

STUDY OF THE ANTIMICROBIAL ACTIVITY OF PHYTOINTMENT FOR TREATMENT OF MECHANICAL DAMAGES OF DAIRY TISSUE

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Introduction. Every period of a modern woman's life requires a special approach, but in each of them one should not forget that the main thing is health.

Medicinal plants, due to the fact that they contain different groups of biologically active substances (phenolic compounds, amino acids, vitamins, saponins, macro- and microelements, etc.), have a polyvalent effect on various parts of the pathological process. In the pathological process of gynaecological diseases can be involved absolutely all the links of the neuroendocrine system, the cardiovascular system, central and autonomic nervous systems, urogenital, immune, as well as the system of metabolism and haemostasis. All these factors lead to the violation of the specific functions of the female body and, therefore, when determining the treatment tactics, this gives grounds for wider use of herbal medicine [3].

One of the common pathologies found in gynaecological practice is cracking nipples, which represent the rupture of an external sensitive epidermis due to its mechanical damage. Cracks have different shapes (straight and stellar), as well as different depths (superficial and deep). The nature of cracks is quite diverse: poor quality personal care products or non-compliance with personal

hygiene rules, low-quality gels and cosmetics, improper breastfeeding, vitamin A and E deficiency, early use of hormonal contraceptives and other medicines, tanning bed, improperly selected linen and other [4].

Diagnosis is performed only when there are complaints, namely: when the nipple swells, blushing, pain and heaviness in the chest are felt.

The treatment is prescribed by the doctor depending on the duration of the disease and its severity. If the disease lasts up to three days, then wound healing, antiseptic and palliative drugs are prescribed. If the disease lasts more than three days, then antibiotic therapy is added to these agents, as there is a secondary infection. In such cases, the breastfeeding of the baby stops.

Taking into account the above, the composition of the soft dosage form for use in gynaecology, which has reparative, wound healing action, was developed.

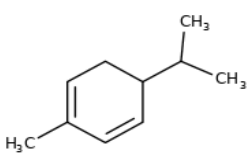
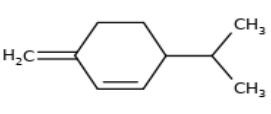
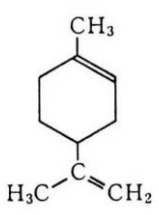
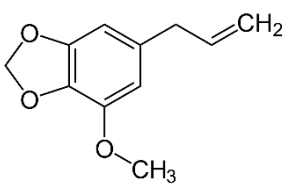
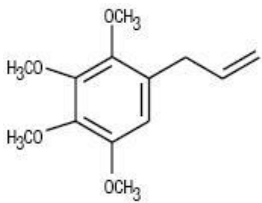
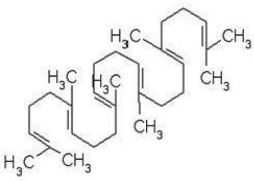
Ointment under the conventional name "Phytolan" contains in its composition vegetable oils: amaranth, sea buckthorn, parsley leaves, melaleuca and lanolin anhydrous as the basis [8].

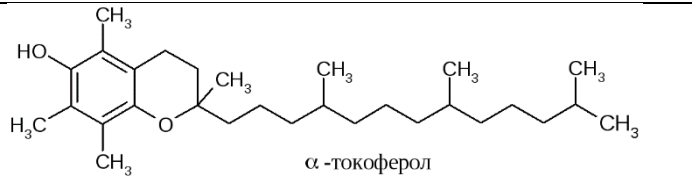
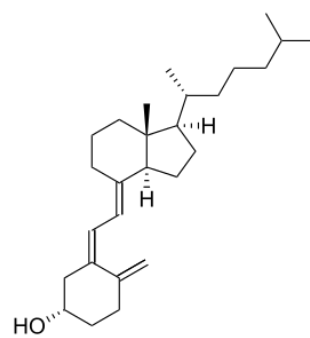
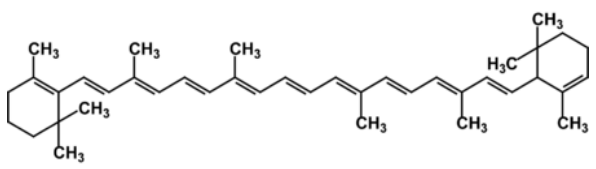
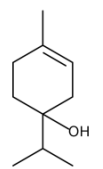
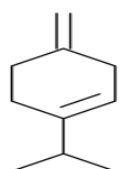
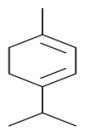
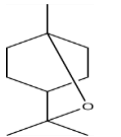
The chemical composition and the pharmacological properties due to it are given in Tab. 1. The unique healing properties of amaranth oil are largely determined by the presence in its composition of two powerful antioxidants - squalene and vitamin E, contained in an amaranth oil in a rare, especially active, tocotrienol form [9].

