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LAVANDULA ANGUSTIFOLIA MILL. OF UKRAINIAN ORIGIN: A COMPARATIVE STUDY OF THE CHEMICAL COMPOSITION AND ANTIMICROBIAL POTENTIAL OF HERB EXTRACTS

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Provide updated data on the antimicrobial activity and chemical composition of original dry extracts from Lavandula angustifolia herb of Ukrainian origin.

The aim *– provide new data on the antimicrobial activity of original dry extracts of lavender herb of Ukrainian origin and their chemical composition..*

Materials and methods. *The objects of the study are dry extracts obtained from the lavender herb with purified water and ethanol solutions (40 and 70 %). The main biologically active substances (BAS) of the extracts were determined by the Thin-layer chromatography and Absorption spectrophotometry methods. The microbiological properties of the test samples of the investigated plant extracts were studied in vitro by the two-fold serial dilutions method. The ability of microorganisms to form a biofilm was determined by the method of adhesion to polystyrene in flat-bottomed plastic plates. The optical density of the initial bacterial suspension was measured on the Densi-La-Meter device, and the density of inoculated bacterial cells on the Multiskan EX photometer at a wavelength of 540 nm. The study of the antimicrobial activity of water and ethanol extracts of lavender herb in a wide range of concentrations was carried out by the agar diffusion method in the "wells" modification, which is commonly used in microbiological practice.*

Results. *Water and water-ethanol extracts of lavender of Ukrainian origin were obtained. Terpenoids (linalool, linylyl acetate and traces of 1,8-cineol), flavonoids (hyperoside, isoquercitrin) and hydroxycinnamic acids (rosmarinic, chlorogenic acids) were identified in the extracts. The total content of phenolic compounds is 2.02–2.60 mg/g, flavonoids – 1.46–3.17 mg/g. The largest amount of BAS was extracted with 70 % ethanol. According to the results of experimental studies, the extracts of the lavender herb, obtained by extraction with a water-ethanol solution (40 and 70 % ethanol) at a concentration of 1 mg/ml, have antimicrobial properties against a wide range of infectious agents (S. aureus, E. coli, K. pneumoniae, P. aeruginosa, C. albicans). Studies of the influence of test samples of lavender extracts at a concentration of 1 mg/ml on the ability of microorganisms (S. aureus, E. coli, K. pneumoniae, P. aeruginosa) to form biofilms demonstrated that the highest inhibitory activity against biofilm formation was found in the case of the action of test of a sample of phytoextract obtained by extraction with a water-ethanol solution (40 % ethanol), which accounted for S. aureus – 57.8 %, P. aeruginosa – 66.7 %. A wide spectrum of antimicrobial action was established for the tested lavender phytoextracts under the conditions of application of the concentration range of 10–60 μg/ml. The best spectrum of antimicrobial action and the highest activity corresponds to the lavender extract, obtained by extraction with 70 % ethanol, with the effect depending on the concentration.*

Conclusion. *The lavender herb of Ukrainian origin is a promising and affordable source of potential antimicrobial active pharmaceutical ingredients (API). Water-ethanol lavender extract (70 % ethanol), according to research results, has shown high antimicrobial and antifungal potential. According to preliminary data, antimicrobial activity correlates with the content of phenolic compounds. The obtained results may be useful for the search for original substances for the complex correction of symptoms of neurological deficits of infectious etiology*

Keywords: *Lavandula angustifolia, extracts, chemical composition, antimicrobial activity, antifungal activity, biofilm formation of microbial cultures*

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1. Introduction

Lavender (*Lavandula angustifolia Mill.*) is one of the main essential oil crops grown in the world. Lavender essential oil products are widely used in the food, perfumery, cosmetic and pharmaceutical industries [1]. It is known that the essential oil has bactericidal and radioprotective properties, contains a wide range of biological active substances (BAS) [2].

In the pharmaceutical industry, lavender flowers are widely used, while the herb is a production waste, despite the large number of phenolic compounds in its composition [2]. One of the reasons for this today, we believe, is that the lavender herb is understudied. Many reviews have been devoted to the pharmacological action of lavender [3, 4], but the information presented mainly concerns lavender essential oil. Nevertheless,

there are modern studies that confirm the perspective of further experimental studies of the pharmacological potential of not only essential oil [5]. As for the terrestrial part of lavender, there are separate data in the literature about the revealed efficiency in treating brain tumors [6], dopaminergic and cholinergic neurotransmitters are associated with ejaculatory habits [7], lavender extracts reduce serum and ovarian cholesterol level, elicit estrogenic and anti-inflammatory properties [8]. Currently, the neurotropic properties of lavender preparations are mostly known. In Chinese traditional medicine, lavender herb extract is used as a neuroprotective agent and is considered effective in the treatment of Alzheimer's disease [9].

