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The Study of the Antibacterial Effect of the Umbellate Wintergreen Extract

Abstract

The article presents the studies on the antimicrobial effect of the umbellate wintergreen herb extract. The study object was the umbellate wintergreen herb extract obtained with 50% ethyl alcohol. The sensitivity of microbial strains to the umbellate wintergreen extract was determined by the well diffusion method in Mueller Hinton agar. Five test strains were used as test cultures: *Staphylococcus aureus* ATCC 6538 gram-positive microorganisms, *Bacillus subtilis* ATCC 6633 spore culture, gram-negative *Proteus vulgaris* ATCC 4636, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 9027. The antifungal effect was determined against *Candida albicans* ATCC 885-653. The antibacterial activity of the test substances was assessed by the diameter of the growth inhibition zones. It was found that the extract of the umbellate wintergreen herb exhibited antibacterial properties against test microorganisms where the diameters of the growth inhibition zones were at the level of 20–28 mm. The antibacterial effect of the umbellate wintergreen herb extract was also determined against such clinical strains of microorganisms as *Staphylococcus aureus* 124, *Enterococcus faecalis* 42, *Pseudomonas aeruginosa* 18, *Klebsiella pneumoniae* 64, *Candida albicans* 69. The growth inhibition zones were within 19–24 mm.

Keywords: umbellate wintergreen; liquid extract; antimicrobial effect

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Дослідження антибактеріальної дії екстракту зимоліюбки зонтичної

Анотація

У статті наведено результати дослідження антимікробної дії екстракту трави зимоліюбки зонтичної. Об'єктом для дослідження обрали екстракт трави зимоліюбки зонтичної, отриманий 50% спиртом етиловим. Чутливість штамів мікроорганізмів до екстракту зимоліюбки визначали методом колодязів на середовищі Мюллера-Хінтона. Як тест-культури використовували 5 тестових штамів: грампозитивні мікроорганізми *Staphylococcus aureus* ATCC 6538, спорову культуру *Bacillus subtilis* ATCC 6633, грамнегативні *Proteus vulgaris* ATCC 4636, *Escherichia coli* ATCC 25922 та *Pseudomonas aeruginosa* ATCC 9027. Антифунгальну дію з'ясовували щодо *Candida albicans* ATCC 885-653. Антибактеріальну активність дослідних речовин оцінювали за діаметром зон затримки зростання. З'ясовано, що екстракт трави зимоліюбки зонтичної проявляв антибактеріальні властивості до тестових мікроорганізмів, де діаметри зон затримки зростання були на рівні 20–28 мм. Антибактеріальну дію екстракту зимоліюбки також визначали щодо клінічних штамів мікроорганізмів: *Staphylococcus aureus* 124, *Enterococcus faecalis* 42, *Pseudomonas aeruginosa* 18, *Klebsiella pneumoniae* 64, *Candida albicans* 69. Зони затримки зростання були в межах 19–24 мм.

Ключові слова: зимоліюбка зонтична; рідкий екстракт; антимікробна дія

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■ Introduction

Today, the use of the therapeutic potential of medicinal plants is considered a physiological method of prevention and treatment, which affects the normalization of metabolic processes and the restoration of the body's functional capabilities. Medicinal products based on plants can be used for a longer period of time, including in the treatment of chronic diseases [1].

Therefore, the role of phytotherapy in modern medicine is constantly growing, which is due, on the one hand, to the insignificant toxicity and biological safety for the human body of a large number of herbal medicines, and on the other hand, to the peculiarities of the clinical effectiveness of herbal medicines, namely: a wide therapeutic spectrum, a gradual increase in the severity of the expected clinical effect, a complex effect on various pathogenetic links of diseases, and relatively infrequent manifestations of side effects, even with prolonged use [1, 2].

At the present stage, pharmaceutical science continues to be enriched with information about the broad antibacterial significance of plant substances in human life, the possibility of their use for the treatment of diseases caused by microorganisms. Plants of the domestic flora deserve special attention in this aspect [3].

Thus, *Chimaphila* is a genus of flowering plants of the *Veresaceae* family, which has about 20 species. The Ukrainian name “umbellate” is due to the fact that representatives of its genus are found with green leaves in winter. Umbellate grows in the temperate and cold zones of the Northern Hemisphere, choosing dry pine and spruce forests for life. The species umbellate wintergreen, or wintergreen, a plant used by the natives of North America, is mainly grown in culture [4].