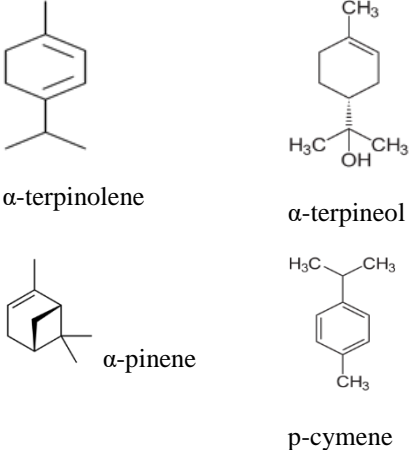
Amaranth oil contains more than 70 % of mono- and polyunsaturated fatty acids (linoleic (omega-6), oleic (omega-9), linolenic (omega-3), arachidonic, palmitoleic acids, etc.).

Tabl.1. Chemical composition and pharmacological properties of components of ointment "Phytolan"

Component	Chemical composition	Pharmacological properties
Parsley oil	<p>In parsley oil, about 45 components have been identified, the main of which: - monoterpenes (up to 80%): α-pinene 17 %, β-pinene 11,8 %, α-phellandrene 4,6 %, β-phellandrene 7,8 %, limonen 2,5 %, 1,3,8-p-Menthatriene; phenolic esters: myristicin 17,7 %, apiol 2 %</p> <div style="text-align: center;"> <p>I II III</p> <p>pinene isomers</p> </div>	<p>Affects the female reproductive system: stimulates the blood flow to the pelvic region and reduces the uterus, restores an irregular cycle (amenorrhea), pain relieves (dysmenorrhea), normalizes the menstrual cycle, enhances lactation. Lowers muscle tension, relieves the condition of mechanical damage to tissues, bruises, reduces muscle pain, restricts blood vessels, and improves local blood circulation, which is very important in case of damage to the skin of the mammary gland and the nodal mastopathy.</p>

	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">  <p>α- phellandrene</p> </div> <div style="text-align: center;">  <p>β phellandrene</p> </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 20px;"> <div style="text-align: center;">  <p>limonen</p> </div> <div style="text-align: center;">  <p>myristicin</p> </div> </div> <div style="text-align: center; margin-top: 20px;">  <p>allyltetramethoxybenzene</p> </div>	
<p>Ama-ranth oil</p>	<p>More than 70% of mono- and polyunsaturated fatty acids (linoleic (omega-6), oleinic (omega-9), linolenic (omega-3), arachidonic, palmitoleic acids, etc.). More than 9% of phospholipids (which contain phosphatidylcholine predominantly), squalene (over 8%), about 2% vitamin E, phytosterols (over 2%), carotenoids (vitamin A precursors), vitamin D, bile acids, various macro- and microelements: potassium, iron, phosphorus, calcium, magnesium, copper, and others. The valuable component of amaranth oil is squalene.</p> <div style="text-align: center;">  </div> <p>2,6,10,15,19,23-hexamethyltetracos-2,6,10,14,18,22-hexaene</p>	<p>Contributes to the oxygenation of organs and tissues, has immunomodulating, antitumor and anticarcinogen effects. Stimulates skin rejuvenation, saturates it with oxygen and restores blood supply. Strengthens the process of protein synthesis, activates the functioning of the skin cells, which helps to quickly heal wounds</p>