On the other hand, the use of lavender is often aimed at fighting infections [10, 11]. And it was this property that attracted our attention in view not only of the great problem of antibiotic resistance in the world [12, 13]. According to many scientists, the antimicrobial effect is also positive for the correction of neurodegenerative conditions, especially those caused by the influence of infections. The role of various microorganisms in the development of neurological diseases is known. In particular, experimental data show that lung infection caused by *Pseudomonas aeruginosa* causes systemic and neuroinflammation [14]. In turn, this is a potential mechanism of increased permeability of the blood-brain barrier (BBB) and behavioral disorders [14]. McLoughlin et al. [15] have demonstrated that brain microvascular endothelial cells permeability was induced due to *S. aureus* infection. It is shown that high mRNA expression levels of several cytokines and macrophage inflammatory proteins in *S. aureus* infected rat brain abscess models, have led to BBB disruption [16]. Authors [17] describe the neurologic and neuroradiologic complications of Shiga toxin producing *Escherichia coli* infection (STEC)-associated hemolytic-uremic syndrome (HUS) in adults. All but 1 of these 52 showed neurologic symptoms. Focal neurologic signs like double vision, difficulties in finding words, or hyperreflexia were present in 23, additional deficits in orientation, attention, memory, or constructive abilities in 9, and marked impairment of consciousness in 15. Appropriate antibiotic treatment is the key to survival in patients with bacterial CNS infection *K. pneumoniae* [18]. In view of the given data, the antimicrobial effect may be useful for correcting the symptoms of neurological deficits, especially those of infectious or unknown etiology.

Antimicrobial activity for lavender essential oil is well-known. Today, it is used in the food industry, which allows you to avoid the use of antibiotics. In our opinion, experimental studies of the effectiveness of lavender oil and its combinations on methicillin-resistant strains of microorganisms deserve special attention [19, 20]. Another advantage of lavender oil is its effectiveness against film-forming strains of pathogenic and opportunistic microorganisms [21, 22]. In the literature, there is also information on the antibacterial activity of extracts obtained from the aerial part of lavender [23, 24], in particular, against resistant strains [25]. And it is this property that can become decisive and promising for the wide use of lavender preparations in medical practice, since film formation is considered one of the additional factors of the pathogenicity of microorganisms [26], and the ability to form biofilms by strains of pathogenic and conditionally pathogenic microorganisms is the basis of increasing antibiotic resistance [27].

These facts prompted us to investigate the antimicrobial activity of lavender herb extracts, as a possible link in the correction of symptoms of neurological deficits of infectious etiology. Since, according to the given experimental data, the extracts obtained from the ground part of lavender to one degree or another, repeat the pharmacological effects of the oil, the determination of their antibacterial potential opens new prospects for the complex use of this type of raw material. In addition, the results of such studies can provide an answer to the question regarding the pharmacological markers of lavender, since earlier scientists noted the leading role of linalool in providing antimicrobial action [10, 28].

The availability of raw materials and standard conditions for their cultivation are of great importance for the widespread introduction of new medicinal products of plant origin. In Ukraine, lavender was traditionally grown industrially in the southern regions – Crimea, Mykolaiv and Kherson regions. At the same time, the "fashion" for lavender prompted the development of the cultivation of this culture almost throughout Ukraine. In addition to the aesthetic, this culture has great consumer value. Taking into account the fact that the raw material base of lavender is currently being expanded in Ukraine, the study of its above-ground part as a source of BAS that can be used for the needs of pharmacy is a promising direction of research that will make it possible to establish other pharmacological effects of BAS of this plant and allow the use in industry of all parts of the plant, reducing the amount of waste [29, 30]. Previously, we conducted a study of the chemical composition of lavender samples grown in different regions of Ukraine [31]. As expected, it varied significantly depending on the climatic conditions of cultivation, as well as the provision of standard cultivation conditions. Based on the results of research, we selected the most promising raw materials in view of the component composition and BAS content. Samples from Lviv, Vinnytsia, Zakarpattia and Kharkiv regions were the richest in phenolic substances. And it is these regions that can be the base for industrial cultivation of raw materials for the purpose of further use in pharmacy [31].

Considering the above, the purpose of this study was to obtain dry extracts from the ground part of narrow-leaved lavender of Ukrainian origin and a comparative experimental study of their chemical composition, antifungal and antimicrobial activity, in particular, their effect on the ability to destroy biofilms of microbial cultures or prevent their formation *in vitro*.

2. Research planning (methodology)

To obtain extracts, we chose raw materials harvested in the city of Lviv as the most promising according to the results of previous studies [31].

For conducting research, it is planned to obtain three extracts – water and water-ethanol (40 and 70 %), in which the content of BAS will be different.

To clarify the role of BAS and further standardization of extracts, it is planned to determine the component composition of BAS (terpenoids and phenolic compounds) by the method of Thin-Layer Chromatofraphy and High-performance thin-layer chromatography (TLC and HPTLC) and the quantitative content of BAS groups (phenolic compounds and flavonoids) by the method of absorption spectrophotometry.

The design of experimental studies of antimicrobial activity involved four series of experiments. In the first series, it was expedient to determine the level and spectrum of antimicrobial activity of dry extracts of lavender at a concentration of 1 mg/ml and to conduct a comparative study of the antimicrobial activity of lavender test samples according to reference strains of microorganisms *in vitro*. In the second series, it was planned to investigate the effect of test samples of extracts of narrow-leaved lavender at a concentration of 1 mg/ml on the ability to destroy biofilms and prevent the formation of biofilms *in vitro* using pure cultures of reference strains of *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*. The third series of experiments is devoted to finding an effective concentration when studying the antimicrobial activity of lavender extracts. In the fourth series of experiments, it is planned to study the influence of lavender extracts on the processes of biofilm formation in a wide range of concentrations (10–40 μg/ml). Microorganisms that can become one of the causes of neurological deficiency symptoms were selected for the study.

