RESEARCH OF ANTIMICROBIC ACTIVITY OF FOAMING PRODUCTS SAMPLES WITH OCTOPIROX

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Introduction. Seborrheic dermatitis (SD) - chronic recurrent skin disease, which is associated with increased cutaneous fatty secretion, change in its qualitative composition and is characterized by localization in the areas of accumulation of sebaceous glands on the scalp, face, upper torso. SD refers to the most common dermatoses, its share in the structure of dermatological incidence is 10% [1,2].

Therefore, the choice of tactics for the treatment of patients with SD depends on the degree of clinical manifestations, duration of the disease, information about the effectiveness of previously conducted therapy. The disease requires regular treatment with systemic and topical therapy for a long time. For external treatment, are used the agents that have anti-inflammatory, antipruritic, antifungal, and in the case of secondary infection - antibacterial and antiseptic action [3].

Particular attention is paid to the choice of antifungal medicines in the treatment of SD. Traditionally, local remedies in the form of ointments or creams are used to treat SD of the scalp, they cause many inconveniences when used. When applied upon the skin, the hair becomes greasy and unattractive. Definitely, when localizing the process on the scalp, among the external use products it is preferable to use therapeutic forms assuring uniform distribution of the product and with good organoleptic properties [4-6]. In this respect, foaming products, in particular shampoos, which include active substances that have certain therapeutic potential regarding the main mechanisms of SD treatment, having self-regulating, anti-inflammatory, antifungal, antibacterial, reparative, moisturizing properties, etc. are very appropriate.

One of the main factors in the development of this disease is the yeastlike fungus of Malassézia fúrfur genus (or Pityrósporum ovále), which is present in the skin of each person. These fungi are localized in the middle and superficial parts of the epidermis corneous layer, inside and between the corneous layer, as well as in the hair follicles on the skin areas characterized by increased sebaceous excretion (chest, back, scalp, auricle area, nasolabial folds, brow arches, large folds of skin) [7].

Most commonly such products in the quality of active agents are:

- octopirox – a component with antifungal activity, which has a pronounced effect on the Malassezia fungi;
- zinc pyrithione - this component has anti-inflammatory and antibacterial properties, it reduces the number of fungi;
- ciclopiroxolamine – it possesses a broad spectrum of action and is effective against dermatomycetes, yeastlike fungi (including Candida genus), it is also active pertaining to some strains of gram-negative and gram-positive bacteria;
- tar - it has antifungal action and in addition it reduces the exfoliation of cells, especially effective in psoriasis and seborrheic dermatitis;
- selenium sulphide - products containing this element help to reduce the amount of the produced yeast fungi, but products containing this element must be thoroughly washed;
- ketoconazole – an antifungal element with a large spectrum of influence on the dandruff originator [2,3, 7].

Octopirox®, which has strong fungicidal (antifungal) and antibacterial actions, was chosen among a number of modern products in this field. However, it is primarily an antifungal agent that effects directly the fungi of Malassézia fúrfur (or Pityrósporum ovále) genus; it also has minimal irritant effect; and reduces sebum formation. Therefore, the main purpose of our experiment was to study the antimicrobial activity of developed samples of foaming agents with octopirox to justify the rational concentration [8,9].

Materials and methods. For this study, we produced a number of experimental samples of foaming bases with octopirox and α-lipoic acid at different concentrations.:

sample № 1 - foaming base;
sample № 2 – foaming base + α-lipoic acid;
sample № 3 – foaming base + octopirox (0,25 %);
sample № 4 – foaming base + octopirox (0,5 %);
sample № 5 – foaming base + octopirox (0,75 %);
sample № 6 – foaming base + octopirox (1,0 %).

Samples with active pharmaceutical ingredients and surface active agents were provided by the public joint-stock company “Chervona zirka” Chemical-pharmaceutical Plant (Kharkiv, Ukraine). Lactic acid was used as a regulator of the foaming bases (Lactic Acid, Galactic, Belgium) which is considered to be an optimal component. Lactic acid is a part of the skin acid mantle and it is also allowed in application of foaming products ((EU) Regulation №1223/2009) [10,11].

The level of pH value of the samples was determined potentiometrically (SPU 1.2, 2.2.3) using the device "pH Meter Metrohm 744 " (Germany) [12].

Some experimental samples were prepared at the Scientific Laboratory of the Department of Commodity Studies at the National University of Pharmacy.

