

## 1. Introduction

Helminths in humans are classified into round (nematodes), tape (cestodes) and flukes (trematodes). The toxic effect of the products of vital activity and disintegration of helminths parasitizing in the human body causes damage to the nervous system, dyspeptic disorders, suppression of the secretory and motor functions of the digestive system. Often there are allergic reactions, expressed by fever, rash, itching, eosinophilia or asthmatic attacks. The mechanical effect of helminths and their larvae vital activities lead to trauma caused by worms' suckers, crests and teeth. For example, ascaride conglomerates can clog the bile ducts and cause intestinal insufficiency [1, 2].

Nematodes (causative agents of ascariasis; trichocephalosis; enterobiosis; ankylostomidosis; strongyloidiasis; trichinosis) are one of the most common groups among helminths of the digestive system (Table 1) [3]. Children under 10 years old are especially susceptible: the share of children helminthiasis of digestive system accounts for 92.3 % of enterobiosis cases, 71.1 % of ascariasis cases, 61.5 % of trichocephalosis cases and 66.2 % of toxocariasis cases [4, 5].

The centuries-old heritage of folk medicine in the treatment of digestive system helminthiasis contains many recipes for mono- and polycomponent remedies. The analysis of this experience, as well as drugs offered by phytomedicine, made it possible to identify a number of medicinal plant raw material, which is promising for the creation of complex herbal medicines with polyvalent pharmacological activity [6, 7].

## RESEARCH OF ANTIMICROBIAL ACTIVITY OF ANTHELMINTHIC MIXTURE FOR NEMATODOSIS TREATMENT

**Kateryna Semchenko**  
PhD, Associate Professor<sup>1</sup>  
tolochko.kv@gmail.com

**Kateryna Romas**  
PhD, Assistant<sup>1</sup>  
ketirom@rambler.ru

<sup>1</sup>Department of Pharmaceutical Technology of Drugs  
National University of Pharmacy  
53 Pushkinska str., Kharkiv, Ukraine, 61002

**Abstract:** Parasitic infections are one of the most acute problems of the modern society as this group of diseases is met in all countries of the world. According to the World Health Organization around 1.2 billion people are infected with diverse parasitic infections each year. Helminthiasis of digestive system, primarily nematodosis, constitute a group of special risk, since a particularly sensitive audience for them are children of younger and middle age.

Phytotherapy, which is based on the centuries-old experience of folk medicine, promises to be a solution to this problem, enabling to create effective complex medicines for nematodosis prevention and treatment. Thus, the herbal mixture was prepared on the basis of the offered composition of medicinal plant raw material.

The research of antimicrobial activity of the studied herbal mixture was conducted in vitro by the method of diffusion into agar at the Biotechnology Department of the National Pharmaceutical University under the direction of Doctor of Pharmaceutical Sciences, prof. Strelets O. P.

The obtained results of the carried out research of the antimicrobial properties of the herbal mixture samples allowed examining the level of antimicrobial activity.

The further research of the investigated herbal mixture and the offered phytocomposition is promising for creation of new effective medicines with the complex action including anthelmintic (specific to nematodes), antiparasitic, anti-inflammatory, antibacterial, antiseptic, hepatoprotective, nephroprotective, laxative, antiallergic, antispasmodic, analgesic, sedative activities.

**Keywords:** nematodosis, helminthiasis, medicines of natural origin, medicinal plant raw material, antimicrobial activity, parasitology, digestive system, phytotherapy, pharmacotherapy.

