IN VITRO ANTIMICROBIAL STUDY OF NEW MODIFICATIONS OF *SALVIA OFFICINALIS* EXTRACTS

Verkhovodovoda Y.V. , Kireyev I.V., Koshovyi O.M. , Osolodchenko T.P.

National university of pharmacy

Introduction. During treatment with antibiotics and up to 12 weeks after its completion, in 5-30% of patients [1, 2] antibiotic-associated diarrhea is recorded. It arises in connection with the treatment of antibiotics, without other obvious causes, including acute violation of normal intestinal flora [3]. The most common cause of antibiotic-associated diarrhea among children and adults is *Clostridium difficile* (*C. difficile*). *C. difficile* infection is associated with significant morbidity and mortality [4, 5].

There is an increasing tendency of the frequency and severity of *C. difficile* associated infection [6], changes in the structure of morbidity [7], the appearance of more toxigenic strains [8]. Clinically, antibiotic associated diarrhea varies from light intestinal discomfort to the development of pseudomembranous colitis with complications [9].

A total of 7711 CDI cases were reported, 5756 of which (74.6 %) were healthcare-associated (HA) CDI. There were 611/7711 (7.9 %) cases classified as 'recurrent' infections, and 921/5499 (16.7 %) cases which had a complicated course of infection. While 4160/5248 (79.3 %) cases with known outcome were reported to have been discharged alive, 1088/5248 (20.7 %) CDI cases had died from various causes. These include 207/5248 (3.9 %) fatal cases in which CDI was reported to have contributed to a fatal outcome [10].

Carrier is observed in up to 57% of cases in patients undergoing long-term in-patient treatment [11]. In the case of clostridial infection, antibacterial therapy with metronidazole, vancomycin, rifaximin, fidaksomycin, which has a number of side effects; there is also a fact of development of microorganism resistance. Actual issue of pharmacy is a development of a safe plant antimicrobial agent.

It is known that *Salvia officinalis* L. (*S. officinalis*) has been used for a long time as an antiseptic and for treatment of diseases of the intestine. For the first time, new modified derivatives of *S. officinalis* extracts were obtained, namely: dry extract on the basis of broth of salvia leaves of (salvia broth) (extract No 1), dry extract of salvia leaves, obtained with 50 % ethanol (50 % salvia extract) (extract No 2), dry extract of salvia leaves, obtained with 96 % ethanol (96 % salvia extract) (extract No 3), a complex of phenolic compounds with L-lysine (lysine complex) (extract No 4), a complex of phenolic compounds with arginine (arginine complex) (extract No 5), polysaccharide complex (extract No 6), purified complex (extract No 7), saponine complex (extract No 8), phenolic complex (extract No 9), flavonoid complex (extract No 10), complex of hydrophilic phenolic compounds (hydroxycholic complex) (extract No 11). For control as a reference, the leaves of the salvia (liktravy, Ukraine) were examined; the solution was prepared according to the instructions (No 12). Phytosubstances No 13, No 14, No 15 - respectively, diluents H_2O, 50 % C_2H_5OH, 96 % C_2H_5OH also as control.

The purpose of the work was to investigate the antimicrobial activity of new modified derivatives of *S. officinalis* extracts: salvia broth, 50 % salvia extract, 96 % salvia extract, saliva decoction and it's derivative extracts.

Materials and methods. Extracts were obtained at the Department of Pharmacognosy of the National University of Pharmacy (Kharkiv, Ukraine) and provided for study. The antimicrobial activity of derivatives of salivary grass extracts was determined in the Laboratory of Biochemistry and Biotechnology of the Mechnikov Institute of Microbiology and Immunology (Kharkiv, Ukraine) under the direction of the candidate of biological sciences T. P. Osolodchenko in an in vitro experiment using the method of diffusion in agar - "the method of wells", which is based on the ability of the active substance to diffuse into agar with a standard test culture. The results obtained with this method allow characterizing the antimicrobial activity of the test sample, as the zones of growth retardation of microorganisms are formed due to the diffusion of biologically active substances into a dense nutrient medium. According to the WHO recommendations, the test strains were used to evaluate the activity of the drug: *Staphylococcus aureus* ATCC 26923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Proteus vulgaris* ATCC 4636, *Candida albicans* ATCC 885/653.

