GALAXY INTERNATIONAL INTERDISCIPLINARY RESEARCH JOURNAL (GIIRJ)
ISSN (E): 2347-6915
Vol. 10, Issue 4, April. (2022)

# COMPARATIVE STUDY OF THE ANTIMICROBIAL PROPERTIES OF ESSENTIAL OILS OF MEDICINAL PLANTS.

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## ABSTRACT

The purpose of this work was to study the microbiological purity and antimicrobial activity of essential oils of medicinal plants. Determination of the antimicrobial activity of essential oils was carried out by diffusion into agar against certain types of bacteria; Escherichia coli, Bacillus subtilis, Staphylococcus aureus and yeast-like fungi of the genus Candida. Essential oil from narrow-leaved lavender inflorescences showed antimicrobial activity against Escherichia coli - 30.0 mm. diameter of the zone of growth suppression, Staphylococcus aureus - 28.0 mm., Candida albicans -22.0 mm. Basil essential oil showed activity against Escherichia coli - there was no growth of bacteria on the dishes, Candida albicans - 36.0 mm., Staphylococcus aureus - 25.0 mm.. Based on the above, we can conclude that from the studied essential oils, the essential oil of basil showed a pronounced antibacterial activity compared with the essential oil from the inflorescences of angustifolia lavender.

**Keywords**: test culture, inhibition, taxonomy, identification, tinctorial properties, strain, bacilli, cocci, bacteria, fungi.

## INTRODUCTION

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

Lavender essential oil has the ability to eliminate nervous tension, relieve pain, disinfect the skin, improve blood circulation and treat respiratory diseases. Medicinal herbs containing complexes of antibiotic substances represent one of the richest sources of antibiotics for medical practice. The essential oil of basil, like the plant from which it is extracted, deserves its title of "royal", which was assigned to it thousands of years ago. In the ancient world, basil was considered the king of all plants. Basil relieves symptoms and promotes recovery from respiratory diseases, including bronchitis, asthma, and difficult breathing, especially if colds are accompanied by high temperature.

The available raw material base and the results of preliminary pharmacological studies, which open up prospects for the use of lavender and basil as effective anti-inflammatory agents, indicate the relevance and expediency of a comprehensive study of these plants. The purpose of this work was a comparative study of additional pharmacological efficacy - the antimicrobial activity of the essential oil from the inflorescences of angustifolia lavender and the essential oil of basil. As is known, medicinal products 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 have studied the index

of microbiological purity of the inflorescences of angustifolia lavender and in order to characterize the quality of domestic raw materials.

Materials and methods The objects of the research were inflorescences of angustifolia lavender. The test for microbiological purity according to the requirements of SP XI [1] 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 raw materials was suspended in a phosphate buffer solution (pH 7.0) so that the final suspension volume was 100 ml.

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

Determination of the fungi total number. 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 $32.5^0$C. After 72 hours and finally after 5 days, the total number of yeast and mold colonies on two dishes was counted, the average value was found and, multiplying by the dilution index, i.e. by 10, the number of fungi per 1 g of the sample was calculated. All fungal colonies were taken into account on the dish, even if their number was less than 30. To detect and identify bacteria of the Enterobacteriaceae family, a sample of raw materials in the amount of 10.0 g was added to 100 ml of nutrient medium No. 3, mixed and incubated at a temperature of 30 to $35^0$C for 24-48 hours. Taking into account the presence of growth, resetting was done with a loop on medium No. 4 (Endo agar) and No. 5 (bismuth sulfite agar), poured into Petri dishes. The inoculations were incubated at a temperature of 30 to $35^0$C for 24-48 hours. Since after incubation on media No. 4 and No. 5 no colonies were observed corresponding to the morphological characteristics of bacteria of the Enterobacteriaceae family, it was concluded that they were absent in the test sample (Table 1).

Table 1 Indicators of microbiological purity of angustifolia lavender inflorescences:

| | Regulatory Requirements | Test results |
|---|---|---|
| 1 | The total number of aerobic bacteria (in 1 g of the sample) - no more than 107 (in total). | 160 CFU |
| 2 | The total number of yeast and mold fungi (in 1g sample) - no more than 105 (in total). | 100 CFU |
| 3 | Bacteria of the family Enterobacteriaceae – must be absent | Missing |
| 4 | Escherichia.coli - must be absent in 1 g. | Missing |
| 5 | Bacteria of the genus Salmonella - must be absent in 10 g. | Missing |

Based on the data obtained, it can be concluded that the inflorescences of angustifolia lavender fully meet the requirements for medicinal plant raw materials in terms of its microbiological purity.

