabouraud's medium. The inoculations were incubated for 5 days at a temperature of 25 to 32.5^0C. After 72 hours and finally after 5 days, the total number of colonies of yeast and mold fungi on two plates was counted, the average value was found and, multiplying by the dilution index, i.e. by 10, the number of fungi in 1 g of the sample was calculated. All fungal colonies were counted on the plate, even if their number was less than 30.

To detect and identify bacteria of the family Enterobacteriaceae, a sample of raw materials in the amount of 10.0 g was introduced into 100 ml of nutrient medium №3, mixed and incubated at a temperature of 30 to 35^0C for 24-48 hours. Taking into account the presence of growth, a loop was subcultured on media №4 (Endo agar) and №5 (bismuth sulfite agar), poured into Petri dishes. The inoculations were incubated at a temperature of 30 to 35^0C for 24-48 hours. Since no colonies corresponding to the morphological characteristics of bacteria of the Enterobacteriaceae family were observed after incubation on media №4 and №5, it was concluded that they were absent in the test sample (Table 1).

Table 1

Indicators of microbiological purity of narrow-leaved lavender inflorescences:

	Requirements of regulatory documents	Test results
1	The total number of aerobic bacteria (in 1 g of the sample) is no more than 107 (in total).	160 CFU

2	The total number of yeasts and molds (per gram of the sample) is no more than 105 (in total).	100 CFU
3	Bacteria of the Enterobacteriaceae family - must be absent	Absent
4	Escherichia.coli - must be absent in 1 g.	Absent
5	Bacteria of the genus Salmonella - must be absent in 10 g.	Absent

Based on the data obtained, it can be concluded that the inflorescences of narrow-leaved lavender fully comply with the requirements for medicinal plant raw materials in relation to its microbiological purity.

Further research was aimed at obtaining essential oil. Essential oil from the inflorescences of narrow-leaved lavender was obtained in cooperation with the staff of the Department of Pharmacognosy of the Tashkent Pharmaceutical Institute. The quantitative content of essential oils was determined according to the requirements of SP XI [2].

For the inflorescences of narrow-leaved lavender, the optimal conditions for the quantitative determination of essential oil were selected. The research results are presented in tables 2 and 3.

Table 2

Essential oil yield depending on the weight,%

Hinge weight, g	Essential oil yield,%
10,0	1,5±0,02
20,0	2,0±0,03
30.0	1,9±0,02

From the results presented in table 2 it can be seen that the maximum yield of essential oil is observed when using a sample of raw materials weighing 20.0 g.

The next stage of research was to determine the dependence of the yield of essential oil on the distillation time. For this, the essential oil was obtained for 1 hour, 1.5 hours, 2.0 hours. The results are shown in Table 3.

Table 3

Essential oil yield depending on the distillation time,%

P/p №	Distillation time, h	Essential oil yield, %
1.	1,0	1,5±0,03
2.	1,5	1,9±0,04
3.	2,0	2,0±0,02
4.	2,5	1,8±0,03

From the results presented in table 3, it can be seen that the maximum yield of essential oil is observed at a distillation time of 2.0 hours. Based on the tests carried out, it was found that the maximum yield of essential oil is observed at a distillation time of 2 hours and at a weight of 20.0. A further increase in the distillation time and the weight of the sample did not lead to a significant increase in the yield of essential oil.

The study of the antimicrobial activity of the obtained essential oil was carried out jointly with the staff of the bacteriological laboratory of LLC Scientific Center for Standardization of Medicines, in accordance with the requirements of the SP XI [2] and the "Guidelines for quality control of laboratory research" [3].Antimicrobial activity was determined by the sensitivity of test cultures of microorganisms by diffusion in a dense nutrient medium [4]. For laboratory studies, an 18-hour agar culture of microorganisms was used, diluted in sterile saline, standardized according to the McFarland standard of 0.5 and additionally diluted 10 times with sterile saline to a concentration of 107 microbial bodies / ml. Sowing on a solid nutrient medium was carried out by the "lawn" method.

We used a modified hole method (A.M. - T. Bektemirov, 2007), after sowing test strains of microorganisms with a lawn on a dense nutrient medium, with a metal punch with a diameter of 5.0 mm. wells were made into which 0.1 ml was added the investigational drug.Stock solutions of control and test samples were prepared in sterile solvents with a concentration of 1 mg/ml. To reduce the influence of fluctuations in time between the instillation of the solutions used in the experiment, after their introduction, the cups were kept at room temperature for 1-2 hours.Petri dishes were placed in a thermostat at 370C for 18-24 hours. The results were recorded visually - according to the size of the inhibition zone of the microorganisms growth around the wells. An isotonic sodium chloride solution was used as a control.

Before use, the strains of microorganisms taken from the preserving nutrient medium were twice subcultured on nutrient media corresponding to each taxonomic group of bacteria. Test cultures were identified by cultural, morphological, tinctorial, enzymatic-biochemical and antigenic properties. The main registration and passport data characterizing the properties of test cultures of microorganisms are presented in Table 4.

Table 4

List of strains of microorganisms used to determine antimicrobial activity

№	Name	Registration number	Morphology, tinctorial properties	Enzymatic properties	A source excretions	Where did the strain come from
1	St. aureus	ATCC 25923	gram-positivecocci	typical	museum culture	Collection of Scientific Center for Standardization of Medicinal Products LLC

2	St. epidermidis	ATCC	gram-positivecocci	typical	the museum	Collection of Scientific Center for Standardization of Medicinal Products LLC
3	Bacillus subtilis	ATCC 6633	gram-positive bacilli, strep-tobacilli, central spores	typical	museum culture	Collection of Scientific Center for Standardization of Medicinal Products LLC
4	E.coli	ATCC 25922	gram-negative sticks	typical	museum culture	Collection of Scientific Center for Standardization of Medicinal Products LLC
5	C albicans	ATCC 885653	Gram-positivebuddingdrusen	typical	museum culture	Collection of Scientific Center for Standardization of Medicinal Products LLC

The diameter of the zone of inhibition of bacterial growth is shown in Table 5

Table 5

The diameter of the zone of inhibition of bacterial growth, mm.

	St. aureus (mm.)	St. epidermidis (mm.)	E.coli (mm.)	C.albi-cans (mm.)	Bacillussubtilis (mm.)
essential oil from the inflorescences of lavender angustifolia	28.0	26.0	30.0	22.0	33.0
The control	6.0	6.0	6.0	6.0	6.0

As can be seen from table 5, the essential oil from the inflorescences of lavender angustifolia has an antimicrobial effect against gram-negative rods, gram-positive cocci, bacilli and yeast-like fungi of the genus Candida.

Conclusions: on the basis of the above, we can conclude that the studied essential oil from the inflorescences of narrow-leaved lavender has a pronounced antimicrobial effect against gram-negative bacilli, gram-positive cocci, bacilli and yeast-like fungi of the genus Candida. Therefore, we can recommend the essential oil from the inflorescences of lavender angustifolia for the manufacture of wound healing medicines.

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