The beneficial properties of umbellate wintergreen were recognized by official medicine during the Civil War in the United States: field doctors used its diuretic and astringent effects. The plant was included in the US Pharmacopoeia in 1820. For centuries, this plant was one of the main medicines of rural residents of America [5, 6].

For the preparation of medicinal products, the herb umbellate wintergreen (*Herbae Chimaphiliae umbellate*) is used, which is harvested during the flowering period. Umbellate wintergreen belongs to unofficial medicinal plants.

The chemical composition is quite rich and specific. The herb contains hyperoside, kaempferol, arbutin, homoarbutin, avicularin, ericolin, the bitter compound ursone, about 20% amylin, up to 5% tannins, as well as gum, resins, organic acids, mucus, etc. [7].

In alternative medicine, umbellate wintergreen is used as a means to improve diuresis, reduce blood sugar, disinfect the urinary tract, increase the excretion of chloride and nitrogenous salts from the body, improve digestion and appetite, normalize menstruation, etc. [8].

The infusion of the umbellate wintergreen herb is used to treat chronic kidney diseases (nephritis, albuminuria, hematuria), as well as to relieve inflammation and remove sand from the bladder, with urethral stricture, chronic gonorrheal urethritis, for the treatment of the prostate gland, gout, dyspepsia and diabetes mellitus. The infusion of umbellate wintergreen helps with dropsy and edema.

The infusion of umbellate wintergreen is also used as an astringent for inflammatory processes of the gastrointestinal tract and for respiratory tract catarrhs. In addition, the herb has a tonic and restorative effect in diseases caused by excessive physical exertion [6].

The aim of the work was to study the antimicrobial effect of the umbellate wintergreen herb extract on test and clinical strains of microorganisms.

■ Materials and methods

The study of antimicrobial action was conducted at the premises of the State Institution “Mechnikov Institute of Microbiology and Immunology” under the supervision of the head of the Laboratory of Biochemistry and Biotechnology, Candidate of Biology, senior researcher Osolodchenko T. P.

The study object was the extract of umbellate wintergreen herb. For the study, a dried umbellate wintergreen herb was used (manufacturer TM “Green Pharmacy”, Zhytomyr). The umbellate wintergreen extract was obtained at the premises of the LLC “Experimental plant “GNCLS” (Kharkiv) in the period of May–June 2023. The umbellate wintergreen extract was obtained with 50% ethyl alcohol described in detail in the article [9].

The sensitivity of microbial strains to the umbellate wintergreen extract was determined in accordance with the methodological guidelines “Determination of the sensitivity of microorganisms to antibacterial drugs” (Order of the Ministry of Health of Ukraine dated 05.04.2007 No. 167) by the well diffusion method in Mueller Hinton agar (“Himedia Laboratories Pvt. Ltd, India), which was prepared according to the manufacturer’s instructions [10].

A suspension of microorganisms with a certain concentration of microbial cells (optical density) was prepared using a turbidity standard (0.5 units on the McFarland scale). A Densi-Lameter device (manufactured by PLIVA-Lachema, Czech Republic; wavelength 540 nm) was used. The suspension was prepared according to the instructions for the device and the information sheet on innovations in the healthcare system No. 163-2006 “Standardization for the preparation of microbial suspensions”, Kyiv [11]. Synchronization of cultures was carried out using low temperature (4 °C). The sensitivity of fungi was determined on the Sabouraud’s medium. The sensitivity of the test substances was determined on two layers of the nutrient medium, which were poured into Petri dishes. The lower layer consisted of agar-agar (10 ml). On it, 3–6 sterile metal cylinders with a diameter of 8 mm and a height of 10 mm were installed. The upper layer (14 ml of the nutrient medium + 1 ml of the microbial solution 0.5 units on the McFarland scale) was poured around the cylinders, which consisted of a nutrient agar medium with the appropriate standard for daily cultivation of the microorganism. After solidification, the wells were removed

with sterile tweezers, and the test substance (0.3 ml) was added to the wells. The antibacterial activity of the test substances was assessed by the diameter of the growth inhibition zones [12, 13]:

- 10 mm – a microorganism insensitive to the test substance;
- 10–15 mm – a microorganism weakly sensitive to the test substance;
- 15–25 mm – a microorganism sensitive to the test substance;
- 25 mm and above – a microorganism highly sensitive to the test substance.

