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THE ANTIBACTERIAL ACTIVITY OF THE TABLETS WITH DRY EXTRACT OF ROUND-LEAVED WINTERGREEN LEAVES

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Abstract

Pyrola rotundifolia L. is a perennial herbaceous plant, commonly known as round-leaved wintergreen. The medicinal properties of this plant have been known since ancient times. These properties are the result of the availability of many groups of biologically active compounds in *Pyrola rotundifolia* L. The tablets with dry extract of round-leaved wintergreen leaves were studied to present antibacterial properties. Phytochemical analysis of the tablets with dry extract of round-leaved wintergreen leaves indicated the presence of flavonoids. The total content of these biologically active substances in recalculation into hyperoside was to sample 1 - 6.1 mg, sample 2 - 5.6 mg, in sample 3 - 6.0 mg. Antibacterial activity of tablets with dry extract of round-leaved wintergreen leaves was determined "wells" method by measuring inhibition zone diameter. Canephron ("Bionorica SE", Germany) and Furagin ("Arterium", Ukraine) tablets were reference drugs. As a result, tablets with dry extract of round-leaved wintergreen leaves were found very active against *Staphylococcus haemolyticus* (20 ± 0.15) mm) and *Escherichia coli* (19 ± 0.12) mm), and active against *Klebsiella spp* (18 ± 0.10) mm). Consequently, tablets with dry extract of round-leaved wintergreen leaves can be used as antibacterial medicines for the treatment of diseases caused by gram-positive and gram-negative bacteria.

Keywords: *Pyrola rotundifolia* L., round-leaved wintergreen, *tablets, flavonoids, antibacterial activity*

Introduction

The experience of using herbal medicines in folk and official medicine is studied to search for promising medicinal plants and create phytopreparations based on them. An alternative to this is to conduct a variety of studies of official MP, combining high physiological activity and polyvalent effects on the body for the introduction of new herbal medicines in the treatment process.

Medicinal plant raw materials, which is used both in the native state and to obtain many valuable drugs is one of the sources of treatment and prevention of modern medicine [1-4]. The use of medicinal plants in folk and scientific medicine has a long history [5-8]. From 1981, 38 molecules have been derived from medicinal plants, out of which 1,130 new therapeutic agents have approved as pharmaceutical drugs [9, 10]. In recent years, there has been a tendency around the world to increase the use of herbal medicines. Phytopreparations are also combined well with foods and synthetic drugs [11]. Due to many groups of biologically active substances in plants with different pharmacological action, herbal medicines can be used to treat many diseases. The biologically active substances complex, which is formed in a living plant cell, has a great resemblance to the human body, so the components of medicinal plants are more easily assimilated by the body and have minor side effects [12-14]. Unlike synthetic drugs, herbal medicines cause fewer complications, great tolerability especially allergic ones, so they can be prescribed for a long time, especially for the rehabilitation of patients [15]. Different methods of treatment using medicinal plants are of interest to our society [16]. Therefore, the use of a wide raw material base of plants and the development of new medicines from herbal raw materials is one of the important tasks of modern pharmacy and medicine [17].

Plants with a large raw material base *Pyrola rotundifolia* L. Medicinal properties of this plant have been known since ancient times.

Pyrola rotundifolia L. is a perennial herbaceous plant, commonly known as round-leaved wintergreen, belongs to the family *Ericaceae* [18, 19]. This plant is widely used in medicine in Europe, Asia and North America. Its leaves are characterized by the following types of pharmacological action: diuretic, astringent, antiseptic, wound healing, hemostatic, anti-inflammatory, antibacterial. The use of the plant is indicated in diseases of the digestive system, mainly stomach, intestines, liver, kidney and urinary tract diseases, rheumatism, prostatitis, purulent wounds and rashes, respiratory infections, ascites and conjunctivitis [20].

In European folk medicine, decoction and tincture of *Pyrola rotundifolia* L. is used orally as a diuretic and bactericidal agent for cystitis, diabetes, heart disease, liver, kidney, gastrointestinal diseases, headaches, sore throats, coughs, furuncles and scurvy. Externally, a decoction of the plant is used to treat wounds and rashes.

It should be noted that the aqueous extract of the plant *in vitro* inhibits the growth of many species of bacilli pathogenic to humans. At microbiological research it is specified that decoction of *Pyrola rotundifolia* L. showed the expressed antimicrobic activity compared with activity of bearberry, in relation to various microorganisms. The highest activity of decoction was found against enterobacteria and staphylococci, less – against pseudomonads and fungi [21].

The choice of the optimal remedy still remains a problem due to the expansion of the arsenal of antibacterial drugs. To date, aspects of the formation of resistance to antibacterial therapy and ways to overcome them are in the center of attention of physicians and society [22]. Taking into account the above proof-points, it can be argued that the use of a new drug, namely tablets based on the extract of *Pyrola rotundifolia* L. leaves for antibacterial therapy is relevant. Therefore, the aim of the study was to investigate the antimicrobial activity of tablets based on the extract of *Pyrola rotundifolia* L. leaves and to establish the content of flavonoids that provide this activity.

