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The Research of Antimicrobial Activity of *Asperula Humifusa* M.Bieb Besser Phenolic Complex

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The antimicrobial activity of phenolic complex obtained from *Asperula humifusa* M.Bieb Besser herb was studied against 6 bacterial cultures: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 4636, *Bacillus subtilis* ATCC 6633, *Candida albicans* 885-663. It was established that phenolic complex of spreading bedstraw's herb has a significant antimicrobial activity against *P.aeruginosa*, *E.coli*, *S.aureus* and moderate antimicrobial activity against *P.vulgaris*, *B.subtilis* and *C.albicans*. The correlation between the content of the sum of the major groups of biologically active substances in fraction and their level of antimicrobial activity against the most significant test bacteria had been established. The results indicate prospect of further research of spreading bedstraw's herb's fractions in order to create on their basis new antimicrobial agents.

Keyword: *Asperula Humifusa*, Phenolic Complex, Antimicrobial Activity

1. Introduction

Recently there has been a tendency of increasing a range of nosocomial infections, modifying of their biological properties, resistance to antibiotics and a high level of morbidity, which creates a ground for searching an alternative agents with wide range of effects on pathogenic and opportunistic pathogenic microflora^[8,9]. of particular interest are plants' extracts that are active against resistant bacterial cultures to antibiotics and synthetic drugs. The representatives of the genus Woodruff (*Asperula* L.) Madder family (Rubiaceae) had been used in folk medicine as antibacterial agents for a long time^[5].

The **aim of our study** was to investigate the antimicrobial activity of phenolic complex of

spreading bedstraw's herb (*Asperula humifusa* M.Bieb Besser) Madder family (*Rubiaceae* Juss.) against the most important in the epidemiological significance bacterial cultures.

2. Materials and Methods

The object of the study was phenolic complex obtained from air-dried spreading bedstraw's herb (*Asperula humifusa* M.Bieb Besser), harvested at flowering stage in summer 2012, near Eupatoria in the AR of Crimea. Substance was obtained by exhaustive sequential circulating extraction of raw materials by ethyl acetate-alcohol mixture (8:2) in a Soxhlet apparatus, after a preliminary degreasing by chloroform. The extract was concentrated to dryness. Yield was 4.58%.

Component composition of terpenoids, free fatty acids and carbohydrates had been studied by chromatography-mass spectrometry^[7], the content of phenolic compounds – by UV-spectrophotometry and HPLC^[3], the content of chlorophylls, carotenoids and flavonoids – by three-dimensional scanning spectrofluorimetry^[4]. Antibacterial activity of this complex was studied *in vitro* using the agar diffusion method ("wells")^[2,6]. The degree of sensitivity of microorganisms was evaluated by measuring zones of growth delay. Microbial load amounted to 10^7 microbial cells per 1 ml of medium and was determined visually by optical opacity standard McFarland.

To assess the activity of the phenolic complex we used the next standard bacterial cultures regulated by WHO to study the antimicrobial activity of drugs: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 4636, *Bacillus subtilis* ATCC 6633, *Candida albicans* 885-663. To determine the antimicrobial activity, the bacterial cultures were cultivated on meat pepton agar at 37 °C for 24 hours. Antimicrobial activity was measured as a radius in mm to give a zone of inhibition.

To determine the antifungal activity of active substance Sabourau medium was used. Each series of medium had been controlled qualitatively and quantitatively in accordance with the regulations.

To quantify the antimicrobial activity of the fraction and determine the minimal inhibitory concentration, the method of serial dilutions was used.

Researched phenolic complex was used as a 2% alcohol solution. The results were processed statistically by Glantz S^[1].

3. Results and Discussion

Analysis of the chemical composition of the obtained phenolic complex showed that it contains 3,86 % of hydroxycinnamic acids, 4,9 % of flavonoids, 0,04 % of other phenolic compounds, 0,02 % of terpenoids, 0,09 % of free fatty acids and esters, 0,03 % of aldehydes and ketones, 1,3 % of chlorophylls, 0,5 % of

carotenoids, 0,03 % of higher hydrocarbons [3]. Hydroxycinnamic acids are represented by 3-caffeoylquinic acid, 3,5- and 4,5-dycaffeoylquinic acids, cynaroside, luteolin-7-O-galactoside, luteolin. In addition, the fraction contains irydoids and steroid compounds.