■ Results and discussion

According to the results of the study, it was found that the extract of the umbellate wintergreen herb exhibited antibacterial properties against test microorganisms where the diameters of the growth inhibition zones were at the level of 20–28 mm (**Table 1**).

The results of the studies showed that the sample had antibacterial properties against all clinical strains of microorganisms where the growth inhibition zones were within 19–24 mm (**Table 2**).

The data in **Table 3** show that the sample (diluted with sterile distilled water) exhibits antibacterial properties against all test microorganisms in the dilution. High indicators remain in a dilution of 1:4 (from 20–28 mm to 18–24 mm), then the inhibition zones gradually decrease to 14–15 mm. In a dilution of 1:32, the antimicrobial activity is observed in *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633.

In **Table 4**, the results of the studies demonstrate that the sample (diluted with sterile distilled water) in a dilution of 1:4 exhibits antibacterial properties against all clinical strains of microorganisms. Dilutions of 1:8 and 1:16 show weak antibacterial activity (diameters of the growth inhibition zones are 12–15 mm). No antimicrobial activity is observed in a dilution of 1:32.

The study was conducted to determine the antibacterial activity of the sample for 28 days (**Tables 5 and 6**).

Table 1. The antibacterial effect of the sample on test microorganisms by the agar diffusion method

Sample	Diameters of the growth inhibition zones of microorganisms, mm					
	<i>Staphylococcus aureus</i> ATCC 25923	<i>Escherichia coli</i> ATCC 25922	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Proteus vulgaris</i> ATCC 4636	<i>Bacillus subtilis</i> ATCC 6633	<i>Candida albicans</i> ATCC 885-653
Umbellate wintergreen extract	27, 27, 28	24, 25, 25	23, 24, 25	23, 24, 24	27, 28, 28	20, 21, 22

Table 2. The antibacterial effect of the sample on clinical microorganisms by the agar diffusion method

Sample	Diameters of the growth inhibition zones of microorganisms, mm				
	<i>Staphylococcus aureus</i> 124	<i>Enterococcus faecalis</i> 42	<i>Pseudomonas aeruginosa</i> 18	<i>Klebsiella pneumoniae</i> 64	<i>Candida albicans</i> 69
Umbellate wintergreen extract	23, 24, 24	22, 22, 23	20, 21, 21	22, 23, 22	20, 20, 19

Table 3. The antibacterial effect of the sample on test microorganisms in the diluted agar diffusion method

Sample with dilution	Diameters of the growth inhibition zones of microorganisms, mm					
	<i>Staphylococcus aureus</i> ATCC 25923	<i>Escherichia coli</i> ATCC 25922	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Proteus vulgaris</i> ATCC 4636	<i>Bacillus subtilis</i> ATCC 6633	<i>Candida albicans</i> ATCC 885-653
Without dilution	27, 27, 28	24, 25, 25	23, 24, 25	23, 24, 24	27, 28, 28	20, 21, 22
1:2	27, 26, 26	23, 23, 23	21, 22, 22	21, 22, 21	24, 25, 25	18, 19, 19
1:4	24, 23, 23	21, 21, 22	20, 19, 18	20, 18, 19	23, 23, 22	17, 16, 16
1:8	21, 21, 20	19, 19, 20	17, 17, 17	17, 17, 17	20, 20, 19	15, 14, 14
1:16	19, 18, 19	17, 16, 16	14, 15, 15	14, 14, 14	17, 16, 16	13, 13, 12
1:32	15, 16, 16	14, 14, 14	growth	growth	14, 15, 15	growth

Table 4. The antibacterial effect of the sample on clinical microorganisms in the diluted agar diffusion method

