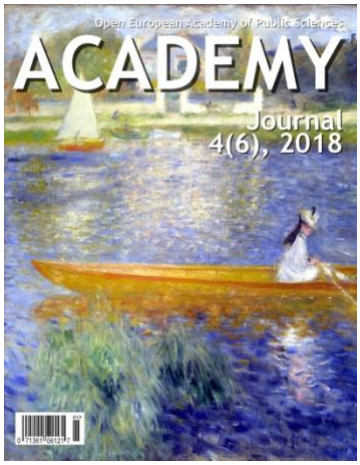

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Семченко Е.В, Вишневская Л.И., Половко Н.П., Ромась Е.П.

ИЗУЧЕНИЕ АНТИМИКРОБНОЙ АКТИВНОСТИ И МИКРОБИОЛОГИЧЕСКОЙ ЧИСТОТЫ ЭКСТЕМПОРАЛЬНОЙ МИКСТУРЫ, РЕКОМЕНДУЕМОЙ В КАЧЕСТВЕ ДОПОЛНИТЕЛЬНОЙ ТЕРАПИИ В ЛЕЧЕНИИ ТРЕМАТОДОЗОВ

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ИЗУЧЕНИЕ АНТИМИКРОБНОЙ АКТИВНОСТИ И МИКРОБИОЛОГИЧЕСКОЙ ЧИСТОТЫ ЭКСТЕМПОРАЛЬНОЙ МИКСТУРЫ, РЕКОМЕНДУЕМОЙ В КАЧЕСТВЕ ДОПОЛНИТЕЛЬНОЙ ТЕРАПИИ В ЛЕЧЕНИИ ТРЕМАТОДОЗОВ

Семченко Екатерина Валентиновна
к.ф.н., доцент кафедры аптечной технологии лекарств,
Национальный фармацевтический университет, Украина, г. Харьков
E-mail: tolochko.kv@gmail.com
Вишневская Лилия Ивановна
д.ф.н., декан фармацевтического факультета № 1,
Национальный фармацевтический университет, Украина, г. Харьков
Половко Наталья Петровна
д.ф.н., профессор, заведующая кафедрой аптечной технологии лекарств,
Национальный фармацевтический университет, Украина, г. Харьков
Ромась Екатерина Петровна
к.ф.н., старший преподаватель кафедры аптечной технологии лекарств,
Национальный фармацевтический университет, Украина, г. Харьков

INVESTIGATION OF ANTIMICROBIAL ACTIVITY AND MICROBIOLOGICAL PURITY OF THE EXTEMPORAL MIXTURE RECOMMENDED FOR SUPPLEMENTARY THERAPY OF TREMATODES

Kateryna V. Semchenko
candidate of pharmaceutical sciences, associate professor,
Department of Pharmaceutical Technology of Drugs,
National University of Pharmacy, Ukraine, Kharkiv,
Liliia I. Vyshnevska
doctor of pharmaceutical sciences, dean of Pharmaceutical Faculty 1,

Department of Pharmaceutical Technology of Drugs,
National University of Pharmacy, Ukraine, Kharkiv,
Natalya P. Polovko
doctor of pharmaceutical sciences, professor,
head of Department of Pharmaceutical Technology of Drugs,
National University of Pharmacy, Ukraine, Kharkiv,
Kateryna P. Romas
candidate of pharmaceutical sciences, seniour lecturer,
Department of Pharmaceutical Technology of Drugs,
National University of Pharmacy, Ukraine, Kharkiv,

АННОТАЦИЯ

Частота трематодозов, вызванных потреблением загрязненных продуктов и воды, достигает более 56 миллионов человек и ежегодно увеличивается. Целью данного исследования является определение качества экстемпоральной микстуры с экстрактами предварительно отобранных лекарственных растений, рекомендованной для вспомогательной терапии трематодозов, по показателям «микробиологическая чистота» и «антимикробная активность», в соответствии с ГФУ. Полученные данные показали, что внешняя смесь микстура соответствует требованиям ГФУ по микробиологической чистоте и показывает слабую антимикробную активность.