Created the most favorable conditions for the cultivation of microorganisms, namely: meat-peptone agar for obtaining a daily culture of bacteria; Saburo environment - for a 48-hour culture of the fungus of the genus *Candida*. Petri dishes of the same size were installed on a horizontal surface (adjusted by the water column) and poured into them 10 ml of a sterile, non-infected test-culture of "hungry" agar. After the first layer of dense nutrient medium was sealed, cylinders made of stainless steel (height 10 mm, outer diameter 8 mm) were installed on its surface and filled them with "infected" medium, and in order to determine the sensitivity of the *Candida* genus - Saburo medium in the amount of 15 ml. For the second layer, a sterile dense nutrient medium was poured into tubes in a quantity of 15 ml, melted in a water bath, cooled to a temperature of 45 ° C, and was isolated by slurries of test cultures. The microbial load was about 1.0×10^5 CFU / ml. Suspensions of bacterial cultures and 48-hour culture of the *Candida* genus were prepared in separate samples in a physiological solution ex tempore. The concentration of microorganisms in the suspension was determined according to the state standard of turbidity No5. After the second layer was sealed, the cylinders were removed, and in the well formed between the first and second layers of dense nutrient media, a sterile graduated sample was introduced into the sample. 10% aqueous solutions of *S. officinalis* extracts were prepared ex tempore in a volume of 0.3 ml. Experimental

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Petri dishes were captured in a thermostat at + 37 °C for 24 hours, for cultivation of bacteria at + 25 °C, for 48 hours - a fungus of the genus *Candida*. The diameter of the test culture growth delay zones was measured in mm, including the diameter of the well. Antimicrobial activity was evaluated according to the following criteria:

- absence of the growth retardation zone of microorganisms around the well, as well as the delay zone with a diameter of 6-10 mm, was assessed as the insensibility of microorganisms to the specimen inserted into the well;

- growth retardation zones with a diameter of 11-20 mm were evaluated as the sensitivity of culture to the investigated sample;

- growth retardation zones with a diameter greater than 20 mm were considered as an indicator of high sensitivity of microorganisms to the specimen.

The data did not obey the normal distribution law. Nonparametric methods were used (Median, upper and lower quartile were calculated). The Kruskal -Wallis test was used to compare the activity of Salvia officinalis extracts. In identifying the differences between the two groups, the Mann-Whitney U - test was used. The use of water, 50% alcohol, 96% alcohol as a control of materials showed a lack of growth retardation zones.

Results of the research and their discussion. In one of the modern studies, the obtained *S. officinalis* essential oils showed remarkable activity against *B. subtillis* and *S. epidermidis.* Essential oil of *S. officinalis* is a potential natural source of antimicrobial activity [12, 13]. In another study, four essential oils of *S. officinalis* could inhibit the growth of *E. coli*, *S. aureus* and *C. albicans* [14, 15, 16, 17]. In our study, essential oils only present in phytoextract № 3, which provides novelty of the data.

The results of the study of antimicrobial activity of the extracts studied are given in Table 1.

It was found that extract № 1 had high activity and delayed the growth of *S. aureus* to 22-23 mm. At the same time, this extract was maximum effective towards the representatives of gram-positive microflora, and reduced the activity of the gram-negative microflora, the growth retardation zone in *E. coli* and *P. vulgaris* is 16-17 mm. The smallest sensitivity among the studied strains was *P. aeruginosa*.

Effect on pathogenic mushrooms, which was *C. albicans*, was also sufficiently sensitive. When modifying the extract by increasing the amount of phenolic compounds and amino acids, we received a multi-directional action. Thus, the inclusion of arginine in the complex of phenolic compounds did not significantly affect the antimicrobial action. The inclusion of amino acid L-lysine to phenolic complex increased the antimicrobial and antifungal activity and slightly decreased the antimicrobial effect in relation to the representatives of gram-negative flora.

A comparative analysis of the efficacy of various concentrates on S. aureus showed the greatest efficacy of the extract of decoction of saliva officinalis dissolved in water, 50% alcohol, 96% alcohol (p_w = 0.04). The analysis showed that its efficiency is significantly higher than other investigated phytoextracts of salvia officinalis (p_u = 0,04).

The greatest effect of extracts № 9, 10, 11 with the same efficiency compared with other extracts (p_w = 0.03).

The study of the salvia officinalis phytosubstances show the effectiveness of 3 extracts (№9, 10, 11) (p_w = 0.03). The action of those salvia officinalis extracts was significantly different (p_u = 0.1). The greatest influence on Proteus had alcohol extracts (p_w = 0.03), with the most pronounced effect of decoction diluted in 96% alcohol (p_u = 0.02).

Conclusions. 1) For the first time, extracts of *S. officinalis* in most have antimicrobial activity at the level of weakly active or active substances.

2) The inclusion of L-lysine phenolic complex provided more pronounced antimicrobial effect on most strains in comparison with phenolic complex.

3) Derivatives of *S. officinalis* extracts are promising objects for further study of anti-inflammatory activity as potential effective substances for the treatment and prevention of infectious intestinal diseases.

