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STUDY OF ANTIMICROBIAL EFFECT OF *CHRYSANTHEMUM×HORTORUM* BAILEY PECTORAL VARIETY FLOWERS AND LEAVES

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Abstract

The aim. The aim of our study was to determine the antimicrobial effect of 10% and 20% aqueous solutions of *Chrysanthemum x hortorum* Bailey flowers and leaves Pectoral variery. **Materials and methods.** 10 % and 20 % native aqueous solutions of flowers and leaves of *Chrysanthemum x hortorum* Bailey Pectoral variery were studied. The antibacterial action of the samples was determined regarding 9 test cultures: gram-positive microorganisms *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 14990, *Micrococcus luteus* NCTC 2665, *Corunebacterium spp* ATCC 373, *Serratia marcescens*, gram-negative microorganisms *Klebsiella pneumoniae* ATCC13883, *Escherichia coli* ATCC 25922, spore culture *Bacillus cereus* NCTC 74. The antifungal effect was studied against *Candida albicans* ATCC 855/653. The bacteriostatic effect of the studied samples was determined by the results of growth of reference strains in native aqueous solution of the corresponding samples and in dilutions of 1:1, 1:2 in meat-peptone broth; bactericidal action – in the absence of growth of the contents of test tubes with native solutions of the samples and in a 1:1 dilution in dense nutrient media. The following media were used to determine the bactericidal effect: meat-peptone agar for all cocci and bacilli, blood sugar agar – for *Klebsiella* and corynebacteria, Endo medium – for enterobacteria, Saburo medium – for yeast-like fungi of the *Candida* genus. **Results.** It was established that the native 10% aqueous solution of *Chrysanthemum x hortorum* Bailey Pectoral variery flowers had a bactericidal effect against *Staphylococcus epidermidis* and *Micrococcus luteus*, delayed growth and reproduction (bacteriostatic effect) of *Staphylococcus aureus* and *Escherichia coli* and and did not affect the growth of *Klebsiella*, corynebacteria, sera, and *Candida* fungi. Dilution of 10% solution of chrysanthemum flowers 1:1 had a bacteriostatic effect only on the test culture of staphylococci. Antibacterial effect of 10% aqueous solution of *Chrysanthemum x hortorum* Bailey Pectoral variery leaves was active only in the native form, it was not manifested when diluted. 20% aqueous solution of flowers and leaves of *Chrysanthemum x hortorum* Bailey Pectoral variery proved to have antimicrobial activity similar to the previous 10% solutions. **Conclusions.** It has been established that all the studied samples of flowers and leaves of chrysanthemums have antimicrobial activity. Native 10 % and 20 % aqueous solutions of chrysanthemum flowers and leaves has shown antibacterial activity against gram-positive and gram-negative microflora, so they are promising for development of a drug with antimicrobial properties.

Keywords: extracts of flowers and leaves of *Chrysanthemum x hortorum* Bailey Pectoral variery, 10% and 20 % aqueous solution, antimicrobial activity, test-cultures

Introduction

Over the last decade, the use of medicinal plants (MP) and phytopreparations as adjuvants for treatment and prevention of somatic and infectious diseases has significantly increased. Herbal medicines contain some groups of biologically active substances (BAS) or combinations, therefore they exhibit various medicinal properties [1, 2]. Natural substances, related to the body, contribute to metabolism, have a positive effect on the physiological stability of human organs and systems, have a polyvalent effect as well as antibacterial, significantly reduce the risk of side effects [3-7].

In scientific and practical aspects, research on new therapeutic and prophylactic agents with antiviral, antimicrobial, anti-inflammatory effects is promising [8, 9].

It is established that many medicinal plants have antimicrobial effect [10, 11], but in the literature there is no information on the study of antimicrobial properties of the *Chrysanthemum* genus *(Chrysanthemum L.) Asteraceae* family, which has been used in traditional medicine around the world as a plant that was personification of longevity and health since a long time ago [12].

Decoction of chrysanthemums reduces high blood pressure, normalizes coronary circulation, regulates myocardial function, decreases blood cholesterol, has antitoxic effect, kills cancer cells, improves functioning of autonomic nervous system [13, 14]. The infusion of chrysanthemum petals is very effective in cases of cold (bronchitis), conjunctivitis, blepharitis, abscesses, and furuncles as a natural antibiotic [15, 16]. Infusion of chrysanthemum leaves is used to rinse the mouth for prevention of periodontitis [17].