The antimicrobial activity of prototype gels was studied in vitro by diffusion in agar (“wells” method) [13,14]. This method is based on the ability of active substances to diffuse in the agar medium, which was

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previously inoculated by bacterial crops. The results of the studies make it possible to characterize both the antimicrobial activity of the samples and the release of antimicrobial substances from the base, because the growth inhibition zones of microorganisms are formed as a result of the diffusion of these substances into a dense nutrient medium.

The pure cultures from the American Collection of Crops (ATCC) were used as test cultures: gram-positive bacteria *of Staphylococcus of aureus* of ATCC 25293, spore culture *of Bacillus of subtilis* of ATCC 6633, gram-negative cultures *of Escherichia of coli* of ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. The antifungal effect was elucidated with respect to the yeast-like fungus of the genus Candida - *Candida albicans* ATCC 885-653 and the fungus *Aspergillus brasiliensis* ATCC 16404 [13,14]. One-day suspensions of bacterial microorganisms in physiological solution, a two-day culture of a yeastlike fungus and five-day Aspergillus niger culture were used in these experiments. The microbial load was 107 colony-forming units of microorganisms in 1 ml of nutrient medium (CFU / ml).

In Petri dishes, which were installed on a horizontal surface, 10 ml of melted "hungry" agar were added. After solidification of this lower layer of agar, 3 sterile steel cylinders (inner diameter - 6.0 ± 0.1 mm, height - 10.0 ± 0.1 mm) were placed on its surface at equal distance from each other and from the edge of the dish. Around the cylinders, an upper layer was filled, consisting of 14 ml of melted and cooled to 45-48°C agar mixed with the seed dose of the test microorganism. When working with bacterial cultures, meat-peptone agar (MPA) was used for the second layer, while working with fungal crops - agar Saburo. After cooling the upper layer, the cylinders were removed with sterile forceps and the test samples were added to the resulting wells until they were completely filled. Petri dishes were held for 30-40 minutes at room temperature and placed in a thermostat - bacterial cultures at a temperature of 30-35° C for 18-24 hours and culture of a fungus at a temperature of 20-25 ° C for 48 hours [13].

The results were recorded by measuring the growth inhibition zone of microorganisms, including the diameter of the wells. The measurements were carried out with an accuracy of 1 mm, while focusing on the complete absence of visible growth.

The diameter of the growth inhibition zone of microorganisms characterized the antimicrobial activity of the experimental samples:

1. the absence of growth inhibition zone of microorganisms around the well, as well as a inhibition zone with a diameter of up to 10 mm, was assessed as insensitivity of microorganisms to the sample introduced into the well;
2. the growth inhibition areas 11-15 mm in diameter were assessed as a weak sensitivity of the culture to the concentration of the active antimicrobial substance that was being studied;
3. growth inhibition zones with a diameter of 16-25 mm - as an indicator of the moderate sensitivity of strains of the microorganism to the test sample;
4. growth inhibition zones, the diameter of which exceeded 25 mm, indicate a high sensitivity of microorganisms to the test sample [13,14].

The given researches have been carried out at the Biotechnology Department, National University of Pharmacy under the guidance of prof. Strilets O.P. The processing of the research results was carried out according to the method given by State Pharmacopoeia of Ukraine in the section "Statistical analysis of the results of biological tests and quantitative determinations" [14].

Results. During the studies of antimicrobial properties of foaming agents regarding different cultures of microorganisms, the following results were obtained, which are shown in table 1.

Table 1 - Results for antimicrobial activity of samples (n=5)

Sample	Cultures of microorganisms					
	S. aureus ATCC 25293	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922	*Ps. aerug.* ATCC 27853	*C. albicans* ATCC 885-653	*Aspergillus niger* ATCC 16404.
	Diameters of growth inhibition zones of microorganisms, mm					
№ 1	-	-	-	-	-	-
№ 2	-	-	-	-	-	-
№ 3	21,4±0,5	19,6±0,5	15,6±0,5	13,8±0,4	33,6±0,5	32,6±0,5
№ 4	29,6±0,5	26,4±0,5	21,4±0,5	18,2±0,4	42,2±0,4	39,4±0,5
№ 5	31,2±0,4	27,4±0,5	22,2±0,4	19,6±0,5	43,2±0,4	40,2±0,4
№ 6	31,6±0,5	27,8±0,4	22,8±0,4	20,2±0,4	43,8±0,4	39,8±0,4

The data obtained experimentally and presented in table 1 confirm that the test samples No. 3, 4, 5 and 6 which have octopirox in different concentrations possess a wide range of antimicrobial action pertaining to the used test strains, namely, to bacterial gram-positive (*Staphylococcus aureus* ATCC 25293 and sporous culture of *Bacillus subtilis* ATCC 6633), gram-negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) cultures. And it also has a fungicidal effect pertaining to yeastlike fungus of the *Candida* genus - *Candida albicans* ATCC 885-653 and fungus *Aspergillus niger* ATCC 16404.