	 <p style="text-align: center;">α -токоферол</p> <p>Tocopheryl acetate (vitamin E)</p>  <p>vitamin D</p>  <p>β-carotene</p>	
<p>Sea buck-thorn oil</p>	<p>Carotenoids, vitamins (E, C, B1, B2, B3, B6, B9, K), macro- and trace elements (magnesium, iron, calcium, manganese, silicon, nickel, molybdenum, etc.), amino acids, mono- and polyunsaturated fatty acids, phytosterols, phospholipids), flavonoids (rutin, isorhamnetin, quercetin, cempferol, etc.), triterpene acids (ursolic, oleanolic, etc.), organic acids (malic, tartaric, salicylic, oxalic, malic, amber), phytoncides, tannins, pectin, coumarin, etc.).</p>	<p>Wound healing, reparative, regenerative</p>
<p>Mela-leuca oil</p>	<p>Terpinen-4-ol, a concentration of 30% to 48% and a component of 1,8-cineole, a concentration of 0 to 15%, and some other components</p> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">  <p>terpinen-4-ol</p> </div> <div style="text-align: center;">  <p>γ-terpinen</p> </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 20px;"> <div style="text-align: center;">  <p>α-terpinen</p> </div> <div style="text-align: center;">  <p>1,8-cineole</p> </div> </div>	<p>Antimicrobial, anti-inflammatory, anti-tumor action</p>

	 <p> <chem>CC1=CC=C(C)C=C1</chem> α-terpinolene </p> <p> <chem>CC1=CC=C(C)C(O)C1</chem> α-terpineol </p> <p> <chem>CC1=CC2=C(C1)C=CC2</chem> α-pinene </p> <p> <chem>CC1=CC=C(C)C=C1C</chem> p-cymene </p>	
Lanolin anhydrous	Palmitine, stearin, behenic, cerotinic, montanic, lignoceric acid, cholesterol, isocholesterine 25-33%, ergosterol 0.1%, carnaubic, ceryl, cetyl alcohol 29.9%	Softening, reparative, and moisturizing

More than 9 % of phospholipids (which contain phosphatidylcholine predominantly), squalene (over 8 %), about 2 % vitamin E, phytosterols (over 2 %), carotenoids (vitamin A precursors), vitamin D, bile acids, various macro- and microelements: potassium, iron, phosphorus, calcium, magnesium, copper, and others. Squalene is part of the sebaceous glands and subcutaneous fatty tissue, is an important participant in the synthesis of steroid hormones, cholesterol and vitamin D in the human body, which promotes the oxygenation of organs and tissues, has a powerful anti-tumour and anticarcinogen effect, increases the resistance of the human body to various viral, fungal, bacterial infections and the effects of harmful radioactive radiation, development and spread of malignant tumors, activates metabolic processes, promotes quick and effective recovery of damaged areas of the skin and mucous membranes. It also stimulates skin rejuvenation, saturates it with oxygen and restores blood supply, strengthens the process of protein synthesis, activates the function of skin cells, which helps to quickly heal wounds.

Anti-inflammatory, bactericidal, antitumor and immunostimulating properties of amaranth oil are also largely due to high content of phytosterol in it.

Parsley contains essential oil in all parts: roots (0.1 %), seeds (2-7 %) and leaves (0.3-0.7 %). In parsley oil, about 45 components have been identified, the main of which: - monoterpenes (up to 80 %): α -pinene 17 %, β -pinene 11,8 %, α -phellandrene 4,6 %, β -phellandrene 7,8 %, limonen 2,5 %, 1,3,8-p-Menthatriene; phenolic esters: myristicin 17,7 %, apiol 2 % [3].