Methods

The object of the study was tablets with dry extract of round-leaved wintergreen which containing dry extract of round-leaved wintergreen (300 mg), VIVAPUR®112 (114 mg), PROSOLV® EASYtab SP (72 mg), Croscarmellose sodium (42

mg), Tablettose® 80 (42 mg), Neusilin® US 2 (24 mg), Magnesium stearate (6 mg) [23].

The tablets were developed at the Department of Pharmacy Management, Economics and Technology (I. Horbachevsky Ternopil National Medical University) [24].

Chemicals and reagents

Acetic acid, acetone, aluminium chloride, anhydrous sodium sulphate, hydrochloric acid, sodium molybdate, hexamethylenetetramine, sodium hydroxide, methanol were of the highest purity. Chemicals and reagents were purchased from the Ltd. Sfera Sim (Lviv, Ukraine).

Microorganisms

Antimicrobial activity of tablets with dry extract of *Pyrola rotundifolia* L. leaves were investigated against clinical strains, namely, *Klebsiella spp*, *Escherichia coli*, *Staphylococcus haemolyticus*. Cell concentration was 0.5 McFarland.

Identification reactions of flavonoids

In the presence of flavonoids, tablets with dry extract of round-leaved wintergreen leaves were investigated. For this 1.00 g of the powdered tablets was placed in a 100 ml flask, poured with 35 ml of 70 % ethanol P and heated in a boiling water bath for 20 min, stirring periodically. After cooling, the obtained solution was filtered and purified. To do this, the filtrate was applied to a column with a diameter of 1 cm, filled with 1 g of polyamide, washed with 50 ml of water and washed the flavonoids from the column with 70 % ethanol P, selecting fractions colored in yellow. The purified extract was evaporated to 1/2 volume and used for qualitative reactions to detect flavonoids. The work was carried out in comparison with 0.1 % solution of rutin:

1. Shinoda's test: 2-3 drops of chloride acid and a pinch of metal magnesium powder was added to 1 ml of purified extract (and 0.1 % solution of rutin).

2. A reaction with alkal isolution: 1-2 drops of 10% hydroalcoholic potassium hydroxide solution were added to 1 ml of the extract.

3. Reaction with iron (III) chloride: 1-2 drops of 10 % solution of ferrum (III) chloride were added to 1 ml of the extract.

4. Reaction with Lead (II) acetate: 3-5 drops of 10 % solution of plumbum acetate were added to 1 ml of extract [25].

Total flavonoids content

Stock solution:

1.00 g (exact weight) of the powdered tablets was placed in a 100 ml volumetric flask, added 1 ml of a 5 g/l solution of hexamethylenetetramine R, 20 ml of acetone R 2 ml of hydrochloric acid R1, and it is mixed. Boil the mixture under a reflux condenser for 30 min and filtered into a 100 ml flask. Add the plug of absorbent cotton to the residue in the flask and extract with 2 quantities, each of 20 ml, of acetone R, each time boiling in a water bath under reflux condenser for 10 min, cooled. Filtered the liquid through a plug of absorbent cotton then through a filter paper into a 100 ml volumetric flask and added acetone R to the mark by rinsing the flask and filter paper. Introduced 20 ml of the obtained solution into a separating funnel, added 20 ml of water R, extract the mixture with one quantity of 15 ml and then with three quantities, each of 10 ml, of ethyl acetate R. Combined the ethyl acetate extracts in a separating funnel, rinsed with two quantities, each of 50 ml, of water R, filtered the extract over 10 g of anhydrous sodium sulphate R into a 50 ml volumetric flask and added acetone R to the mark.

Test solution:

10 ml of the stock solution was placed in a 25 ml volumetric flask, added 1 ml of aluminium chloride reagent R, added 5% (*vol/vol*) solution of glacial acetic acid R in methanol R to the mark, and stirred.

Compensation liquid:

10 ml of the stock solution was placed in a 25 ml volumetric flask and added 5% (*vol/vol*) solution of glacial acetic acid R in methanol R to the mark, stirred.

Measured the absorbance of the test solution after 30 min, by comparison with the compensation liquid.

The quantitative content of flavonoids in the tablets was made by the absorption spectrophotometry on a spectrophotometer Lambda 25 UV Perkin Elmer (USA) at a wavelength of 425 nm [26, 27].

Used a specific absorption of hyperoside equal to 500 [28].