It should be noted that sample number 1 – a foaming base and sample number 2 (a foaming base + alpha lipoic acid) do not have antimicrobial action

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pertaining to all cultures of test strains of both gram-positive and gram-negative bacteria and fungal cultures.

It has been made certain experimentally that test strains of *Candida albicans* and *Aspergillus niger* fungi microorganisms are the most sensitive to all test samples No. 3,4,5,6 (the diameter of the inhibition zones exceeds 25 mm).

Sample No. 3 demonstrated moderate activity pertaining to gram-positive bacterial test cultures - *Staphylococcus aureus* (21.4 ± 0.5 mm), *Bacillus subtilis* (19.6 ± 0.5 mm), but regarding the gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* this sample demonstrated weak activity (15.6 ± 0.5 mm) and (13.8 ± 0.4 mm), respectively (microbial inhibition zones 11-15 mm).

Sample No. 4, as compared to other samples No. 5 and 6, demonstrated the greatest antimicrobial effect pertaining to all bacterial test cultures used in the experiment, high antimicrobial activity pertaining to gram-positive bacteria *Staphylococcus aureus* (29,6 ± 0,5 mm), *Bacillus subtilis* (26,4 ± 0,5 mm) and moderate activity pertaining to gram-negative cultures of *Escherichia coli* (21,4 ± 0, 5 mm), *Pseudomonas aeruginosa* (18.2 ± 0.4 mm). Cultures of *Candida albicans* and *Aspergillus niger fungi* are highly sensitive to the action of foaming agent (sample No. 4) which has octopirox 0.5% - 42.2 ± 0.4 mm and 39.4 ± 0.5 mm, respectively.

Samples No. 5 and No. 6 also have a broad spectrum of antimicrobial activity, but the values of antibacterial and antifungal activity are not significantly different from the activity of sample No. 4, pertaining to *Staphylococcus aureus* culture, the diameter of the inhibition zone is 31.2 ± 0.4; 31.6 ± 0.5 and 29.6 ± 0.5 mm, respectively, *Bacillus subtilis* 27.4 ± 0.5; 27.8 ± 0.4 and 26.4 ± 0.5 mm, respectively. Pertaining to gram-negative bacteria, activity of samples No. 5, No. 6 and No. 4 was *Escherichia coli* 22.2 ± 0.4; 22.8 ± 0.4 and 21.4 ± 0.5 mm, *Pseudomonas aeruginosa* 19.6 ± 0.5; 20.2 ± 0.4 and 18.2 ± 0.4 mm, respectively. Antifungal activity pertaining to *Candida albicans* for samples No. 4, No. 5 and No. 6 is 42.2 ± 0.4, 43.2 ± 0.4, and 43.8 ± 0.4 mm, respectively; for *Aspergillus niger* culture, 39.4 ± 0.5, 40.2 ± 0.4, and 39.8 ± 0.4 mm, respectively.

Therefore, based on the experimental data, in the development of the foaming product composition with octopirox AFI concentration of 0.5% is optimal and further increase it to 0.75% (sample No. 5) and 1.0% (sample No. 6) is impractical.

It should be noted that the studies showed that foaming samples had high antifungal activity and moderate effect on gram-negative bacteria, so in the development of the optimal composition of the tool and its long-term storage and use, it is necessary to consider the introduction of preservatives and conduct appropriate research.

Conclusion. Thus, the results of the experiments showed that the test samples No. 1 and No. 2 did not have any antimicrobial action pertaining to gram-positive, gram-negative bacterial cultures and antifungal activity. Samples No. 3, No. 4, No. 5 and No. 6 of the foaming product with octopirox at concentrations of 0.25%, 0.5%, 0.75% and 1.0% have a broad spectrum of antimicrobial action pertaining to gram-positive (*Staphylococcus aureus* ATSC 25293 and spore cultures of *Bacillus subtilis* ATSS 6633), gram-negative (*Escherichia coli* ATSS 25922 and *Pseudomonas aeruginosa* ATCC 27853) bacterial cultures, as well as in relation to the cultures of *Candida albicans fungi* ATSS 885-653 and *Aspergillus niger fungus* ATCC 16404.

However, the experimental results showed that sample No 4 (octopirox concentration of 0.5%) was the most promising for further work on the development of antimicrobial activity of the foaming product.