Parsley's oil has a powerful effect on the female reproductive system: stimulates the blood flow to the pelvic region and reduces the uterus, restores an irregular cycle (amenorrhoea), pain relieves (dysmenorrhoea), normalizes the menstrual cycle, enhances lactation. Lowers muscle tension, relieves the condition of mechanical damage to tissues, bruises, reduces muscle pain, restricts blood vessels, and improves local blood circulation, which is very

important in case of damage to the skin of the mammary gland and the nodal mastopathy [7].

In the parsley essential oil, there are natural phytoestrogens, which is why it is actively used in pregnancy, puberty, climacteric disorders. Apiol enhances blood circulation, stimulates the reduction of inflammation and the disappearance of cysts.

Oil of sea buckthorn is a source of carotenoids, vitamins (E, C, B1, B2, B3, B6, B9, K), macro- and trace elements (magnesium, iron, calcium, manganese, silicon, nickel, molybdenum, etc.), amino acids, mono- and polyunsaturated fatty acids, phytosterols, phospholipids), contains also flavonoids (rutine, isorhamnetin quercetin, cempferol, etc.), triterpic acids (ursolic, oleanolic, etc.), organic acids (wine, salicylic, oxalic, malic, amber), phytoncides, tannins, pectin, coumarin, etc.) providing reparative and wound healing effect.

The composition of tea tree oil is determined by the international standard ISO 4730. Among more than 98 compounds contained in the oil, terpinen-4-ol is responsible for most of the antimicrobial activity. As part of the oil, the component is terpinen-4-ol, a concentration of 30% to 48% and a component of 1,8-cineole, a concentration of which is 0 to 15%, and some other components. Tea tree essential oil provides anti-inflammatory and anti-tumour (in vitro) action [1].

Lanolin anhydrous - well absorbed into the skin and has a softening effect. By properties, it is close to human sebum (chemical similarity with eleidin - a substance in the glossy layer of the epidermis).

Lanolin has a very high emulsification capacity, improves the absorption of ointment, promotes deeper penetration of active substances into the breast tissue, promotes the repair of nipple cracks and softens the skin of the mammary gland. The expressed moisturizing properties of lanolin are due to the ability to absorb and retain a large amount of moisture. Penetrating deeply into the skin, lanolin not only saturates the skin with moisture, but also prevents its loss for a long time.

An important characteristic of the created drug is the presence of antimicrobial activity in relation to the agents of "hospital" infections that can accompany a woman in the hospital, or during the treatment of various gynaecological diseases.

The aim of the research was comparative study of the spectrum and level of antimicrobial activity in vitro ointment "Phytolan", the comparator preparation - the analogue by the action of the cream "Mama care" and specimens containing ointment base - anhydrous lanolin and a separate phytooil in a concentration of 10 %.

Material&methods. As test strains we took standard strains from the American standard collection of microorganisms: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 885-653. In their cultivation and research, they used the appropriate nutrient media: Müller-Hinton agar (in determining antibacterial activity) and Saburo agar (in determining antifungal activity). The purity of each culture of the microorganism was confirmed by the typical morphological, tinctorial, cultural and biochemical properties. The antimicrobial activity of the drugs was studied in vitro in commonly accepted in microbiological practice method of diffusion in agar in the modification of wells. This method is based on the ability of active substances to diffuse into agar medium, which is pre-sown with the test culture [5,

6].

The molten agar nutrient medium was cooled to 45° C, poured into the bottom layer into Petri dishes in a volume of 10 ml. After hardening of the agar on it were placed six sterile cylinders of stainless steel 10 mm in height and an internal diameter of 8 mm, around which the second layer of medium was poured into a volume of 15 ml, seeded with appropriate cultures of microorganisms. The microbial load was 0,5 units. Mc Farland turbidity per 1 ml of medium. After clamping the upper layer of agar, the cylinders were removed with sterile tweezers and in the formed wells, the test specimens were administered. Petri dishes were kept for one hour at room temperature, after which they were placed in a thermostat and incubated for 24 hours at a temperature of 37° C with Muller - Hinton agar 25° C with Sabur agar [2].