3. Materials and methods

Preparation of extracts and study of their chemical composition was carried out in September–December 2023, antimicrobial activity – in March–June 2024.

Raw material. Samples of the herb of *Lavandula angustifolia* Mill. collected in the Lviv Botanical Garden of the Ivan Franko National University of Lviv (Ukraine) at the stage of mass flowering in 2022. Plant material containing leaves, flowers, and inflorescence stems together was air-dried and ground before analysis. The samples were verified by Dr. M. Skibytska and deposited in the Herbarium of the Ivan Franko National University of Lviv (herbar sample LW0056626).

Obtaining dry extracts. The raw material was crushed to a particle size of 2 mm, placed in a flask and the extractant (distilled water, 40 % ethanol, 70 % ethanol) was added at the rate (1:10); each was separately extracted in a reflux water bath for 1.5 hours, filtered through a vacuum filter. The meal from each extract was again poured with the appropriate solvent (1:10), boiled in a water bath for 1.5 hours, filtered. The obtained extracts were accordingly combined and evaporated on a rotary evaporator to 100 ml. The obtained extract was transferred to an evaporating dish and placed in an oven at a temperature not higher than 100 °C until complete drying. The dried extracts were ground into powder using a mortar and pestle. Stored at a temperature of 4 °C.

In further studies:

– test sample No. 1 – an extract of the lavender herb, obtained by extraction with purified water;

– test sample No. 2 – the lavender herb extract, obtained by extraction with a water-ethanol solution (40 % ethanol);

– test sample No. 3 – the extract of the lavender herb, obtained by extraction with a water-ethanol solution (70 % ethanol).

The quality control of dry extracts consisted in determining the loss mass in during drying according to the Ph.Eur. 11.0, 2.2.32 method. Loss on drying [32], total ash content according to the method EP 11.0 Lavender flowers, total ash (2.3.16) [32], qualitative analysis of terpenoids and phenolic compounds in dry extracts of lavender were carried out by the HPTLC and TLC methods, respectively, as well as in the determination of the total content of flavonoids and phenolic compounds by the spectrophotometric method.

Component composition. The identification of compounds of terpenoid nature was carried out by the TLC method according to the Ph.Eur. 11.0 Lavender flower method [32]. TLC Siliga Gel 60, glass plates 20×20, Merck (Darmstadt, Germany) chromatographic plates were used for analysis; solvent system – toluene: ethyl acetate (95:5), linalyl acetate, linalool and 1,8-cineole were used as standards. After chromatography, the plates were treated with anisaldehyde solution and dried in a Memmert UE 300 oven at 100–105 °C for 5 minutes.

Identification of phenolic compounds was carried out by the HPTLC method [32] on HPTLC Silica gel 60 F_{254} plates, 20×10 cm (Merck). Standards of chlorogenic, caffeic and rosmarinic acids, hyperoside were used as reference standards. The research was carried out in the solvent system ethyl acetate: formic acid: water (68:8:8) in daylight; at 254, 365 nm after derivatization with diphenylboric acid aminoethyl ether, then macrogol 400 and heating at 105 °C for 5 min.

Contents of BAS groups. The total content of phenolic compounds was determined with the Folin–Ciocâlteu reagent by the spectrophotometric method [33, 34] in terms of gallic acid. The optical density was measured on a spectrophotometer SPECORD 200 (Germany) at a wavelength of 765 nm.

Determination of the total content of flavonoids was carried out using a solution of aluminum chloride hexahydrate 10 % in terms of rutin at a wavelength of (410±5) nm, standard rutin was used as a comparison solution [34, 35]. To obtain statistically reliable results, experiments were performed at least five times. Statistical processing of data on the content of phenolic compounds was carried out in accordance with the requirements of the State Pharmacopoeia of Ukraine (SPhU) [34] using software (Microsoft Office Excel 7.0).

Antibacterial activity. The microbiological properties of the samples of the investigated plant extracts at a concentration of 1 mg/ml were studied *in vitro* by the method of two-fold serial dilutions. Solvents are physiological saline, 40 and 70 % ethanol solutions. Test strains are reference strains from the American standard culture collection: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* NCTC 5055, *Candida albicans* ATCC 885-653. The determination

was carried out in accordance with the requirements [36, 37], as described by us earlier [38].

Study of the effect on the ability to destroy biofilms and prevent the formation of biofilms in vitro (test samples in a concentration of 1 mg/ml). To study the ability of strains to form biofilms, pure cultures of reference strains *S. aureus, E. coli, K. pneumoniae, P. aeruginosa* were sown on nutrient agar and incubated in a thermostat for 24 h at a temperature of 37 °C (*S. aureus, E. coli, P. aeruginosa*). Washes from agar cultures were carried out by adding 1 ml of isotonic sodium chloride solution and adjusted to the turbidity standard, considering the amount of 10^9 m.t./ cm^3. The study was conducted according to the methodology given in the literature [36, 39, 40].