Research of antimicrobic activity of foaming products samples with octopirox

Zaika S.V., Strilets O. P., Baranova I.I., Bezpala Yu. O., Martyniuk T. V.

Introduction. Seborrheic dermatitis (SD) - chronic recurrent skin disease, which is associated with increased cutaneous fatty secretion, change in its qualitative composition and is characterized by localization in the areas of accumulation of sebaceous glands on the scalp, face, upper torso. Therefore, the choice of tactics for the treatment of patients with SD depends on the degree of clinical manifestations, duration of the disease, information about the effectiveness of previously conducted therapy. Particular attention is paid to the choice of antifungal medicines in the treatment of SD. Traditionally, local remedies in the form of ointments or creams are used to treat SD of the scalp, they cause many inconveniences when used. In this respect, foaming products, in particular shampoos, which include active substances that have certain therapeutic potential regarding the main mechanisms of SD treatment, having self-regulating, anti-inflammatory, antifungal, antibacterial, reparative, moisturizing properties, etc. are very appropriate. One of the main factors in the development of this disease is the yeastlike fungus of Malassézia fúrfur genus (or Pityrósporum ovále), which is present in the skin of each person. Octopirox®, which has strong fungicidal (antifungal) and antibacterial actions, was chosen among a number of modern products in this field. Therefore, the main purpose of our experiment was to study the antimicrobial activity of developed samples of foaming agents with octopirox to justify the rational concentration. **Materials and methods.** For this study, we produced a number of experimental samples of foaming bases with octopirox and α-lipoic acid at different concentrations: sample № 1 - foaming base; sample № 2 – foaming base + α-lipoic acid; sample № 3 – foaming base + octopirox (0,25 %); sample № 4 – foaming base + octopirox (0,5 %); sample № 5 – foaming base + octopirox (0,75 %); sample № 6 – foaming base + octopirox (1,0 %). The antimicrobial activity of prototype gels was studied in vitro by diffusion in agar ("wells" method). This method is based on the ability of active substances to diffuse in the agar medium, which was previously inoculated by bacterial crops. The results of the studies make it

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possible to characterize both the antimicrobial activity of the samples and the release of antimicrobial substances from the base, because the growth inhibition zones of microorganisms are formed as a result of the diffusion of these substances into a dense nutrient medium. **Results.** Based on the experimental data, in the development of the foaming product composition with octopirox AFI concentration of 0.5% is optimal and further increase it to 0.75% (sample No. 5) and 1.0% (sample No. 6) is impractical. It should be noted that the studies showed that foaming samples had high antifungal activity and moderate effect on gram-negative bacteria, so in the development of the optimal composition of the tool and its long-term storage and use, it is necessary to consider the introduction of preservatives and conduct appropriate research. **Conclusion.** Thus, the results of the experiments showed that the test samples No. 1 and No. 2 did not have any antimicrobial action pertaining to gram-positive, gram-negative bacterial cultures and antifungal activity. Samples No. 3, No. 4, No. 5 and No. 6 of the foaming product with octopirox at concentrations of 0.25%, 0.5%, 0.75% and 1.0% have a broad spectrum of antimicrobial action pertaining to gram-positive (*Staphylococcus aureus* ATSC 25293 and spore cultures of *Bacillus subtilis* ATSS 6633), gram-negative (*Escherichia coli* ATSS 25922 and *Pseudomonas aeruginosa* ATCC 27853) bacterial cultures, as well as in relation to the cultures of *Candida albicans fungi* ATSS 885-653 and *Aspergillus niger fungus* ATCC 16404. However, the experimental results showed that sample No 4 (octopirox concentration of 0.5%) was the most promising for further work on the development of antimicrobial activity of the foaming product.

Key words: seborrheic dermatitis, antimicrobial activity, octopirox, foaming product, product for men.