The level of antimicrobial activity of the developed ointment, experimental specimens and the comparison preparation was evidenced by the diameter of zones of growth retardation of microorganisms around the well.

The research was conducted in six-time repetitions for each test culture. Statistical processing of the results of the study was carried out according to Student's criterion ($P < 0.5$). (Tab. 2).

Table 2. Indicators of spectrum and level of antimicrobial activity of experimental samples

№	Samples	Diameter of the zone of growth retardation of microorganisms, mm				
		<i>S. aureus</i> ATCC 25923	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 9027	<i>B. subtilis</i> ATCC 6633	<i>C. albicans</i> ATCC 885-653.
1.	«Phytolan» ointment	13.1±0.71	12.2±0.15	12.2±0.12	12.2±0.11	12.2±0.12
2.	Comparison drug	12.1±0.13	12.1±0.14	12.1±0.14	12.1±0.11	12.2±0.13
3.	Lanolin + amaranth oil	12.1±0.12	12.1±0.12	12.1±0.15	12.1±0.11	12.2±0.12
4.	Lanolin + oil of sea buckthorn	12.1±0.14	12.2±0.12	12.2±0.15	12.2±0.12	12.1±0.13
5.	Lanolin + parsley oil	12.2±0.14	12.2±0.13	12.2±0.15	12.2±0.11	12.1±0.12
6.	Lanolin + tea tree oil	12.8±0.14	12.2±0.12	12.2±0.16	12.2±0.21	12.2±0.15

Results&discussion. According to the results of the research, shown in Tab. 2, ointment samples (# 1) exhibited a moderate level of antimicrobial activity against *Staphylococcus aureus* culture and practically no activity compared to other cultures. Thus, the growth retardation zone of *S. aureus* culture around the ointment samples was 13.6 mm, around other cultures 12.1 - 12.2 mm.

Other study samples: the comparison drug "Mama care" (analogue by effect) and individually samples with

lanolin and amaranth, sea buckthorn, parsley, tea tree almost did not exhibit antimicrobial activity to used test cultures.

Thus, the zones of growth inhibition of bacterial cultures around the wells with the above mentioned samples ranged from 12,1 to 12,8 mm, cultures of the fungus *C. albicans* 12,1 – 12,2, indicating that there is practically no antimicrobial action. Despite the known antimicrobial

properties of essential oils, in particular tea tree (6), the absence of a pronounced effect in vitro may be due to the technological difficulties of creating an effective antimicrobial concentration in the ointment. The tendency towards the manifestation of antistaphylococcal activity of the ointment may be related to the effect of synergy of essential oils in the composition, in particular the influence of tea tree oil and requires further study of the mechanism of this phenomenon.

Conclusions

1. The use of essential oils in the treatment of mastopathy is substantiated.
2. Antimicrobial activity of the developed ointment in relation to *S. aureus* culture was established and the synergistic effect of essential oils in the composition of the designed drug was revealed.
3. The promise of the application of the developed preparation for the treatment of mechanical damage to the tissues of the mammary gland, complicated by bacterial infections, and the prevention of mastopathy has been proven.

STUDY OF THE ANTIMICROBIAL ACTIVITY OF PHYTOOINTMENT FOR TREATMENT OF MECHANICAL DAMAGES OF DAIRY TISSUE

Zuikina S. S., Vyshnevskaya L. I., Silaeva L. F.