On the basis of the conducted research [8, 9] the promising medicinal plant raw materials for the development of a new phytomedicines with complex anthelmintic action (specific to nematodes) are:

– Tansy flowers (Flores Tanaceti vulgaris, Tanacetum vulgare L., Asteraceae);

– Flax seeds (Semina Lini, Linum usitatissimum L., Linaceae);

– Horsetail herb (Herba Equiseti arvensis, Equisetum arvense L., Equisetaceae);

– Wormwood herb (Herba Artemisii absinthii, Artemisia absinthium L., Asteraceae);

– Centaury Herb (Herba Centaurii, Centaurium erythraea Rafn., Gentianaceae);

– Birch buds (Gemmae Betulae, Betula pendula Roth., Betulaceae);

– Willow bark (Cortex Salicis, Salix acutifolia Willd., Salicaceae);

– Cornflower herb (Flores Centaureae Cyani, Centaurea cyanus, Asteraceae)

– Valerian rhizomes with roots (Rhizomata cum radicibus Valerianae, Valeriana officinalis L., Valerianaceae);

– Dandelion herb with roots (Radices cum herba Taraxaci, Taraxacum officinale Wigg., Asteraceae).

Based on the offered composition the herbal mixture containing aqueous and water-glycerine extracts of the said medicinal plant raw material was prepared. Such composition provides anthelmintic (specific to nematodosis), antiparasitic, anti-inflammatory, antibacterial, antiseptic, hepatoprotective, nephroprotective, laxative, antiallergic, antispasmodic, analgesic and sedative activities.

**Table 1**  
Classification of common nematodes that cause disease in humans

Helminth	Path of infection	Disease	Larvae location	Helminth location
Pinworm	Oral	Enterobiosis	Hands, surrounding objects	Small intestine
Roundworm	Oral-faecal	Ascariasis	Hands, vegetables, flies' legs, soil	Intestine, moves to the liver, pancreas
Toxocara worm (larva)	Oral-faecal	Toxocariasis	Hands, vegetables, flies' legs, soil	Heart, liver, muscles, lungs, eyes, brain
Whipworm	Oral-faecal	Trichocephalosis	Hands, vegetables, flies' legs, soil	Small intestine

Thus, the aim of our research was to evaluate the antimicrobial activity of the proposed herbal mixture in order to determine the prospects of the further specified studies and development of new medicine with complex anthelmintic activity (specific to nematodosis).

## 2. Methods

The research of the antimicrobial activity was carried out at the Biotechnology Department of the National Pharmaceutical University, under the direction of Doctor of Pharmaceutical Sciences, prof. Strelets O.P. in May-June 2017.

The antimicrobial activity of the studied herbal mixture was investigated *in vitro* by the method of diffusion into agar (the method of "wells") [10, 11]. This method 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.

Research was carried out under aseptic conditions, using a laminar box (biosecurity cabinet AC2-4E1 "Esco", Indonesia).

The following pure cultures were used as the test cultures: gram-positive microorganisms *Staphylococcus aureus* ATCC 25293, spore culture *Bacillus subtilis* ATCC 6633, gram-negative cultures *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Fungicidal activity was examined towards yeast fungi of the genus *Candida albicans* ATCC 885-653. During the studies, one-day suspensions of bacterial microorganisms in physiological saline and a two-day culture of yeast fungi were used. The microbial load was  $10^7$  CFU/ml.

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 –  $6.0 \pm 0.1$  mm, height –  $10.0 \pm 0.1$  mm). Around the cylinders, the top layer was filled, which consisted of 14 ml of molten and cooled up to  $45-48^\circ\text{C}$  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  $32.5 \pm 2.5^\circ\text{C}$  for 18–24 h, culture of yeast fungi – at the temperature  $22.5 \pm 2.5^\circ\text{C}$  for 48 h.

The interpretation of the results was performed by the 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 wall, 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.

## 3. Results

The obtained results of the carried out research of the antimicrobial properties of the investigated mixture samples are represented in Table 2.

**Table 2**  
Results of antimicrobial activity research (n=5)

Sample	Microorganisms cultures				
	<i>S. aureus</i> ATCC 25293	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> ATCC 25922	<i>Ps. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 885-653
	Diameters of microorganisms' growth inhibition zones, mm				
Herbal mixture	13.6±0.5	19.8±0.4	17.8±0.4	17.6±0.5	–

Note: "–" – zone of growth inhibition of microorganisms is absent

## 4. Discussion

Research of antimicrobial activity of herbal mixture with the composition described above is original for such combination of active substances. However, it is known that an increased content of coagulase-positive staphylococci and enterococci is observed in patients with helminthiasis, namely, ascariasis [12].