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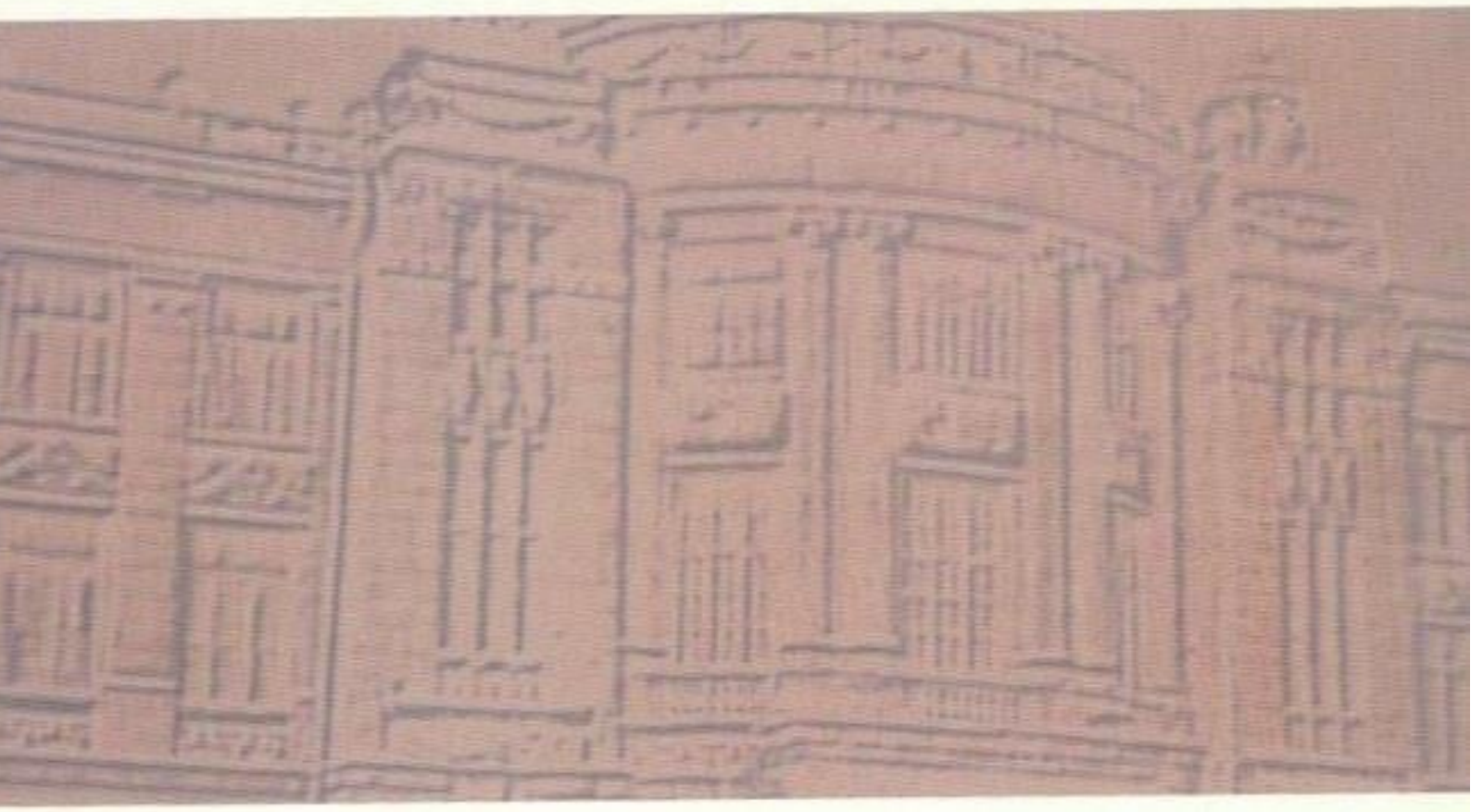

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extraction of herbal material with methanol, followed by concentration of the extract to the ratio of HD-test solution 1: 2, 1: 5, 1: 8); the choice of application volume of the test solution ; 5) the choice of the detection method (review of unified reagents and/or solutions for derivatization of chromatograms). Following conditions for identification have been chosen: the test solutions of HD (1: 5 in methanol), standard solution *CRS SPhU* alantholactone and isoalantholactone (0.1% solutions in methanol), TLC plates with a thin layer of silica gel treated with 5% silver nitrate solution, a solvent system toluene-ethyl acetate (9: 1), detection is carried out after treatment the plate with anise aldehyde solution and followed by heating. **Conclusion.** A procedure for identification of elecampane by for the national monograph of the SPhU "Elecampane roots and rhizomes" has been developed. It allows to identify such biologically active substances of the elecampane, as sesquiterpene lactones, which are markers of this species The developed chromatographic conditions allow to reliably chromatographically identify HD of elecampane in the presence on chromatograms of 3 zones of lactones – alantolactone, isoalantolactone and dihydroisoalantolactone.

Keywords. Elecampane roots and rhizomes, sesquiterpene lactones, monograph of the SPhU, TLC method, alantolactone, isoalantolactone and dihydroisoalantolactone.

Research of antimicrobic activity of foaming products samples with octopirox 54-57

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