ABSTRACT

Today, frequency of trematodes infections caused by contaminated food and water intake reaches more than 56 million of people worldwide and shows the tendency to increase. The aim of this research is to determine the quality of the extemporal mixture with extracts of the pre-selected medicinal plants, recommended for supplementary trematodes treatment, by indicators “microbiological purity” and “antimicrobial activity” in accordance with SPhU methods. The findings showed that the extemporal mixture meets the SPhU requirements by microbiological purity and shows week antimicrobial activity.

Ключевые слова: антигельминтные препараты; микстура; антимикробная активность; микробиологическая чистота; трематоды.

Keywords: anthelminthic medicines; mixture; antimicrobial activity; microbiological purity; trematodes.

INTRODUCTION

According to the WHO, at least 56 million people in the world suffer from one or more trematode infections of food origin, which are the most common in East Asia and South America [8, 9].

Trematodes are any parasitic flatworm of the class Trematoda.

Trematodes have organs of fixation – muscle suckers (oral and abdominal). Under the investing tissue of trematodes there is a double layer of muscles; Trematodes have nervous, excretory, reproductive and digestive systems. Almost all trematodes are hermaphrodites, while only schistosomes of different sexes [10].

The life cycle of the trematode is closely linked to freshwater reservoirs. Some trematodes (opisthorchiasis, clonorhosis, metagonimous, etc.) are infected with the use of raw or freshly salted fish; others - when drinking water from standing water bodies and consuming wild aquatic plants (fasciolosis). Schistosoma larvae swim in water and actively penetrate into the circulatory system through the skin. The dissemination of trematodes contributes to the contamination of water bodies with feces of patients with larvae [7].

Prevention methods to combat this group of pathogens are:

- protection of water supply sources from fecal contamination;
- sanation of infected animals;
- in the foci of infection – do not consume water plants;
- molluscs destruction;
- sanitary control over the manufacture of fishery products.

Among the trematodes that occur in Ukraine, the most common are opisthorchiasis, fasciolysis and schistosomiasis.

Opisthorchiasis is one of the most common human helminthiasis. In Ukraine, quite large centers of opisthorchiasis are found in the basins of the Dnipro, Desna, Southern Bug, Seversky Donets. The cases of infection with opisthorchiasis are registered more than in 20 regions of Ukraine.

Human fasciolosis and schistosomiasis are usually recorded as sporadic cases, however, in tropical climatic zones, the damage to humans can reach a high level [1 – 3].

General characteristics of the above-mentioned helminthiasis is given in Table 1 [1 – 5, 10].

The analysis of existing presciptions of folk medicine, as well as the experience of trematodes treatment with herbal remedies, allowed to indicate the most promising plant raw material for the creation of phytocomposition with anthelminthic action with a predominant effect on trematodes [10, 11]:

- ✓ Tansy flowers (Flores Tanaceti vulgaris, Tanacetum vulgare L., Asteraceae);
- ✓ Wormwood herb (Herba Artemisii absinthii, Artemisia absinthium L., Asteraceae);
- ✓ Flax seeds (Semina Lini, Linum usitatissimum L., Linaceae);
- ✓ Walnut leaves (Folia Juglandis, Juglans regia L., Juglandaceae);
- ✓ Chamomile flowers (Flores Chamomillae, Chamomilla recutita L., Asteraceae);
- ✓ Centaury grass (Herba Centaurii, Centaurium erythraea Rafn., Gentianaceae);
- ✓ Peonies rhizomes and roots (Rhizomata cum radices Paeoniae anomalae, Paeonia anomala L., Paeoniaceae);
- ✓ Ginger rhizome (Rhizoma Zingiberi officinalis, Zingiber officinal L., Zingiberaceae);

✓ Ginseng roots (Radices Ginseng, Panax ginseng C. A. Mey, Araliaceae).