Instead of test samples, sterile water (200 µl) was added to the control variants (wells). After 24 h of exposure, the wells were washed three times with 200 µl of distilled water and the number of adhered cells was determined by the spectrophotometric method. The degree of biofilm destruction (%) was determined as the difference between the adhesion of cells in untreated and treated with the tested compounds wells of a polystyrene tablet.

Determination of the effective concentration during the study of antimicrobial activity of test samples (agar diffusion method). Test samples were studied in a wide range of concentrations (10, 30 and 60 µg/ml). Experiments were carried out by the agar diffusion method in the modification of "wells" generally accepted in microbiological practice [36]. According to WHO recommendations [41], reference strains from a typical collection of cultures were used as test microorganisms: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Klebsiella pneumoniae* NCTC 5055, *Candida albicans* ATCC 885-653. Preparation of the suspension of microorganisms was carried out on a Densi-La-Meter device (PLIVA-Lachema, Czech Republic; wavelength – 540 nm) according to instruction No. 163 – 2006 "Standardization of preparation of microbial suspensions". The microbial load was 10^7 microbial cells per 1 ml of medium and was determined according to the McFarland standard [42]. An 18–24-hour culture of microorganisms was used for experiments. Synchronization of cultures was carried out at a temperature of 4 °C. Appropriate nutrient media specified in the national part of the SPhU [34] were used for growing cultures of microorganisms and conducting research. The purity of each microorganism culture was confirmed by typical morphological, tinctorial, cultural and biochemical properties.

The molten agar nutrient medium was cooled to 45 °C, the bottom layer was poured into Petri dishes in a volume of 10 ml. After solidification of the agar, five sterile stainless-steel cylinders with a height of 10 mm and an internal diameter of 8 mm were placed on it, around which a second layer of 15 ml medium inoculated with appropriate cultures of microorganisms was poured. The microbial load was 1×10^7 colony-forming units (CFU) per 1 ml of medium. After solidification of the upper layer of agar, the cylinders were removed with sterile tweezers and a test sample was introduced into the formed wells, considering their volume (0.25–0.3 ml). Petri dishes were kept for 1 hour at room temperature, after which they were placed in a thermostat and incubated for 24 hours at a temperature of 37 °C. The level of antimicrobial activity of the substances was recorded by the diameter of the growth retardation zone of microorganisms around the well with the applied drug, evaluating it according to the scale: diameter of the growth retardation zone of the microorganism<14–15 mm – resistant strain; 15–18 mm – weakly sensitive strain; >18 is a sensitive strain. The obtained data were analyzed by methods of variational statistics.

Determination of the effect of test samples on the processes of biofilm formation was carried out in a wide range of concentrations (10–40 µg/ml). Statistical processing of the obtained results was carried out using a package of application programs for Microsoft Excel 2003. Analysis of qualitative data was carried out using the $\chi 2$ criterion.

The obtained data were expressed in the form of the average (M) and the minimum and maximum values (min-max) of the sample. The assessment of reliability of differences between samples was carried out according to the Mann-Whitney test. Comparisons were made with control 1 and control 2. Differences were considered statistically significant at $p<0.05$ [43].

4. Research results

Dry extracts were obtained from the lavender herb with different extractants. The yield of dry extracts (test samples 1, 2, 3) depending on the extractant was (18.50±0.33) %; (16.00±0.28) %, (14.30 %±0.25) %, respectively (Table 1). Extracts after drying are amorphous colored powders. The moisture content in dry extracts of lavender was for test samples 1, 2 and 3: 2.07, 3.90, 2.17 %, respectively (Table 1). The level of moisture content in the raw material was 4.86 %, which does not exceed the permissible limit of 9 % [32].

TLC chromatograms of lavender herb extracts in the toluene: ethyl acetate (95:5) system during daylight viewing identified brown zones at the level of linalool (Rf 0.29), linalyl acetate (Rf 0.58) and 1,8-cineole (Rf 0.39). In the solvent system ethyl acetate: formic acid: water (68:8:8) after derivatization and when viewing the chromatographic plate at 366 nm in the middle part of the chromatogram there are: a zone in the form of yellow-orange fluorescence corresponding to hyperoside (Rf 0.40) and isoquercitrin (Rf 0.45), the blue zone corresponding to chlorogenic acid (Rf 0.38) and the dark blue zone of rosmarinic acid (Rf 0.82). There are other zones on the chromatogram of the tested solutions that gave the appropriate fluorescence.

The quantitative determination of BAS in the extracts, namely the total content of phenolic compounds and flavonoids, was carried out by the spectrophotometric method (Table 1).

The results of microbiological studies are shown in Table 2, show that the extracts of the lavender herb showed different degrees of antimicrobial activity. Gram-negative *E. coli* and *P. aeruginosa* cultures showed the highest sensitivity to the test samples, as evidenced by the minimum inhibitory concentration levels of 62.5 µg/ml, as well as *Candida fungi* (MIC – 31.25 µg/ml).