Chrysanthemums in the house improve the microclimate because these plants emit very useful substances that have bactericidal properties similar to conifers [18].

Study of the chemical composition of *Chrysanthemum x hortorum* Bailey showed that the plant contains essential oils, organic acids, phenolic compounds (flavonoids, hydroxycinnamic acids, tannins) [19, 20].

Thus, the aim of the research is to determine the antimicrobial effect of 10 % and 20 % aqueous solutions of *Chrysanthemum x hortorum* Bailey flowers and leaves Pectoral variery.

Methods

Plant materials

Flowers and leaves of Chrysanthemum x hortorum Bailey Pectoral variety were harvested in the phase of mass flowering of a plant at the research sites of the Department of flower and ornamental plants of M. M. Hryshko National Botanic Garden of the National Academy of Sciences of Ukraine in Kyiv [21-26]. It is collected in September-October 2019. The raw material was authenticated by prof. Svitlana Marchyshyn (TNMU, Ternopil, Ukraine) [27-29]. A voucher specimen was deposited in the herbarium at the Department of Pharmacognosy and Medical Botany, TNMU, Ternopil, Ukraine [30-32]. The study plant material was dried using the conventional method and stored in paper bags in a dry place [33-36].

Preparation of solutions

About 1000 g of dried flowers and leaves of the *Chrysanthemum x hortorum* Bailey Pectoral were powdered with the help of an appropriate grinder [37]. It was taken in an extractor and extracted using water as a solvent. The extract was concentrated under vacuum to half under volume and dried at a temperature of 50±2° C.

Microorganisms

The antibacterial action of the samples was determined regarding 9 test cultures: gram-positive microorganisms *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 14990, *Micrococcus luteus* NCTC 2665, *Corunebacterium spp* ATCC 373, *Serratia marcescens*, gram-negative microorganisms Klebsiella pneumoniae ATCC13883, *Escherichia coli* ATCC 25922, spore culture *Bacillus cereus* NCTC 74. The antifungal effect was studied against *Candida albicans* ATCC 855/653 [38, 39]. Cell concentration was 0.5 McFarland (used to compare the standard turbidity) [40, 41].

Antibacterial and antifungal test

Experimental study of antimicrobial action was performed at the Laboratory of Microbiological and Parasitological Studies of I. Horbachevsky Ternopil National Medical University in 2019.

10 % and 20 % native aqueous solutions of flowers and leaves of *Chrysanthemum x hortorum* Bailey Pectoral variery were studied (sample No. 1 - Chrysanthemum flowers 10 % aqueous solution, sample No. 2 - Chrysanthemum leaves 10 % aqueous solution, sample No. 3 - Chrysanthemum flowers 20 % aqueous solution, and sample No. 4 Chrysanthemum leaves 10 % aqueous solution.

The bacteriostatic effect of the studied samples was determined by the results of growth of reference strains in native aqueous solution of the corresponding samples and in dilutions of 1:1, 1:2 in meat-peptone broth; bactericidal action – in the absence of growth of the contents of test tubes with native solutions of the samples and in a 1:1 dilution in dense nutrient media. The following media were used to determine the bactericidal effect: meat-peptone agar for all cocci and bacilli, blood sugar agar – for Klebsiella and corynebacteria, Endo medium – for enterobacteria, Saburo medium – for yeast-like fungi of the *Candida* genus [42].

Results and Discussion

The results of the study of the antimicrobial activity (regarding gram-positive and gram-negative flora) and antifungal effect of sample No. 1 - Chrysanthemum flowers 10 % aqueous solution by serial dilutions are presented in Table 1.

The results of the study of the antimicrobial activity (regarding gram-positive and gram-negative bacteria) and antifungal effect of sample No. 2 - Chrysanthemum leaves 10 % aqueous solution are presented in Table 2.

The results of the study of the antimicrobial activity (regarding gram-positive and gram-negative flora) and antifungal effect of sample No. 3 - Chrysanthemum flowers 20 % aqueous solution by serial dilutions are presented in Table 3.