Based on this phytocomposition, an extemporal mixture containing the appropriate aqueous and aqueous-glycerol extracts was proposed and offered for supplementary therapy in the treatment of trematodes.

The objective of our study was to study the quality of the prepared extemporal mixture at the Department of Biotechnology of the National University of Pharmacy (NUPh) under the supervision of Doctor of Pharmaceutical Sciences prof. Strelets O. P. there was investigated its microbiological purity and antimicrobial activity.

Table 1

Characteristics of the most common trematodes (opisthorchiasis, fasciolosis, schistosomiasis)

Indicator	***Opisthorchiasis***	***Fasciolosis***	***Schistosomiasis***
1	**2**	**3**	**4**
Causative agent	*Opisthorchis felineus* (Cat flukes)	*Fasciola hepatica* (Liver flukes)	*Schistosoma heamatobium* (pathogens of schistosomiasis of the genitourinary system), *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma mekongi*, *Schistosoma intercalatum*– pathogens of intestinal schistosomiasis
Epidemiology	Natural focal zoonosis. Biogelminthis. End-owner – human and animals (cats, dogs, pigs) Intermediate owner – freshwater mollusk from family Bithynia; fish from the carp family. The transmission factor is fish (raw or salted) containing living larvae (metacercarium).	Natural focal zoonosis. Oral biogelminthosis. The source of the infection and the final owner - human, large and small cattle, horses, rodents. Intermediate owner – mollusks (small pond). Factors of transmission are aquatic plants, vegetables.	Anthroponosis, (S. japonicun - natural-focal zoonosis). The final owner is a human. The source of infection is a human (when S. japonicum – domestic and wild animals (cows, goats, pigs, horses, cats, dogs, rodents, etc.). Intermediate owner - mollusks. Transmission factor - hit on the skin and mucous membranes of living cercariae when bathing, washing clothes, washing vegetables, working in reservoirs, as well drinking

Continuation of table 1

1	2	3	4
			water from them, (for S. japonicum – in contact with grass and soil on the shores of freshwater ponds).
Pathogenesis	Metacercarium → Bile ducts → Gall bladder → Bile ducts and ducts of the pancreas.	Penetration of the larvae of fascia in the mucous membrane of the small intestine → the liver and gallstones, the pancreas, the brain, eyes, etc.	Cercariae → skin and mucous membranes of man → schistosomules → heart, lungs, large circulatory circle → liver, urinary bladder, intestine.
Clinical manifestations	Diarrhea, allergic rash, pain in the liver of people who came to the endemic area, asymptomatic course of endemic areas inhabitants, pain in the liver and gall bladder, dyspeptic symptoms, hepatomegaly, icterus sclera.	General toxic and allergic signs: fever, skin rash, arthralgia, hepatomegaly, jaundice. Chronic phase: abdominal pain in the liver, gall bladder, pancreas, liver enlargement, jaundice, diarrhea, body weight loss, anemia, adherence of the secondary microflora.	Common symptoms: allergic reactions, weakness, fever. Chronic phase: connective tissue formations, hematuria, dysuria. In schistosomiasis of the genitourinary system: inflammation of the bladder, urinary tract, deformation of the renal pelvis, anuria, uremia. In schistosomiasis of the intestine: pain in the abdomen of indeterminate localization and of varying intensity, dyspeptic symptoms, possible lesions of the genitals.

