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Characterisation of Antimicrobial Properties of Extracts of Selected Medicinal Plants

MONIKA CIOCH, PAWEŁ SATORA, MAGDALENA SKOTNICZNY, DOROTA SEMIK-SZCZURAK and TOMASZ TARKO

Department of Fermentation Technology and Technical Microbiology, Faculty of Food Technology, University of Agriculture, Kraków, Poland

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Abstract

The scope of the experiments included analysis of the antimicrobial activity of ethanolic, methanolic and aqueous extracts against bacterial and fungal cultures and determination of the minimum inhibitory concentration of plant extracts tested microbial growth. Analysis of the antifungal and antibacterial activity was carried out by the disc diffusion method using paper discs. In the experiment 11 species of microorganisms – 8 bacterial and 3 fungal strains were used. The highest antimicrobial activity against the tested strains was demonstrated by black elder (*Sambucus nigra* L.), black locust (*Robinia pseudoacacia* L.) and lingonberry (*Vaccinium vitis-idaea* L.) extracts. The study showed the diverse morphological activity of specific parts of elderberry and quince, which is the effect of different polyphenolic profile of these plants. The yeast *Saccharomyces cerevisiae*, *Escherichia coli*, *Pseudomonas putida* and *Bacillus subtilis* showed the highest sensitivity to the effect of extracts of the analysed plants. As a positive control three antibiotics – amphotericin B, vancomycin and amoxicillin with clavulanic acid were used.

Key words: *Robinia pseudoacacia* L., *Sambucus nigra* L., *Vaccinium vitis-idaea* L., antimicrobial activity, medicinal plants

Introduction

Medicinal plants contain a number of valuable substances, which can support the prevention and treatment of various diseases. Due to differences in the chemical composition of the mixture, biologically active substances exhibit the activity of a different type than acting separately, which is the result of synergism or antagonism of their various components.

The main components responsible for the antimicrobial properties of plants are polyphenolic compounds. They exhibit anti-inflammatory activity *in vitro* and *in vivo* and their mechanism of action is the inhibition of enzymes (phospholipase oxygenase) *i.e.* by binding with hydrosulfide groups and inactivation of bacterial proteins (Kim *et al.*, 1995; Cowan, 1999).

Antimicrobial activity is attributed to flavonols, quinones and flavonoids. These substances exhibit lipophilic properties and cause the destruction of the cell wall and cytoplasmic membrane of microorganisms. Furthermore, they cause inhibition of nucleic acid synthesis, structural and enzymatic proteins as well as saccharides. It has been shown that the antimicrobial activity of flavonoids may be dependent on their structure. It is believed that unsubstituted flavones are characterized by the highest antifungal activity, and flavanones by lower. The introduction to these compounds of hydroxyl or methyl groups reduces their antifungal properties (Małolepsza and Urbanek, 2000).

Anthocyanins form complexes with metals, participating in this way in shaping the colour of flowers (Mitek and Gasik, 2009; Quina *et al.*, 2009). The bioavailability and biological activity of these compounds is closely related to their chemical structure, which affects a large variety of them. Studies have shown that plant extracts rich in these substances have antibacterial and antitumor activity (Middleton *et al.*, 2000; Olejnik *et al.*, 2009).

Secondary metabolites of plants responsible for their antimicrobial properties can also include alkaloids (berberine) which damage the DNA of microbial cells, leading to their death (Omulokoli *et al.*, 1997).

Antimicrobial activity of the protein rather consists of the formation of ion channels in the cell membrane of the microorganism, increasing its permeability. The mechanism of action may also involve blocking the

* Corresponding author: M. Cioch, Department of Fermentation Technology and Technical Microbiology, Faculty of Food Technology, University of Agriculture, Kraków, Poland; **e-mail: monika.cioch@urk.edu.pl**

metabolism of the essential compounds for the bacteria and the inhibition of adhesion microorganism to the surface of the plant cell (Cowan, 1999).

Tannins protect a plant against the effects of microorganisms by the formation of complexes with proteins, while terpenes interfere with the incorporation of the lipophilic compounds in the bacterial cell membrane (Mendoza *et al.*, 1997).

On the basis of phytochemical analysis the presence of many biologically active chemical compounds in flowers, leaves, fruits and rhizomes of medicinal plants was discovered. As a natural source of valuable substances for health and readily available ones, they are an object of interest of pharmacological studies, showing tremendous therapeutic potential.

The aim of the study was to determine the antimicrobial effects of extracts of selected medicinal plants and their minimum dose that inhibits growth of 11 species of microorganisms, both bacterial and fungal.