The results of antimicrobial activity are shown in Table 1.

Table 1: Specific activity of phenolic complex of spreading bedstraw' herb

Bacterial strains	Diameter of growth delay zones, mm	*MBsC, mkg/ml	**MBcC, mkg/ml
<i>S. aureus</i> ATCC 25923	28,7±0,3	31,25	62,5
<i>E. coli</i> ATCC 25922	30,3±0,3	31,25	62,5
<i>P. aeruginosa</i> ATCC 27853	35,3±0,1	31,25	62,5
<i>P. vulgaris</i> ATCC 4636	24,1±0,2	125	250
<i>B. subtilis</i> ATCC 6633	16,2±0,3	62,5	125
<i>C. albicans</i> ATCC 885-663	15,0±0,2	250	500

Note: * MBsC - minimum bacteriostatic concentration; ** MBcC - minimum bactericidal concentration;

The obtained results showed that phenolic complex of spreading bedstraw's herb revealed antibacterial activity against all bacterial cultures. The highest sensitivity was demonstrated by *P.aeruginosa*, *E. coli*, *S.aureus* and moderately sensitive to the chosen fraction's concentration were *P.vulgaris*, *B.subtilis*, *S.albicans*.

Perhaps the antimicrobial activity of this complex is due to hydroxycinnamic acids and flavonoids.

There is a direct correlation between the content of hydroxycinnamic acids and flavonoids in the studied complex and the degree of antimicrobial activity against *P.aeruginosa*, *E. coli*, *S.aureus*.

4. Conclusion

The antimicrobial activity of phenolic complex of spreading bedstraw's herb (*Asperula humifusa* M.Bieb Besser) against different test strains had been studied for the first time. It was found that phenolic complex exhibits significant antimicrobial activity against *P. aeruginosa*, *E. coli*, *S.aureus*, moderate activity against

P.vulgaris B.subtilis and S.albicans. The degree of antibacterial and antifungal activity of this fraction had been established. The high antimicrobial activity of the studied complex is due to hydroxycinnamic acids and flavonoids.

The results suggest the prospect of further microbiological testing of phenolic complex of spreading bedstraw's herb in order to create new antibacterial agents.

5. References

1. Гланц С. Медико-биологическая статистика. Пер. с англ. Практика, Москва, 2001, 459 с.
2. Волянський Ю.Л., Гриценко І.С., Широбоков В.П. і співавт. Вивчення специфічної активності протимікробних лікарських засобів. Київ, 2004, 40с.
3. Ільїна Т.В., Ковальова А.М., Горяча О.В., Гриценко О.М. Хроматографічне дослідження фенілпропаноїдів трави *Galium humifusum* Vieb. Зб. наук. праць співробітників НМАПО ім. П.Л. Шупика, вип.19., кн.3, Київ, 2010, 952 с.
4. Ковальова А.М., Ільїна Т.В., Горяча О.В. Компоненти ефірної олії *Galium humifusum* Vieb. Український біофармацевтичний журнал, 2010; 1(6):49-52.
5. Растительные ресурсы СССР: Цветковые растения, их химический состав, использование; Семейства Caprifoliaceae-Plantaginaceae. Наука, Ленинград 1990, 326 с.
6. Решедько Г.К., Стецюк О.У. Особенности определения чувствительности микроорганизмов диско-диффузным методом. Клиническая микробиология и антимикробная химиотерапия. 2001; 3(4):348-355.
7. Юрченко Н.С., Ільїна Т.В., Ковальова А.М. Хромато-мас-спектрометричне дослідження етилацетатно-спиртової фракції трави *Asperula humifusa* (M.B.) Bess. (*Galium humifusum* Vieb.). Український медичний альманах 2012; 3(15):245-246.
8. Ngono N.A., Ebelle E.R., Ndifor F., Biyiti L., Amvam Zollo P.H., Bouchet P. Chemotherapy 2006; 52(2):103-106.
9. Hoenigl M., Zollner-Schwetz I., Linkesh W. Epidemiology of invasive fungal infections and rationale for antifungal therapy in patients with hematological malignancies. Mycoses 2010; 6:57-61.