Further research was aimed at obtaining essential oil. Essential oil from the inflorescences of angustifolia lavender was obtained jointly 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 [1]. For the inflorescences of angustifolia lavender, the optimal conditions for the quantitative determination of essential oil were selected. The results of the study are presented in tables 2 and 3.

Table 2 Yield of essential oil depending on sample, %

| Sample, 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 time of distillation. For this, the essential oil was obtained within 1 hour, 1,5 hours, 2 hours. The results are shown in table 3.

Table 3 Yield of essential oil depending on distillation time, %

| No. | 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 hours. Based on the tests carried out, it was found that the maximum yield of essential oil is observed with a distillation time of 2 hours and with a sample of 20.0. A further increase in the distillation time and sample weight did not lead to a significant increase in the yield of essential oil. Basil essential oil is obtained from the leaves and flowers of the spicy Basil plant by steam distillation. To obtain 1 kg of essential oil, 100 kg of raw materials are required. The studied preparation of basil essential oil for research was provided to us by the staff of the Department of Pharmacognosy of the Pharmaceutical Institute.

The study of the antimicrobial activity of the obtained essential oil was carried out jointly with the employees of the bacteriological laboratory of the LLC "Scientific Center for Standardization of Medicinal Products", in accordance with the requirements of SP XI [1] and the "Guidelines for Quality Control of Laboratory Research". Antimicrobial activity was determined by the sensitivity of test cultures of microorganisms by diffusion in a dense nutrient medium [2]. 15 ml each was poured into Petri dishes placed on tables with a strictly horizontal surface 3% meat-peptone agar (MPA) [3]. After the agar solidified, the dishes were dried in a

thermostat, then poured by 5 ml. For laboratory studies, an 18-hour agar culture of microorganisms 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 $10^7$ microbial bodies/ml was used. Inoculation on a dense nutrient medium was carried out by the "lawn" method. [4]

A modified hole method was used (A.M.-T.Bektemirov, 2007), after inoculation of 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 of the studied drug was added. Stock solutions of control and test samples were prepared in sterile solvents at a concentration of 1 mg/mL. To reduce the effect of fluctuations in time between instillation of the solutions used in the experiment, after their introduction, the dishes were kept at room temperature for 1-2 hours. Petri dishes were placed in a thermostat at $37^0$ C for 18-24 hours. The results were recorded visually - by the size of the zone of inhibition of the microorganisms growth around the holes. An isotonic sodium chloride solution was used as a control. Before use, microorganism strains taken from the retaining nutrient medium were subcultivated twice on nutrient media appropriate for 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 | Allocation source | Where did the strain come from |
|---|---|---|---|---|---|---|
| 1 | St. aureus | ATCC 25923 | Gram-positive cocci | typical | museum culture | Collection LLC "Scientific Center for Standardization of Medicinal Products" |
| 2 | St. epidermidis | ATCC | Gram-positive cocci | typical | museum | Collection LLC "Scientific Center for Standardization of Medicinal Products" |
| 3 | Bacillus subtilis | ATCC 6633 | gram-positive bacilli, strep-tobacilli, central spores | typical | museum culture | Collection LLC "Scientific Center for Standardization of Medicinal Products" |
| 4 | E.coli | ATCC 25922 | gram-negative bacilli | typical | museum culture | Collection LLC "Scientific Center for Standardization of Medicinal Products" |
| 5 | C albicans | ATCC 885653 | Gram-positive buddingdrusen | typical | museum culture | Collection LLC "Scientific Center for Standardization of Medicinal Products" |

The study of the antimicrobial activity of basil essential oil was carried out jointly with the staff

of the Institute of Microbiology of ASRUz. The studies were carried out in accordance with the requirements of the SP of the XI edition, [1] and the "Guidelines for Quality Control of Laboratory Research" (Tashkent, 2000). Antimicrobial activity was determined by the sensitivity of test - cultures of microorganisms by diffusion in a dense nutrient medium ("Determination of the sensitivity of microorganisms to antibacterial agents" SMC 4.2.1890-04 RF).

The studied sample: the object of the study was the essential oil of basil native and in 50% concentration (solvent-sterile distilled water)

Test cultures: Museum cultures were used as test strains: Bacillus subtilis, Pseudomonas aeruginosa, Enterobateraerogenes, Escherichia coli, Candida albicans, Staphylococcu saureus - from the collection of microorganisms of the laboratory "Genetics of lactic acid bacteria" Institute of Microbiology ASRUz.