Antibacterial test

Antimicrobial activity of tablets was studied *in vitro* [18, 29]. The tablets with dry extract of round-leaved wintergreen leaves were researching. Canephron ("Bionorica SE", Germany) and Furagin ("Arterium", Ukraine) tablets were reference drugs. Studies of the antibacterial effect of tablets with dry extract of round-leaved wintergreen leaves on the vital activity of microorganisms were performed by the method of diffusion into agar ("wells" method). Standardization of research conditions during agar diffusion was ensured by a medium thickness of 10 mm and a diameter of a "well" in it of 6 mm. Clinical strains of *Staphylococcus haemolyticus Escherichia coli*, *Klebsiella spp*, were used for seeding. Cells concentration in them was 0.5 McFarland (1.5×10^8 colony forming units (CFU/ml). Afterward, "wells" were filled with powdered tablets of round-leaved wintergreen, Furagin, and Canephron soaked in meat-peptone broth. After, Petri dishes were placed into a heating block at 37 ℃ [30]. The antibacterial activity was determined by measuring of inhibition zone. Results of the study were evaluated in 24 hours according to the parameters suggested by Alves et al. (2000) [31]:

- <9 mm, inactive ;
- 9–12 mm, less active ;
- 13–18 mm, active;
- >18 mm, very active.

Statistical analysis

All the tests were carried out five times. Results were represented as M ± SEM. Statistical significance of differences between mean values was assessed by the Student's t-test [32]. The level of significance was set at *p<0.05 [33, 34].

Results and Discussion

Experimental studies revealed that tablets with dry extract of round-leaved wintergreen leaves showed indication of flavonoids at the phytochemical screening which is correlated with the activity of the obtained extract.

The detection of flavonoids in study tablets was carried out with the help of generally known qualitative reactions.

The raspberry color of products of Shinoda's test indicates the presence of flavonoids in the tablets - derivatives of the flavonol. As a result of the reaction with iron (III) chloride, a dark green color was observed. Upon interaction with the alkali solution, the appearance of yellow color was observed. As a result of the reaction with Lead (II) acetate, a yellow precipitate was formed.

The above qualitative reactions testify to the presence of flavonoids in the studied tablets.

Flavonoids are the greatest group of natural phenolic compounds and are a practical mandatory component of plants. They are distributed in nature in a free or bound state. One of the most common glycosides in plants are quercetin derivatives (rutin-3-O-rutinoside, hyperoside-3-O-galactoside, quercitrin-3-O-rhamnoside, avicular-3-O-arabinoside). It is believed that -OH groups in phenolic compounds are related to their inhibitory activity [35]. The greater the number of -OH groups in the compound, the more toxic it is for the microorganism [11, 36]. Flavonoids are effective antimicrobial biological active substances against a wide variety of microorganisms. Researchers have founded flavonoid-rich plant extracts have antibacterial activity. Some flavonoids, namely flavonol glycosides, isoflavones, flavanones, flavone glycosides, and others have strong antibacterial activity [37]. Many flavonoids have multiple cellular targets. One of their molecular actions is to establish a complex with proteins due to hydrophobic activity and nonspecific forces like hydrogen bonding, as well as by covalent bond formation [38, 39].

The results of determining the quantitative content of flavonoids in tablets with dry extract of round-leaved wintergreen leaves are shown in Table 1.

The results of the study of antimicrobial activity of tablets with dry extract of round-leaved

wintergreen leaves "wells" method are shown in Table 2.

The results of the studies showed that the researches object has a pronounced antimicrobial activity on clinical strains namely *Staphylococcus haemolyticus, Klebsiella spp* and *Escherichia coli*.

Tablets with dry extract of round-leaved wintergreen leaves were very active against *Escherichia coli* and *Staphylococcus haemolyticus* and active against *Klebsiella spp*.

Conclusions

Phytochemical analysis of the tablets with dry extract of round-leaved wintergreen leaves indicated the presence of flavonoids. The quantitative content of flavonoids was determined by the absorption spectrophotometry at a wavelength of 425 nm. The total content of these biologically active substances in recalculation into hyperoside was to sample 1 - 6.1 mg, sample 2 - 5.6 mg, in sample 3 - 6.0 mg. Antibacterial activity of tablets with dry extract of round-leaved wintergreen leaves was determined "wells" method. Tablets with dry extract of round-leaved wintergreen leaves were found very active against *Staphylococcus haemolyticus* (20 mm) and *Escherichia coli* (19 mm) and active against *Klebsiella spp* (18 mm).

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Table 1. The results of determination of flavonoids in tablets with dry extract of round-leaved wintergreen leaves (M ± SEM, n = 5)

Sample No.	The content of total flavonoids in the recalculation of hyperoside, mg
1	6.1 ± 0.02
2	5.6 ± 0.04
3	6.0 ± 0.02

Table 2. The results of the study of antimicrobial activity of tablets with dry extract of round-leaved wintergreen leaves "wells" method (M ± SEM, n = 5)

The diameter of the growth retardation of microorganisms, mm			
Test culture of microorganisms	Tablets with dry extract of round-leaved wintergreen leaves	«Canephron »	«Furagin»
Staphylococcus haemolyticus	20±0.15	19±0.11	21±0.14
Escherichia coli	19±0.12	18±0.12	20±0.13
Klebsiella spp.	18±0.10	15±0.09	18±0.12

Figure 1. UV spectrum of hyperoside of tablets with dry extract of round-leaved wintergreen leaves