In the second series of experiments, the influence of test samples of dry extracts of lavender on the ability to destroy biofilms and prevent the formation of biofilms of microbial cultures *in vitro* (Tables 3–6) was studied three times.

Values of OD<0.5 were regarded as low film-forming ability, 0.5–1 as average, and OD>1 as high.

According to the results of the study, the ability to inhibit the formation of biofilms was shown by test sample No. 2 in relation to gram-positive and gram-negative microorganisms: *S. aureus* and *P. aeruginosa*. The highest antibiofilm-forming activity was also registered in test sample No. 2 and amounted to *S. aureus* – 57.8 %, *P. aeruginosa* – 66.7 %. Test samples No. 1 and 3 did not slow down the formation of biofilms.

During the study of the effect on the destruction of biofilms (2nd day), it was established that the introduction of test samples into the plates with formed biofilms was not accompanied by a change in the light transmission coefficient, which indicated the inability of the test samples of the extracts of the lavender herb at a concentration dose of 1 mg/ml destroy biofilms of microorganisms.

Therefore, the addition of sample No. 2 to the cultures of *S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* is significantly ($p<0.001$) accompanied by a slowdown in the formation of biofilms of *S. aureus* and *P. aeruginosa* at the studied concentration.

To find an effective concentration in the determination of antimicrobial activity, in the next series of work, test samples of lavender herb extracts were investigated in concentrations of 10, 30 and 60 μg/ml.

The results of microbiological studies showed that the studied test samples revealed a wide spectrum of antibacterial activity (Table 7).

Table 1

Yield and component composition of the obtained lavender dry extracts

Test sample	Yield, %, *n*=5	Moisture, %, *n*=5	Terpenoid presence (TLC)	Presence of phenolic compounds (HPTLC)	Total content of phenolic compounds, mg/g, *n*=5	Total content of flavonoids, mg/g, *n*=5
1	18.5±0.33	2.07±0.04	Linalool, linalyl acetate, 1,8-cineole (traces)	Hyperoside, isoquercitrin, rosemary acid, chlorogenic acid	2.02±0.04	1.46±0.03
2	16.0±0.28	3.90±0.07			2.35±0.04	2.06±0.04
3	14.3±0.25	2.17±0.04			2.60±0.05	3.17±0.06

Table 2

Antimicrobial activity of test samples of lavender herb extracts, *M* (min–max)

Test samples	Minimum inhibitory concentration, μg/ml				
	S. aureus ATCC 25923	*E. coli* ATCC 25922	*K. pneumoniae* NCTC 5055	*P. aeruginosa* ATCC 27853	*C. albicans* NCTC 885-65
No. 1	125 (62.5–250)	At the control level	At the control level	At the control level	At the control level
No. 2	125 (62.5–250)	62.5 (31.25–125)	At the control level	62.5 (31.25–125)	31.25 (15.6–62.5)
No. 3	125 (62.5–250)	62.5 (31.25–125)	125 (62.5–250)	125 (62.5–250)	31.25 (15.6–62.5)
Control 1	1:2	1:2	1:1	1:1	1:4
Control 2	1:2	1:2	1:1	1:1	1:4

Note: The given data are the average values of the results of triplicate determinations of the antimicrobial activity of the drugs against each microorganism culture. Control 1–40 % ethanol solution; control 2–70 % ethanol solution.

Table 3

The coefficient of permeability of the nutrient medium in the case of exposure of test samples of extracts of the lavender herb No. 1–3 to bacterial biofilms of *S. aureus* (%, *M*±*m*)

Control 1 phosphate buffer	Control 2 cultures	Test samples, OD		
		No. 1	No. 2	No. 3
0.06±0.01	0.19±0.01	In 1 day		
		0.26±0.03*	0.11±0.02*	0.24±0.03*
		In 2 days		
		0.32±0.02*	0.22±0.01*	0.28±0.01*

*Note: * – p<0.001 compared to control 1.*

Table 4

The coefficient of permeability of the nutrient medium in the case of exposure of test samples of extracts of the lavender herb No. 1–3 to bacterial biofilms of *E. coli,* (%, *M*±*m*)

Control 1 phosphate buffer	Control 2 cultures	Test samples, OD		
		No. 1	No. 2	No. 3
0.06±0.01	0.15±0.01	In 1 day		
		0.27±0.03*	0.17±0.02*	0.25±0.01*
		In 2 days		
		0.32±0.02*	0.16±0.01*	0.26±0.03*

*Note: * – p<0.001 compared to control 1.*

The conducted studies became the basis for further study of extracts of lavender on the processes of biofilm formation in a wide range of concentrations (10–40 μg/ml) (Tables 8–10).