Experimental

Materials and Methods

Plant material and preparation of extracts. In this study ethanolic, methanolic and aqueous extracts made of fruits of dogwood (*Cornus mas* L.), black mulberry (*Morus nigra* L.), lingonberry (*Vaccinium vitis-idaea* L.), flowers of hawthorn (*Crataegus oxyacantha* L.), black locust (*Robinia pseudoacacia* L.), fruits and leaves of quince (*Cydonia oblonga* Mill.) and leaves and flowers of black elder (*S. nigra* L.) were used. Analysed medicinal plants were collected from organic farming in Lesser Poland Voivodeship. Suitable for analysis parts of plants were harvested and frozen at –80°C, in order to inhibit transformation of the antioxidant activity of the compounds. Frozen research material (10.0 g) was weighed into conical flasks with ground glass joint. Fruits of quince and dogwood were divided after thawing into smaller parts. To the analysed material (in a flask) 90 ml of a suitable solvent (ethanol 80% vol., methanol 80% vol. or hot water) was poured over and extracted with a magnetic stirrer (6 h, room temp.). After extraction, the solutions were filtered, adjusted to 100 ml with a suitable solvent for the determination and stored at –20°C. Extraction of each material was conducted with 3 replications.

Microorganisms. For the tests 11 species of microorganisms (8 bacteria and 3 fungi) from the Pure Culture Collection of the Department of Fermentation Technology and Technical Microbiology, University of Agriculture in Krakow were used. Bacterial cultures: *Bacillus subtilis* subsp. *subtilis* DSM 10, *Bifidobacterium* sp. DSM 20104, *Clostridium* sp. DSM 756, *Escherichia coli* DSM 4261, *Micrococcus luteus* DSM 20030, *Proteus myxofaciens* DSM 4482, *Pseudomonas putida* DSM 291, *Serratia marcescens* DSM 1636. Fungal cultures: *Aspergillus niger* CBS 10930, *Penicillium chrysogenum* DSM 844, *Saccharomyces cerevisiae* DSM 1333.

Microbiological media. Aerobic bacterial strains were cultivated at 32°C for 24 hours and tested on tryptic soy agar (Merck, Germany), and fungal cultures at 28°C for 48 hours on Sabouraud agar (Merck, Germany). Anaerobic *Bifidobacterium* sp. was cultivated at 37°C for 18 hours and grown on the Bifidobacterium-Medium (g/l water: casein peptone, 10; yeast extract, 5; beef extract, 5; soybean hydrolyzate, 5; glucose, 10; K_2HPO_4, 2; $MgSO_4 \cdot 7H_2O$, 0.20; $MnSO_4 \cdot H_2O$, 0.05; Tween 80, 1; NaCl, 5; L-cysteine, 0.50; salt solution, 40; Resazurin (25 mg/100 ml), 4 and *Clostridium* sp. on Pyx-agar medium (g/l water: tryptone, 5; peptone, 5; yeast extract, 10; glucose, 5; resazurin (25 mg/100 ml), 1; salt solution, 40; L-cysteine, 0.50). The composition of the salt solution in g/l of water: $CaCl_2 \cdot 2H_2O$, 0.25; $MgSO_4 \cdot 7H_2O$, 0.50; K_2HPO_4, 1; KH_2PO_4, 1; $NaHCO_3$, 10; NaCl, 2).

Determination of antimicrobial activity of extracts of analysed medicinal plants. The analysis of the antifungal and antibacterial activity was carried out by using a diffusion paper-disc assay. 24-hour age bacterial (10^9 CFU/ml) or fungal (10^8 CFU/ml) culture was streaked into appropriate media, next the paper discs with a diameter of 14 mm were placed and soaked with 4 mg/ml of the analysed extract. The 80% vol. solutions of ethanol and methanol were the negative control, while the positive were discs with vancomycin (30 mg, Bioanalyse, Turkey) or amoxicillin with clavulanic acid (30 mg, Biolab Zrt., Hungary) for bacteria and amphotericin B (10 mg, Sigma-Aldrich, Germany) for fungi. Plates were incubated for 18 h at 37°C (bacteria) and 48 h at 28°C (fungi). Anaerobic microorganisms were plated on Petri dishes by submerged method. Subsequent procedure was analogous to the above. The strains were incubated under anaerobic conditions for 48 h at 37°C. The antimicrobial activity was defined as the diameter of growth inhibition zone around the disks of blotting paper soaked with plant extracts.

Determination of the minimum inhibitory concentration of the plant extracts (MIC). MIC was determined by the classical method in the solid growth medium. 24-hour old bacterial cultures (10^9 CFU/ml) cultivated at 28°C and 48-hour old fungal cells (10^8 CFU/ml) at 37°C and were plated into growth media on which paper disks with a diameter of 14 mm, soaked with 1, 2, 3 or 4 mg/ml respectively of the appropriate extract were placed. Negative control were 80% vol. ethanol and methanol solutions. The plates were incubated for 24 h at 28°C (bacteria) and for 48 h at 37°C (fungi). Anaerobic microorganisms were plated

on Petri dishes by submerged method. Subsequent procedure was analogous to the above. The strains were incubated under anaerobic conditions for 48 h at 37°C. MIC was defined as the lowest extract concentration that inhibited the growth of microorganisms on a solid growth medium.