End of table 1

1	2	3	4
Possible complications	Liver abscesses, gall bladder phlegmon, biliary peritonitis, primary liver cancer, chronic cholangitis, cholecystitis, hepatitis, parasitic cyst rupture, stomach ulcer and duodenal ulcer, biliary sclerosis, large papillary duodenum, mechanical jaundice.	Anemia, cachexia, purulent cholangitis, cholecystitis, phlegmon of the gall bladder, liver abscess, mechanical jaundice, liver fibrosis, acute pancreatitis, myocarditis.	The lesions of the genitals in women (colitis, cracks, polyps of the mucous membrane of the vagina and uterus, menorrhagia, amenorrhea) and men (epididymitis, prostatitis). The link between schistosomiasis of the bladder and cancer of this organ was proved.
Diagnostics	Hemogram: leukocytosis, eosinophilia; biochemical methods: increase of bilirubin, AlAT, AsAT, LF, amylase, lipase, trypsin, dysproteinemia, hyperglycemia, amylase increase in urine, hypo-or achillia of gastric juice, ovarian bile, serological reactions (chronic opisthorchiasis): RNGA, RFA, etc.	Hemogram: leukocytosis, anemia, eosinophilia; biochemical methods: increased activity of AlAT AsAT, bilirubin, dysproteinemia, specific diagnostics.	Hemogram: leukocytosis, anemia, eosinophilia; Diagnosis in the chronic phase: the detection of eggs in the urine, feces, biopsy of the mucous membrane of the bladder and intestine.

MATERIAL AND METHODS

In the analysis of microbiological purity, the method proposed by State Pharmacopoeia of Ukraine (SPhU) 2 ed. (ar. 2.6.12, p. 251) was used [6]. In accordance with the requirements of the SPhU, the samples to be tested must meet the criteria for acceptability of microbiological purity of non-sterile ready-made herbal remedies for oral use (category C) (SPhU 2 ed., p. 795): the total amount of viable aerobic microorganisms (TAMC) should not exceed 105 CFU/g (colony-forming units in 1.0 g of samples) and yeast and mold fungi (TYMC) 104 CFU/g. There should also be no bacteria Escherichia coli, Salmonella (SPhU 2 ed., ar. 5.1.8, p. 795).

The antimicrobial activity was investigated in vitro by the method of diffusion into agar [6, 12]. This method, also known as the method of "wells", is based on the ability of the active substances to diffuse into the agar medium, previously inoculated with microorganisms' cultures. The results of the research characterize both the antimicrobial activity of the medicine and the release of antimicrobial substances from the base as zones of microorganisms' growth inhibition are formed as a result of the diffusion of these substances into a dense nutrient medium.

In a Petri dish set on a horizontal surface, 10 ml of melted "hungry" agar was added. After solidification of this lower agar layer, 3-6 sterile thin-walled steel cylinders were placed on its surface at equal distance from each other and from the edge of the cup (inner diameter – 6.0±0.1 mm, height – 10.0±0.1 mm). Around the cylinders, the top layer was filled, which consisted of 14 ml of molten and cooled up to 45-48 °C agar, which was previously mixed with the seed dose of the test microorganism. When working with bacterial cultures for the second layer, meat-peptone agar (MPA) was used; when working with yeast fungi agar Saburo was used. After cooling of the upper layer the cylinders were removed with sterile tweezers and the test samples of the medicine were added to the obtained wells until they were completely filled. The Petri dishes were

held for 30-40 minutes at room temperature and placed in a thermostat – bacterial cultures at the temperature 32.5±2.5 °C for 18-24 h, culture of yeast fungi – at the temperature 22.5±2.5 °C for 48 h.

Interpretation of results was performed by examination of microbial growth inhibition zone including the diameter of the wells. The measurement was carried out with an accuracy of 1 mm, while focusing on the complete absence of visible growth.

The diameter of microorganisms' growth inhibition zones characterizes the antimicrobial activity of the samples:

- the absence of microorganisms' growth inhibition zones around the well, as well as the growth inhibition area up to 10 mm in diameter, was assessed as insensitivity of microorganisms to the introduced sample;
- growth inhibition areas 11-15 mm in diameter were evaluated as a weak sensitivity of the culture to the concentration of active substances in the sample;
- zones of growth inhibition with a diameter of 16-25 mm were assessed as an indicator of moderate sensitivity of microorganisms to the test sample;
- zones of growth inhibition, the diameter of which exceeded 25 mm, indicates a high sensitivity of microorganisms to the test sample.
- Research was carried out under aseptic conditions, using a laminar box (biosecurity cabinet AC2-4E1 "Esco", Indonesia).