Nutrient media: Nutrient agar was used to reconstitute the cultures, Muller-Hinton agar was used to test for antimicrobial activity. [5]

The study was carried out by the disk-diffusion method.

Inoculum preparation.To prepare the inoculum, we used the method of direct suspension in a sterile isotonic solution of colonies of a pure 18-hour culture of bacteria grown on nutrient agar. To do this, several morphologically similar colonies were collected with a sterile bacteriological loop and the resulting material was suspended in a sterile isotonic solution. The bacterial suspension was brought to a density of 0.5 according to the McFarland turbidity standard by adding microbial mass to the suspension or diluting it with a sterile isotonic solution. The suspension was used no later than 60 minutes after preparation. [6]

Dishes inoculation. The bacterial suspension was inoculated onto the agar no later than 60 minutes after preparation. To do this, 100 µl of the suspension was placed on the surface of a dish with Muller-Hinton agar using a dispenser and evenly rubbed over the surface of the agar with a glass bacteriological spatula to obtain a continuous lawn. [7]

Agar holes method. After complete diffusion of the suspension into the nutrient medium, holes were cut out in the thickness of the nutrient medium using a sterile metal hollow tube with a hole diameter of 8 mm. 100 µl of the test liquid was added to the formed hole. After the diffusion of the liquid into the agar, the dish was placed in a thermostat for 24 hours at 37$^0$C. The diameter of the growth inhibition zone around the holes with the test liquid was measured. [8]

Results and discussion

The diameter of the zone of inhibition of bacterial growth by essential oil from the inflorescences of angustifolia lavender is presented in Table 5.

Table 5 Diameter of the bacterial growth inhibition zone, in mm.

| | St. aureus (mm.) | St. Epidermidis (mm.) | E.coli (mm.) | C albi-cans (mm.) | Bacillus subtilis (mm.) |
|---|---|---|---|---|---|
| essential oil from the nflorescences of lavender angustifolia. | 28.0 | 26.0 | 30.0 | 22.0 | 33.0 |
| 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 angustifolia lavender has an antimicrobial effect against gram-negative bacilli, gram-positive cocci, bacilli and yeast-like fungi of the genus Candida.

It has been ascertained that the essential oil of basil has a pronounced antimicrobial activity against all test cultures under study. On the surface of the dishes with inoculated B. subtilis and E. coli, no growth of test cultures was observed, which shows the sensitivity of these strains to essential oil vapors (Table 6).

Table 6 Diameter of the inhibition zone of the test – cultures growth with essential oil of basil (in mm.)

| № | Test - culture | Suppression zone diameter, mm. | |
|---|---|---|---|
| | | 100% solution | 50% solution |
| 1 | Bacillus subtilis | no rise on the dish | |
| 2 | Pseudomonas aeruginosa | 35 | 23 |
| 3 | Enterobacteraerogenes | 30 | 25 |
| 4 | Escherichia coli | no rise on the dish | |
| 5 | Candida albicans | 50 | 36 |
| 6 | Staphylococcus aureus | 25 – b/c<br>35 – b/c | 20 – b/c<br>25 – b/c |

Despite the incomplete diffusion of the essential oil in the holes, high antimicrobial activity was observed and the diameter of the inhibition zones growth exceeded 20 mm. in all cases. In relation to Staphylococcus aureus, the sample has both bactericidal and bacteriostatic effects.

The diameter of the bacterial growth inhibition zone is presented in Table 7.

Table 7 Comparison of the diameters of the inhibition zone of bacterial growth, in mm.

| | St. aureus (mm.) | E.coli (mm.) | C albi-cans (mm.) | Bacillus subtilis (mm.) |
|---|---|---|---|---|
| essential oil from the inflorescences of lavender angustifolia. | 28.0 | 30.0 | 22.0 | 33.0 |
| Essential oil of basil | 25.0 | No growth on the dish | 36.0 | No growth on the dish |
| Control | 6.0 | 6.0 | 6.0 | 6.0 |

As can be seen from Table 7, the studied essential oils have a pronounced antimicrobial effect against gram-negative bacilli, gram-positive cocci, bacilli and yeast-like fungi of the genus Candida.

## CONCLUSION

based on the above, we can conclude that among the studied essential oils, basil essential oil showed a pronounced antimicrobial effect against gram-negative bacilli, bacilli and yeast-like fungi of the genus Candida compared to the essential oil from the inflorescences of angustifolia lavender. Against St. aureus essential oil of basil showed a pronounced antimicrobial effect.

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