The results of the study of the antimicrobial activity (regarding gram-positive and gram-negative flora) and antifungal effect of sample No. 4 - Chrysanthemum leaves 20 % aqueous solution by serial dilutions are presented in Table 4.

We established that the native 10 % aqueous solution of *Chrysanthemum x hortorum* Bailey Pectoral variery flowers had a bactericidal effect against *Staphylococus epidermidis* and *Micrococcus luteus*, delays growth and reproduction (bacteriostatic effect) of *Staphylococcus aureus* and *Escherichia coli* and did not affect Klebsiella, corynebacteria, sera, and *Candida* fungi. Dilution of 10 % solution of chrysanthemum flowers 1:1 had a bacteriostatic effect only on the test culture of staphylococci.

Antibacterial effect of 10% aqueous solution of *Chrysanthemum x hortorum* Bailey Pectoral variery leaves was active only in the native form, it was not manifested when diluted. As for the first sample, bactericidal activity was detected against *Staphylococus epidermidis* and *Micrococcus luteus.*, bacteriostatic – against *Staphylococus aureus*, *Corynebacterium spp., Escherichia coli.* Similarly, sample No.2 has no antibacterial action against *Klebsiella*, fungi of the *Candida* genus, bacilli and sera.

20 % aqueous solution of flowers and leaves of *Chrysanthemum x hortorum* Bailey Pectoral variery proved to have antimicrobial activity similar to the previous 10 % solutions. Thus, the native 20 % aqueous solution of chrysanthemum flowers (sample No. 3) stopped growth of staphylococci, micrococci (bactericidal action), delayed growth of *Escherichia coli* and *Bacillus cereus* (bacteriostatic action), and did not affect the growth of Klebsiella, corynebacteria, sera, and *Candida* fungi. At a dilution of 1:1, the bacteriostatic effect was only for *Staphylococus aureus* and *Micrococcus luteus*.

20 % aqueous solution of flowers and leaves of *Chrysanthemum x hortorum* Bailey Pectoral variery proved to have antimicrobial activity similar to the previous 10% solutions. Thus, the native 20 % aqueous solution of chrysanthemum flowers (sample No. 3) stopped growth of staphylococci, micrococci (bactericidal action), delayed growth of *Escherichia coli* and bacilli (bacteriostatic action) and did not affect the growth of Klebsiella, corynebacteria, sera, and *Candida* fungi. At a dilution

of 1:1, the bacteriostatic effect was only for *Staphylococus aureus and Micrococcus luteus.*

Bactericidal action of 20 % aqueous solution of *Chrysanthemum x hortorum* Bailey Pectoral variery leaves (sample No.4) was observed against *Staphylococus epidermidis*, *Bacillus cereus*, *Escherichia coli*, and *Micrococcus luteus*; bacteriostatic – against *Staphylococus aureus*, other cultures were resistant. The bacteriostatic effect of sample No.4 in a dilution of 1:1 was manifested only for *Bacillus cereus* and *Micrococcus luteus*.

Thus, 10 % and 20 % native aqueous solutions of *Chrysanthemum x hortorum* Bailey Pectoral variery leaves flowers and leaves have antibacterial activity against gram-positive staphylococci, micrococci, bacilli, and gram-negative *Escherichia coli*. At a dilution of 1:1 and 1:2 antibacterial effect is less significant or absent. These solutions do not affect Klebsiella, corynebacteria, sera and fungi of the *Candida* genus.

The results of phytochemical studies showed that in Chrysanthemum x hortorum Bailey flowers and leaves Pectoral variery identified 26 and 27 components of volatile compounds, the total content of which was 1290 mg / kg and 2688 mg / kg respectively. It is known that essential oils possess antibacterial activity [43].

It was found out from scientific sources [44] that such components as β-bisabolene and bisabolol, α-curcumene [45] show pronounced antibacterial activity. *Chrysanthemum x hortorum* Bailey flowers and leaves Pectoral variery had been studied concerning these compounds which resulted in - bisabolene 3.5 mg / kg in flowers, α-curcumene in flowers - 22.7 mg / kg, in leaves - 132.5 mg / kg.