RESULTS AND DISCUSSION

The obtained results of the carried out research of the microbiological purity of the extemporal mixture samples are represented in Table 2.

Table 2

Results of extemporal mixture samples study by the indicator "microbiological purity"

Samples	Two-layer sowing method		Microorganisms	
	Number of CFU/ml			
	aerobic micro-organisms (TAMC)	yeast and mold fungi (TYMC)	*E. coli*	*Salmonella*
Extemporal mixture	860	420	-	-

Legend: CFU/ml – colony-forming units in 1.0 ml of samples.
«-» means the complete absence of bacteria in the sample.

Incubation of the prepared samples of mixture with herbal extracts (1:20 dilution) on McConkie agar (temperature 30-35ºC – 72 hours), deoxycholate agar with xylose and lysine (temperature 30-35 ºC – 48 hours) showed the absence of colonies corresponding to the result "the absence of bacteria Escherichia coli and Salmonella in 1 ml of the studied samples".

Determination of microbiological purity of samples by a method of two-layer sowing showed that the total number of viable aerobic microorganisms (TAMC) does not exceed 860 CFU/ml (notm 105 CFU/ml) and the total number of yeast and mold fungi (TYMC) does not exceed 420 CFU/ml (norm 104 CFU/ml).

The results of the further study of the antimicrobial activity of samples of the prepared mixture are given in Table 3.

Table 3

Results of antimicrobial activity of extemporal mixture samples (n=5)

Sample	Microorganisms cultures				
	S. aureus ATCC 25293	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922	*Ps. aeruginosa* ATCC 27853	*C. albicans* ATCC 885-653
	Diameters of the zone of growth retardation of microorganisms, mm				
Extemporal	13,8±0,4	17,8±0,4	17,6±0,5	19,6±0,5	-

mixture					

Legend: «-» – the zone of growth retardation of microorganisms is absent.

The above data indicate that the samples of the extemporal mixture have an antibacterial activity spectrum in relation to the used test strains of bacteria, namely, bacterial gram-positive (Staphylococcus aureus ATCC 25293 and spore culture Bacillus subtilis ATCC 6633), gram-negative (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853) cultures. Antifungal activity in relation to the yeast-like fungus of the genus Candida – Candida albicans ATCC 885-653, is absent.

Weak activity is detected in relation to the test culture Staphylococcus aureus, growth retardation diameter is less than 15 mm, namely 13,8±0,4 mm.

Samples of the studied extemporal mixture showed moderate antimicrobial activity (the diameter of the growth retardation zones of the test culture is 16-25 mm) in relation to bacterial cultures Bacillus subtilis (17,8±0,4) and to gram-negative cultures Escherichia coli (17,6±0,5), Pseudomonas aeruginosa (19,6±0,5).

CONCLUSION

On the basis of conducted research concerning the determination of the microbiological purity of the analyzed samples of the extemporal mixture, it was established that they fully comply with the requirements of the SPhU, which are put forward to the ready-made non-sterile medicinal herbal remedies for oral use (group C) in relation to the content of the total number of viable aerobic mesophilic bacteria (TAMC, not more than 105 CFU/ml) and fungi (TYMC, not more 104 CFU/ml) and the absence of bacteria Escherichia coli and Salmonella.

The study of antimicrobial activity showed that samples of the extemporal mixture have an antimicrobial spectrum in relation to the gram-positive (Staphylococcus aureus ATCC 25293, Bacillus subtilis ATCC 6633), gram-negative (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853) bacterial cultures of microorganisms and are characterized by moderate

activity in relation to these test cultures. It should be noted that fungicidal activity in relation to yeast-like fungus Candida albicans ATCC 885-653 is not set.

In order to ensure the microbiological stability of the dosage form in the process of usage and storage, consideration should be given to the addition of antimicrobial preservatives or active pharmaceutical ingredients with antimicrobial activity.

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