Introduction. One of the common pathologies found in gynaecological practice is cracking nipples, which represent the rupture of an external sensitive epidermis due to its mechanical damage. Cracks have different shapes (straight and stellar), as well as different depths (superficial and deep). The nature of cracks is quite diverse: poor quality personal care products or non-compliance with personal hygiene rules, low-quality gels and cosmetics, improper breastfeeding, vitamin A and E deficiency, early use of hormonal contraceptives and other medicines, tanning bed, improperly selected linen and other. Diagnosis is performed only when there are complaints, namely: when the nipple swells, blushing, pain and heaviness in the chest are felt. The treatment is prescribed by the doctor depending on the duration of the disease and its severity. If the disease lasts up to three days, then wound healing, antiseptic and palliative drugs are prescribed. If the disease lasts more than three days, then antibiotic therapy is added to these agents, as there is a secondary infection. In such cases, the breastfeeding of the baby stops. Taking into account the above, the composition of the soft dosage form for use in gynaecology, which has reparative, wound healing action, was developed. Ointment under the conventional name "Phytolan" contains in its composition vegetable oils: amaranth, sea buckthorn, parsley leaves, melaleuca and lanolin anhydrous as the basis. The aim of the research was comparative study of the spectrum and level of antimicrobial activity in vitro ointment "Phytolan", the comparator preparation - the analogue by the action of the cream "Mama care" and specimens containing ointment base - anhydrous lanolin and a separate phytooil in a concentration of 10%. **Material&methods.** As test strains we took standard strains from the American standard collection of microorganisms: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC

6633, *Candida albicans* ATCC 885-653. Cultivation of cultures was carried out by traditional methods in nutrient media with subsequent confirmation of morphological, cultural and biochemical properties (3). In the course of research, appropriate nutrient media indicated in the national part of the SPHU were used: medium # 1 - in the study of antibacterial activity and medium # 2 - in the study of antifungal activity of the samples. The antimicrobial activity of the drugs was studied in vitro in commonly accepted in microbiological practice method of diffusion in agar in the modification of wells. This method is based on the ability of active substances to diffuse into agar medium, which is pre-sown with the test culture. The molten agar nutrient medium was cooled to 45° C, poured into the bottom layer into Petri dishes in a volume of 10 ml. After hardening of the agar on it were placed six sterile cylinders of stainless steel 10 mm in height and an internal diameter of 8 mm, around which the second layer of medium was poured into a volume of 15 ml, seeded with appropriate cultures of microorganisms. The microbial load was 0,5 units. Mc Farland turbidity per 1 ml of medium. After clamping the upper layer of agar, the cylinders were removed with sterile tweezers and in the formed wells, the test specimens were administered. Petri dishes were kept for one hour at room temperature, after which they were placed in a thermostat and incubated for 24 hours at a temperature of 37° C with Muller - Hinton agar and 25° C with Sabur agar. The level of antimicrobial activity of the developed ointment, experimental specimens and the comparison preparation was evidenced by the diameter of zones of growth retardation of microorganisms around the well. The research was conducted in six-time repetitions for each test culture. Statistical processing of the results of the study was carried out according to Student's criterion ($P < 0.5$). **Results&discussion.** According to the results of the research, ointment samples exhibited a moderate level of antimicrobial activity against *Staphylococcus aureus* culture and practically no activity compared to other cultures. Thus, the growth retardation zone of *S. aureus* culture around the ointment samples was 13,6 mm, around other cultures 12,1 – 12,2 mm. Other study samples: the comparison drug "Mama care" (analogue by effect) and individually samples with lanolin and amaranth, sea buckthorn, parsley, tea tree almost did not exhibit antimicrobial activity to used test cultures. Despite the known antimicrobial properties of essential oils, in particular tea tree (6), the absence of a pronounced effect in vitro may be due to the technological difficulties of creating an effective antimicrobial concentration in the ointment. The tendency towards the manifestation of antistaphylococcal activity of the ointment may be related to the effect of synergy of essential oils in the composition, in particular the influence of tea tree oil and requires further study of the mechanism of this phenomenon. **Conclusion.** 1. The use of essential oils in the treatment of mastopathy is substantiated. 2. Antimicrobial activity of the developed ointment in relation to *S. aureus* culture was established and the synergistic effect of essential oils in the composition of the designed drug was revealed. 3. The promise of the application of the developed preparation for the treatment of mechanical damage to the tissues of the mammary gland, complicated by bacterial

infections, and the prevention of mastopathy has been proven.

Key words: mastopathy, ointment, antimicrobial activity, synergism, phytooil.

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