Table 5

The coefficient of permeability of the nutrient medium in the case of exposure of test samples of the lavender herb extracts No. 1–3 to bacterial biofilms of *P. aeruginosa*, (%, *M*±*m*)

Control 1 phosphate buffer	Control 2 cultures	Test samples, OD		
		No. 1	No. 2	No. 3
0.06±0.01	0.18±0.01	In 1 day		
		0.24±0.03*	0.12±0.01*	0.23±0.01*
		In 2 days		
		0.31±0.02*	0.20±0.03*	0.27±0.03*

*Note: * – p<0.001 compared to control 1.*

Table 6

The coefficient of permeability of the nutrient medium in the case of exposure of test samples of extracts of the lavender herb No. 1, 2, 3 to bacterial biofilms of *K. pneumoniae*, (%, *M*±*m*)

Control 1 phosphate buffer	Control 2 cultures	Test samples, OD		
		No. 1	No. 2	No. 3
0.06±0.01	0.15±0.01	In 1 day		
		0.29±0.03*	0.19±0.01*	0.33±0.03*
		In 2 days		
		0.36±0.02*	0.22±0.03*	0.24±0.03*

*Note: * – p<0.001 compared to control 1.*

Table 7

Antimicrobial activity of test samples of lavender herb extracts in concentrations of 10, 30 and 60 μg/ml

Test sample	Concentration, μg/ml	Diameter of the growth retardation zone of microorganisms, mm					
		Gram-positive bacteria		Gram-negative bacteria			Fungi
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*C. albicans*
No. 1	10	17	18	0	0	0	16
	30	19	17	0	0	0	19
	60	20	22	14	0	0	20
No. 2	10	18	17	14	14	0	21
	30	20	19	14	14	15	20
	60	22	20	16	15	16	24
No. 3	10	18	20	14	14	14	22
	30	23	22	15	15	16	23
	60	25	24	15	17	16	26
Control 1	–	15	14	15	14	14	17
Control 2	–	16	15	15	15	15	19

Note: The given data are the average values of the results of triplicate determinations of the antimicrobial activity of the drugs against each microorganism culture. Control 1–40 % ethanol solution; control 2–70 % ethanol solution.

Table 8

The coefficient of permeability of the nutrient medium in case of exposure of test laveder samples – No. 1–3 to bacterial biofilms of *S. aureus* (%, *M*±*m*)

Control 1 phosphate buffer	Control 2 cultures	Test samples	Optical density, OD			
			10 μg/ml	20 μg/ml	30 μg/ml	40 μg/ml
In 1 day						
0.06±0.001	0.17±0.006	No. 1	0.080±0.003*	0.075±0.004*	0.071±0.002*	0.080±0.006*
		No. 2	0.090±0.01*	0.072±0.002*	0.082±0.01*	0.101±0.005*
		No. 3	0.081±0.009*	0.086±0.008*	0.082±0.008*	0.087±0.003*
In 2 days						
0.06±0.001	0.17±0.006	No. 1	0.1±0.01*	0.093±0.007*	0.089±0.005*	0.088±0.006*
		No. 2	0.1±0.005*	0.095±0.005*	0.101±0.005*	0.102±0.004*
		No. 3	0.115±0.008*	0.115±0.008*	0.116±0.005*	0.156±0.048*

*Note: * – p <0.001 compared to control 1.*

Table 9

The coefficient of permeability of the nutrient medium in case of exposure of test laveder samples – No. 1–3 to bacterial biofilms of *E. coli* (%, *M*±*m*)

Control 1 phosphate buffer	Control 2 cultures	Test samples	Optical density, OD			
			10 μg/ml	20 μg/ml	30 μg/ml	40 μg/ml
In 1 day						
0.06±0.01	0.16±0.004	No. 1	0.072±0.005*	0.080±0.007*	0.068±0.005*	0.074±0.002*
		No. 2	0.076±0.001*	0.087±0.023*	0.085±0.007*	0.087±0.003*
		No. 3	0.071±0.002*	0.072±0.005*	0.068±0.005*	0.074±0.002*
In 2 days						
0.06±0.01	0.16±0.004	No. 1	0.073±0.005*	0.063±0.002*	0.084±0.005*	0.079±0.005*
		No. 2	0.077±0.002*	0.072±0.009*	0.069±0.003*	0.069±0.002*
		No. 3	0.094±0.0008*	0.110±0.006*	0.103±0.002*	0.104±0.002*

*Note: * – p<0.001 compared to control 1.*

Table 10

The coefficient of permeability of the nutrient medium in case of exposure of test laveder samples – No. 1–3 to bacterial biofilms of *P. aeruginosa*, (%, *M*±*m*)

Control 1 phosphate buffer	Control 2 cultures	Test samples	Optical density, OD			
			10 μg/ml	20 μg/ml	30 μg/ml	40 μg/ml
In 1 day						
0.06±0.01	0.18±0.01	No. 1	0.076±0.002*	0.075±0.004*	0.079±0.001*	0.081±0.003*
		No. 2	0.077±0.002*	0.076±0.002*	0.088±0.005*	0.095±0.002*
		No. 3	0.068±0.003*	0.067±0.003*	0.068±0.001*	0.076±0.006*
In 2 days						
0.06±0.01	0.18±0.01	No. 1	0.074±0.005*	0.075±0.012*	0.079±0.007*	0.072±0.004*
		No. 2	0.082±0.008*	0.086±0.009*	0.088±0.015*	0.095±0.011*
		No. 3	0.072±0.008*	0.066±0.007*	0.069±0.005*	0.076±0.007*