Data obtained experimentally and presented in Table 2 indicate that the investigated samples of prepared herbal mixture show an antimicrobial action spectrum in relation to the used bacteria test cultures, namely, bacterial gram-positive (*Staphylococcus aureus* ATCC 25293 and spore culture of *Bacillus subtilis* ATCC 6633), gram-negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) cultures. The antifungal activity in relation to the yeast fungi of the genus *Candida* (*Candida albicans* ATCC 885-653) was not found.

It should be noted that the samples revealed weak antimicrobial activity in relation to the test culture of *Staphylococcus aureus*: the diameters of growth inhibition are less than 15 mm, namely  $13.6 \pm 0.5$  mm.

The studied mixture showed a moderate antimicrobial activity (diameter of growth inhibition zones of test culture is 16–25 mm) in relation to bacterial cultures of *Bacillus subtilis* ( $19.8 \pm 0.4$ ) and to gram-negative cultures of *Escherichia coli* ( $17.8 \pm 0.4$ ) and *Pseudomonas aeruginosa* ( $17.6 \pm 0.5$ ).

Thus, the obtained findings showed that samples of herbal mixture for oral administration have an antimicrobial spectrum in relation to 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 can be characterized by moderate activity towards test cultures. It should be noted that fungicidal activity towards the yeast fungi *Candida albicans* ATCC 885-653 was not found.

The investigated samples of the herbal mixture and the offered phytocomposition are promising for further work on the development of the new dosage form with plant extracts for oral administration and require continuation of the research.

### References

1. Bodnya, E. I. (2016). Vormil – a comprehensive solution to the problem of helminthiasis and their consequences. *News Medicine and Pharmacy*, 5 (574), 8–10.
2. All about helminthiasis. *Herbalist*. Available at: <http://www.herbalist.ru/articles-vsjo-pro-glisty.html>
3. Samura, B. A., Babak, O. Ya., Kolesnik, Yu. M. et. al. (2010). *Pharmacotherapy*. Kharkiv: Golden pages, 240.
4. Shadrin, O. G., Kovalchuk, A. A., Dyukareva, S. V., Polkovnichenko, L. M. (2015). Condition of gastrointestinal tract and ways of its correction by helminthiasis at children. *Sovremennaya pediatriya*, 72, 88–91.
5. Ershova, I. B., Osychnyuk, L. M., Mochalova, G. O. (2013). Helminthiasis in children. *Perinatologiya i pediatriya*, 2 (54), 125–131.
6. Minedian, G. Z. (Ed.) (2003). *Collection of folk medicines and non-traditional methods of treatment*. Moscow: Address-Press.
7. Samura, B. A., Chernyh, V. P., Bannyi, I. P. et. al. (2003). *Phytotherapy in the clinic of internal diseases*. Kharkiv: NUPh PH: Golden Pages, 416.
8. Tolochko, K. V., Vyshnevskaya, L. I. (2016). Basic approaches to pharmacotherapy of helminthiasis and prospects of phyto-medicines development for their treatment. *Clinical Pharmacy*, 20 (4), 4–10.
9. Tolochko, K. V., Vyshnevskaya, L. I. (2017). Scientific justification of anthelmintic medicines based on medicinal plant material. *International Journal of Green Pharmacy*, 11 (3), 154–159. doi: 10.22377/ijgp.v11i03.1119
10. *State Pharmacopoeia of Ukraine*. Vol. 1. (2015). Kharkiv: State Enterprise “Ukrainian Scientific Pharmacopoeial Center for Drug Quality”, 1128.
11. Volyanskiy, Yu. L., Gritsenko, I. S., Shirobokov, V. P. et. al. (2004). Study of specific activity of antimicrobial drugs. *Kyiv*, 38.
12. Akhmetova, S. B., Koteneva, E. N., Kabduova, A. K. et. al. (2013). Epidemiology of ascariasis and influence of ascaride invasion on microflora of the thick intestine. *Epidemiological studies in clinical medicine: achievements and prospects*. Kharkiv: Digital printing-house, 1, 10–12.