Volatile compounds also contain sesquiterpenoids (α-cadinol (505.4 mg / kg), β-caryophyllene (166.8 mg / kg), α-caryophyllene (49.1 mg / kg) and sesquiteprene lactone - caryophylene oxide - 436,7 mg/kg), that is also characterized by antibacterial activity.

The antibacterial activity of 10 % and 20 % native aqueous solutions of flowers and leaves of Chrysanthemum x hortorum Bailey Pectoral variery has been tightly related to its content of phenolic compounds (flavonoids, hydroxycinnamic acids, tannins) [46].

Conclusions

Research results showed that all the studied samples of flowers and leaves of chrysanthemums have antimicrobial properties.

Native 10 % and 20 % aqueous solutions of chrysanthemum flowers and leaves have shown antibacterial activity against gram-positive and gram-negative microflora, so they are promising for development of a drug with antimicrobial properties.

These properties of 10 % and 20 % native aqueous solutions of flowers and leaves of Chrysanthemum x hortorum Bailey Pectoral variery are the result of the presence of essential oils and phenolic compounds.

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Table 1. Study of antimicrobial activity of 10 % aqueous solution of flowers of *Chrysanthemum* x *hortorum* Bailey Pectoral variery by serial dilutions

Test culture	Sample No. 1		
	Native solution	1:1	1:2
S. aureus	Bacteriostatic action	Bacteriostatic action	Resistant
S. epidermalis	Bactericidal action	Bacteriostatic action	Resistant
K. pneumoniae	Resistant	Resistant	Resistant
Corinebacterium spp	Resistant	Resistant	Resistant
C. albicans	Resistant	Resistant	Resistant
E. coli	Bacteriostatic action	Resistant	Resistant
B. cereus	Resistant	Resistant	Resistant
S. marcescens	Resistant	Resistant	Resistant
M. luteus	Bactericidal action	Resistant	Resistant

Table 2. Study of antimicrobial activity of 10 % aqueous solution of leaves of *Chrysanthemum* x *hortorum* Bailey Pectoral variery by serial dilutions

Test culture	Sample No. 2		
	Native solution	1:1	1:2
S. aureus	Bacteriostatic action	Resistant	Resistant
S. epidermalis	Bactericidal action	Resistant	Resistant
K. pneumoniae	Resistant	Resistant	Resistant
Corinebacterium spp	Bacteriostatic action	Resistant	Resistant
C. albicans	Resistant	Resistant	Resistant
E. coli	Bacteriostatic action	Resistant	Resistant
B. cereus	Resistant	Resistant	Resistant
S. marcescens	Resistant	Resistant	Resistant
M. luteus	Bactericidal action	Resistant	Resistant

Table 3. Study of antimicrobial activity of 20 % aqueous solution of flowers of *Chrysanthemum* x *hortorum* Bailey Pectoral variery by serial dilutions

Test culture	Sample No. 3		
	Native solution	1:1	1:2
S. aureus	Bactericidal action	Resistant	Resistant
S. epidermalis	Bactericidal action	Bacteriostatic action	Resistant
K. pneumoniae	Resistant	Resistant	Resistant
Corinebacterium spp	Resistant	Resistant	Resistant
C. albicans	Resistant	Resistant	Resistant
E. coli	Bacteriostatic action	Resistant	Resistant
B. cereus	Bacteriostatic action	Resistant	Resistant
S. marcescens	Resistant	Resistant	Resistant
M. luteus	Bactericidal action	Bacteriostatic action	Resistant

Table 4. Study of antimicrobial activity of 20 % aqueous solution of leaves of *Chrysanthemum* x *hortorum* Bailey Pectoral variery by serial dilutions

Test culture	Sample No. 4		
	Native solution	**1:1**	**1:2**
S. aureus	Bacteriostatic action	Resistant	Resistant
S. epidermalis	Bactericidal action	Resistant	Resistant
K. pneumoniae	Resistant	Resistant	Resistant
Corinebacterium spp	Bacteriostatic action	Resistant	Resistant
C. albicans	Resistant	Resistant	Resistant
E. coli	Bactericidal action	Resistant	Resistant
B. cereus	Bactericidal action	Bacteriostatic action	Resistant
S. marcescens	Resistant	Resistant	Resistant
M. luteus	Bactericidal action	Bacteriostatic action	Resistant