Sample with dilution	Diameters of the growth inhibition zones of microorganisms, mm				
	<i>Staphylococcus aureus</i> 124	<i>Enterococcus faecalis</i> 42	<i>Pseudomonas aeruginosa</i> 18	<i>Klebsiella pneumoniae</i> 64	<i>Candida albicans</i> 69
Without dilution	23, 24, 24	22, 22, 23	20, 21, 21	22, 23, 22	20, 20, 19
1:2	21, 20, 20	19, 20, 20	19, 18, 19	20, 20, 19	18, 17, 18
1:4	17, 18, 18	17, 16, 17	17, 16, 17	18, 17, 17	16, 15, 16
1:8	15, 14, 14	14, 15, 15	15, 14, 15	15, 16, 16	13, 14, 14
1:16	13, 13, 14	13, 12, 12	13, 13, 12	13, 13, 13	12, 12, 12
1:32	growth	growth	growth	growth	growth

Table 5. The antibacterial activity of the sample for 28 days against test microorganisms

Sample of the umbellate wintergreen extract	Number of days	Diameters of the growth inhibition zones of microorganisms, mm					
		<i>Staphylococcus aureus</i> ATCC 25923	<i>Escherichia coli</i> ATCC 25922	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Proteus vulgaris</i> ATCC 4636	<i>Bacillus subtilis</i> ATCC 6633	<i>Candida albicans</i> ATCC 885-653
Sample of the umbellate wintergreen extract	primary	27, 27, 28	24, 25, 25	23, 24, 25	23, 24, 24	27, 28, 28	20, 21, 22
	2 days	27, 27, 27	25, 25, 25	24, 24, 24	24, 24, 24	28, 28, 28	20, 21, 21
	7 days	27, 28, 28	24, 24, 25	23, 24, 24	24, 24, 23	27, 28, 28	22, 21, 21
	14 days	27, 27, 27	25, 24, 25	23, 23, 24	23, 23, 23	27, 27, 28	21, 21, 21
	28 days	27, 27, 28	25, 24, 24	23, 23, 24	24, 23, 23	27, 27, 27	20, 21, 20

Table 6. The antibacterial activity of the sample for 28 days against clinical microorganisms

Sample of the umbellate wintergreen extract	Number of days	Diameters of the growth inhibition zones of microorganisms, mm				
		<i>Staphylococcus aureus</i> 124	<i>Enterococcus faecalis</i> 42	<i>Pseudomonas aeruginosa</i> 18	<i>Klebsiella pneumoniae</i> 64	<i>Candida albicans</i> 69
Sample of the umbellate wintergreen extract	primary	23, 24, 24	22, 22, 23	20, 21, 21	22, 23, 22	20, 20, 19
	2 days	24, 24, 24	22, 22, 23	21, 21, 21	22, 22, 23	20, 20, 20
	7 days	24, 24, 24	23, 23, 22	21, 21, 21	23, 23, 22	19, 20, 20
	14 days	23, 23, 24	23, 22, 22	20, 21, 20	22, 22, 22	19, 19, 20
	28 days	24, 23, 23	21, 22, 23	20, 20, 21	22, 22, 21	20, 20, 19

The data in **Tables 5** and **6** indicate that the antibacterial properties of the sample of the umbellate wintergreen extract studied in relation to test and clinical strains of microorganisms do not change within 28 days.

■ Conclusions

It has been experimentally determined that the sample of the umbellate wintergreen herb liquid extract studied exhibits the antibacterial activity against test strains – *Staphylococcus aureus* ATCC 6538 gram-positive microorganisms, *Bacillus subtilis* ATCC 6633 spore culture, gram-negative *Proteus vulgaris* ATCC 4636, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 885-653 and clinical strains – *Staphylococcus aureus* 124, *Enterococcus faecalis* 42, *Pseudomonas*

aeruginosa 18, *Klebsiella pneumoniae* 64, *Candida albicans* 69.

In dilution, the umbellate wintergreen extract exhibited antibacterial properties against all test microorganisms. High indicators remained in a dilution of 1:4 (from 20–28 mm to 18–24 mm), then the inhibition zones gradually decreased to 14–15 mm. In a dilution of 1:32, the antimicrobial activity was observed against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633.

The results of the studies on clinical strains show that the sample in a dilution of 1:4 exhibits antibacterial properties against all clinical strains of microorganisms. Dilutions of 1:8 and 1:16 exhibit a weak antibacterial activity (diameters of the growth inhibition zones is 12–15 mm). No antimicrobial activity is observed in a dilution of 1:32.

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