Table 1. Results of the study of antimicrobial activity of the studied extracts

Phyto-substan-ce	Solvent	Diameter of the growth delay zones (average and standard deviation, mm)					
		E. coli	*S. aureus*	*B. subtilis*	*C. albicans*	*P. aeruginosa*	*P. vulgaris*
1	**H_2O**	**17±1**	**21±1**	**15±1**	**15±1**	**13±1**	**15±1**
	50 %C_2H_5OH	**18±2**	**20±2**	**18±1**	**16±1**	**16±1**	**16±1**
	96 %C_2H_5OH	**18±1**	**18±2**	**20±2**	**19±2**	**17±2**	**17±1**
2	**H_2O**	**14±1**	**17±1**	**14±1**	**14±1**	**15±1**	**15±1**
	50 %C_2H_5OH	**13±2**	**12±1**	**14±2**	**16±1**	**14±1**	**15±1**

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	96 %C_2H_5OH	**15±1**	**16±2**	**16±1**	**16±2**	**15±2**	**15±1**
3	**H_2O**	**16±1**	**17±2**	**15±2**	**14±1**	**14±2**	**14±1**
	50 %C_2H_5OH	**19±1**	**12±1**	**14±1**	**14±2**	**16±1**	**16±1**
	96 %C_2H_5OH	**15±1**	**16±1**	**15±1**	**15±1**	**12±1**	**15±1**
4	**H_2O**	**18±2**	**17±1**	**13±2**	**16±1**	**17±1**	**15±2**
	50 %C_2H_5OH	**20±2**	**18±1**	**15±1**	**17±2**	**15±1**	**16±1**
	96 %C_2H_5OH	**20±2**	**15±1**	**15±1**	**15±1**	**16±1**	**16±2**
5	**H_2O**	**16±2**	**15±1**	**14±2**	**15±2**	**14±2**	**16±2**
	50 %C_2H_5OH	**14±1**	**18±2**	**17±1**	**15±1**	**14±2**	**14±1**
	96 %C_2H_5OH	**16±2**	**16±2**	**15±2**	**19±1**	**12±1**	**15±1**
6	**H_2O**	**18±1**	**16±1**	**15±2**	**17±1**	**16±2**	**14±2**
	50 %C_2H_5OH	**16±1**	**15±2**	**15±1**	**15±2**	**15±1**	**15±2**
	96 %C_2H_5OH	**15±1**	**15±1**	**14±1**	**16±1**	**15±2**	**14±2**
7	**H_2O**	**14±2**	**16±1**	**16±1**	**14±1**	**14±2**	**14±1**
	50 %C_2H_5OH	**14±1**	**13±2**	**17±2**	**14±1**	**15±1**	**15±2**
	96 %C_2H_5OH	**14±2**	**14±1**	**14±1**	**15±1**	**13±1**	**16±1**
8	**H_2O**	**14±2**	**16±2**	**15±2**	**16±2**	**16±1**	**14±1**
	50 %C_2H_5OH	**16±2**	**14±2**	**14±2**	**13±1**	**15±2**	**16±2**
	96 %C_2H_5OH	**14±2**	**15±1**	**13±1**	**17±1**	**16±2**	**13±1**
9	**H_2O**	**20±1**	**16±1**	**14±1**	**18±2**	**17±2**	**15±2**
	50 %C_2H_5OH	**15±1**	**14±2**	**18±2**	**16±1**	**20±1**	**16±2**
	96 %C_2H_5OH	**17±1**	**19±1**	**16±2**	**18±1**	**16±1**	**14±2**
10	**H_2O**	**15±1**	**14±2**	**14±1**	**15±1**	**14±2**	**14±2**
	50 %C_2H_5OH	**15±1**	**15±2**	**18±2**	**17±1**	**20±1**	**16±2**
	96 %C_2H_5OH	**17±2**	**18±2**	**16±1**	**16±2**	**17±1**	**15±1**
11	**H_2O**	**15±1**	**14±2**	**14±1**	**15±1**	**14±2**	**14±2**
	50 %C_2H_5OH	**15±1**	**15±2**	**18±2**	**17±1**	**20±1**	**16±2**
	96 %C_2H_5OH	**17±2**	**18±2**	**16±1**	**16±2**	**16±1**	**16±1**
12	**H_2O**	**16±1**	**19±1**	**14±1**	**13±2**	**13±1**	**14±1**
	50 %C_2H_5OH	**16±1**	**18±1**	**15±1**	**14±2**	**13±1**	**14±1**
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Keywords: Salvia officinalis, antimicrobial, extracts, in vitro

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