*Note: * – p <0.001 compared to control 1.*

5. Discussion

The obtained extracts are characterized by the presence of terpenoids and phenolic substances, which are considered markers of antimicrobial activity [23, 24]. The TLC method established the presence of compounds of the terpene nature of linalool and linalyl acetate in all samples. It should be noted that 1,8-cineole was determined only in trace amounts, so its role in antimicrobial activity is unlikely to be decisive. The study of phenolic compounds by HPTLC method made it possible to determine the presence of hydroxycinnamic acids (rosmarinic and chlorogenic) and flavonoids (hyperoside and isoquercitrin) in all extracts. At the same time, the extract of lavender grass obtained with 70 % ethanol (test sample No. 3) had more intense zones, which indicates a higher content of all components in the raw material. The study of numerical indicators and chemical composition of dry extracts of lavender herb showed that the total content of phenolic compounds and flavonoids is higher in the extract obtained with 70 % ethanol from lavender herb (test sample No. 3). Data of spectrophotometric determination of the total content of phenolic compounds were correlated with the total content of flavonoids. The highest number of phenolic compounds was expected to be contained in test sample No. 3 (2.60 mg/ml), the lowest – in the aqueous extract (test sample No. 1), the total content of phenolic compounds was 2.02 mg/ml (Table 1).

A comparative analysis of the antibacterial activity of the test samples showed that the most active was sample No. 3, which has antifungal activity at the level of 31.25 μg/ml, antibacterial activity of 62.5 μg/ml against *E. coli* and 125 μg/ml against *S. aureus*, *K. pneumoniae*, *P. aeruginosa*. Sample No. 2, to which the capsular microorganism *K. pneumoniae* showed no sensitivity, is somewhat inferior in the spectrum of antimicrobial activity. At the same time, the tested sample No. 2 showed antimicrobial activity against *E. coli* and *P. aeruginosa* at the level of 62.5 μg/ml, and in relation to *S. aureus* – 125 μg/ml. Fungi of the genus Candida were most sensitive to sample No. 2 (31.25 μg/ml). The lowest activity was registered in sample No. 1, which revealed background antistaphylococcal activity at the level of 125 μg/ml (Table 2).

So, the results of the research show that the studied test samples of lavender reveal a wide spectrum of antibacterial activity against gram-positive, gram-negative bacteria and fungi of the genus Candida. The obtained results are correlated with the data of other researchers regarding extracts of *Lavandula angustifolia*, which were obtained from dry flower stalks of lavender and cultivated in Poland and Australia, they were evaluated for their antimicrobial properties. The results of these studies demonstrate that gram-positive bacteria and Candida spp. were more sensitive to all distilled fractions and lavender extracts than *Escherichia coli* (gram-negative bacteria) [2, 24, 44].

A comparative analysis of the activity confirmed that the highest activity is characteristic of the test sample No. 3, which, for its part, contains the largest amount of BAS. And it can be noted that it depends to some extent on the presence of hydroxycinnamic acids and flavonoids characteristic of lavender.

The results of the study of the ability to inhibit or counteract the formation of biofilms by microorganisms turned out to be somewhat different. In this series of experiments (Tables 3–6), test sample No. 2 showed the greatest activity. It was established that the addition of sample No. 2 to the studied cultures was significantly (p<0.001) accompanied by a slowdown in the formation of biofilms of *S. aureus* and *P. aeruginosa* at the studied concentration. Analyzing the content of phenolic substances and flavonoids, it should be noted that it is intermediate in test sample No. 2. Taking this into account, it can be predicted that either the optimal content of these substances is important for influencing biofilm formation, and this dependence may not be linear, or other, minor, components of the extract play a significant role.

Analyzing the results of the study of the antimicrobial properties of the extracts in different concentrations, it should be recognized that the most pronounced antimicrobial activity is found in the highest concentration. Thus, the antistaphylococcal activity of sample No. 3 at a concentration of 60 μg/ml exceeded 1.4 times the concentration of 10 μg/ml and 1.1 times – 30 μg/ml of the latter. The zones of growth retardation of *B. subtilis* at the maximum concentration exceeded this indicator of the minimum and average concentrations by 1.2 and 1.1 times, respectively. Zones of fungal growth inhibition had an average of 6 mm larger area compared to the test sample at a concentration of 10 μg/ml and 3 mm larger area for the test sample with an intermediate concentration (30 μg/ml). According to the conducted microbiological screening, the highest antimicrobial activity was registered in test sample No. 3 at a concentration of 60 μg/ml (Table 7).

We paid the greatest attention to the analysis of the results regarding the influence of test samples in different concentrations on the ability to form biofilms. The analysis of scientific sources devoted to the issues of formation, functioning, and significance of microbial biofilms allows us to assert that associated microorganisms play an important role in the etiology and pathogenesis of infectious diseases, as well as pathological conditions associated with microorganisms, in particular, dysbacteriosis [45].

The results of our own research on the effect of plant extracts on the ability of microorganisms (*S. aureus, E. coli, P. aeruginosa*) to form biofilms showed that, in contrast to a concentration of 1 mg/ml, the best ability to inhibit the formation of biofilms in relation to gram-positive and test samples No. 1 and 3 showed gram-negative microorganisms.

The highest antibiofilm-forming activity was registered in test sample No. 3 and amounted to *S. aureus* – 56.7 %, *P. aeruginosa* – 61.0 %. Sample No. 2 did not show the ability to slow down the formation of biofilms by gram-positive and gram-negative microorganisms.

Thus, regarding the gram-positive microorganism *S. aureus*, test sample No. 1 slowed down the ability to form biofilms by an average of 17 % (10 μg/ml), 22 % (20 μg/ml) and 26 % (30 μg/ml). The effect of the studied sample No. 1 on gram-negative microorganisms was accompanied by a more pronounced inhibition of biofilm formation. The process of biofilm formation of *E. coli* under the influence of the test sample at a concentration of 10 μg/ml decreased by 22 %, and at a concentration of 30 μg – by 29 %. The most pronounced antibiofilm-forming effect against *P. aeruginosa* was recorded at concentrations of 10 and 20 μg/ml. The studied test sample reduced the process of biofilm formation by 58 %, and at a concentration of 30 μg/ml – by 56 %.

Test sample No. 2 showed the ability to slow down biofilm formation of *P. aeruginosa* in concentrations of 10 and 20 μg/ml by 57 and 58 %, respectively.

Test sample No. 3 revealed the highest antibiofilm-forming activity against *E. coli* and *P. aeruginosa*. Among the studied concentrations, the highest activity against *Escherichia coli* was established at concentrations of 10 and 30 μg/ml – 0.071 and 0.068 OD, respectively. This trend was also preserved in relation to *P. aeruginosa*. It was in concentrations of 10 and 30 μg/ml that test sample No. 3 contributed to slowing down the process of biofilm formation by an average of 62 %. It is worth noting that the increase in concentration to 40 μg/ml was not accompanied by an increase in activity.

During the study of the effect on the destruction of biofilms (2nd day), it was established that the introduction of test samples into the tablet with formed biofilms was not accompanied by a change in the light transmission coefficient, which indicated the inability of the test samples to destroy biofilms of microorganisms.

The addition of the studied samples to the cultures of *S. aureus*, *E. coli* and *P. aeruginosa* was significantly (p<0.001) accompanied by a slowdown in the formation of biofilms of gram-positive and gram-negative microorganisms in the studied concentrations.

Antimicrobial activity and antibiofilm formation may be related to the content of phenolic compounds in the extracts – hydroxycinnamic acids and flavonoids.

Therefore, the conducted research substantiates the expediency of further in-depth pharmacological study of the presented extracts of lavender herb in relation to clinical strains of microorganisms.

Practical significance. The prospects of the Ukrainian raw material of Lavandula angustifolia have been proven, and the optimal technology for obtaining substances with antimicrobial properties has been chosen.

Study limitations. The studies were conducted within the framework of generally accepted methods and do not provide for the expansion of their conditions. Also, the conducted research can be expanded due to the use of other extraction technologies.

Prospects for further research. The conducted research substantiates the expediency of an in-depth pharmacological study of lavender herb extracts, as well as the activity of individual BAS included in their composition, to clarify their role and possible optimization of extraction. Manifestation of antimicrobial activity by extracts is promising as an additional link in the multifactorial response to symptoms of neurological deficits.

6. Conclusions

Obtained and characterized extracts (water, ethanol-water 40 and 70 %) of lavender herb of Ukrainian origin. Their numerical indicators, component composition and total content of phenolic compounds and flavo-

noids were determined. The results of the research show that at a concentration of 1 mg/ml, the extracts have antimicrobial properties against a wide range of infectious agents (*S. aureus, E. coli, K. pneumoniae, P. aeruginosa, C. albicans*). The highest activity is observed for the lavender herb extract obtained by extraction with 70 % ethanol. The highest inhibitory activity on biofilm formation at a concentration of 1 mg/ml was found in the case of the action of the test sample of lavender extract, obtained by extraction with 40 % ethanol, which was 57.8 % for *S. aureus* and 66.7 % for *P. aeruginosa*. It was established that at this dose the studied extracts do not have a biodestructive effect on dense films of strains of *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*.

Comparative analysis of the level of antimicrobial activity according to concentration showed a direct relationship. An increase in the concentration to 60 μg/ml was accompanied by an increase in antimicrobial activity. At the same time, the pronounced fungicidal and bactericidal (in relation to gram-positive) activity of the test samples attracts attention. The studied extracts demonstrated a pronounced anti-biofilm-forming effect against *P. aeruginosa* in different concentrations. It was established that the extracts of lavender grass in the studied concentrations do not have a biodestructive effect on dense films of strains of *S. aureus*, *E. coli*, and *P. aeruginosa*. According to a preliminary assessment, antibacterial activity may be correlated with the content of phenolic substances. The prospects of the obtained results for the comprehensive correction of neurological deficiency symptoms are discussed.

Conflict of interests

The authors declare that they have no conflict of interest in relation to this study, including financial, personal, authorship, or any other, that could affect the study, and its results presented in this article.

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Data availability

Data will be provided upon reasonable request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the presented work.

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