Statistical analysis. Variance analysis was used for the obtained results analysis (Statistica program). Comparisons between groups were performed using the post-hoc test of Scheffe. To performed similarity analysis was used dendritic method. All assays were performed in 9 replications.

Results

The analysed extracts were characterized by diverse antifungal and antibacterial activity. The methanol and ethanol extracts proved to be most effective in inhibiting the growth of tested microbial cultures. Extracts from lingonberry and black locust fruits as well as black elder flowers showed the highest antimicrobial activity with respect to the analysed fungal and bacterial cultures. Other plant species had significantly lower antimicrobial properties.

Antimicrobial activity of analysed plants extracts are presented in Table I. Antibiotic effect depended primarily on the type of solvent used for extraction. After eliminating its influence on the culture of microorganism in all cases the most significant zone of growth inhibition of microorganisms was found in case of ethanol and methanol extracts. Extracts of black elder flowers as well black locust and lingonberry fruits most effectively inhibited the growth of tested microorganisms. Contrary to it the activity of black mulberry fruits extracts was the weakest. It inhibited only 8 cultures.

S. cerevisiae yeast were the most sensitive to the effects of extracts from black elder, hawthorn and common quince. To a much lesser extent, these extracts inhibited the growth of bacterial cultures. The only exception were *P. myxofaciens* and *P. putida* showing high sensitivity to the extract from the fruit of *S. nigra*.

Table II shows the antimicrobial action of antibiotics – vancomycin (30 mg) and amoxicillin (20 mg) for bacteria and amphotericin B (10 mg) for fungi used as positive control. These substances blocked effectively cell proliferation of used microorganisms except amoxicillin, which does not affect the *B. subtilis* bacteria.

Ethanol, methanol and water extracts derived from black elder characterized by a relatively growth broad antimicrobial activity (Table III). The extracts of *S. nigra* flowers were slightly worse inhibitors of microbial growth than those obtained from the fruits.

Methanol extracts were characterized by antibiotic activity only against *S. cerevisiae* yeast and bacteria of *E. coli*, *P. myxofaciens*, *M. luteus*, *P. putida* and *Bifidobacterium* sp. In all cases, the minimum inhibitory dose was 2 mg/ml.

Extracts of hawthorn flower were distinguished by slightly weaker antimicrobial activity (Table IV) but strongly inhibited the development of *S. cerevisiae* and *E. coli* (MIC 2 mg/ml). These extracts impacted the growth of other analysed microorganisms. Aqueous extracts of hawthorn flowers demonstrated efficacy against *S. cerevisiae* yeast (MIC 2 mg/ml).

The highest antimicrobial activity was observed in the case of extracts from black locust flowers (Table V). Like the other analysed plants, they did not affect the growth of *A. niger* and *P. chrysogenum* as well as *P. myxofaciens* and *S. marcescens*. The ethanol extracts of black locust effectively inhibited the growth of other microorganisms used in the experiments. In the case of most microorganisms, their growth was inhibited by concentration of 2 mg/ml.

Extract from lingonberry fruit showed relatively high antimicrobial activity (Table VI), however in some cases such as *E. coli*, *M. luteus*, *P. putida*, *Bifidobacterium* sp. and *Clostridium* sp. higher MIC compared to the other extracts discussed above was detected. Noteworthy, that the growth of *A. niger* was stopped by ethanol and methanol lingonberry extracts. Likewise extracts of elderberry fruit, lingonberry extracts inhibited most effectively development of *P. myxofaciens* bacteria (MIC 2 mg/ml). Aqueous extracts showed antimicrobial activity in higher concentration (> 4 mg/ml).

Elderberry, black locust and black mulberry were characterized by the highest antimicrobial activity against tested bacteria and fungi. The study also showed various activity of different morphological parts of elderberry and quince which might be the effect of different polyphenols profile of these plants. The *S. cerevisiae* yeast and bacterial cultures of *E. coli*, *P. putida* and *B. subtilis* were characterized by the highest sensitivity on the tested plant extracts. As a positive control 3 antibiotics were used – amphotericin B, vancomycin and amoxicillin with clavulanic acid.

It was found that amphotericin B inhibited the growth of all tested fungal cultures (Table II). In the case of *S. cerevisiae* there was observed approximately 2-fold higher inhibition zone compared to *A. niger* and *P. chrysogenum*. Studies on the amphotericin B mechanism of action on yeast strains revealed that this antibiotic induces the stimulation of cells, permeabilization of cell membranes and can lead to death of microorganism.

Statistical analysis. Based on the performed similarity analysis by dendritic method, the sensitivity of tested microorganisms was classified regard to ethanol, methanol and water plants extracts (Fig. 1, 2, 3). Separated groups allow effectively present mutual similarities and significant differences in action of plant extracts against

Table I
Antimicrobial activity of ethanol (E), methanol (M) and water (W) extracts of analyzed plants against selected strains (inhibition zones in mm)

Plant material[y]		*Saccharomyces cerevisiae*	*Penicillium chrysogenum*	*Aspergillus niger*	*Escherichia coli*	*Serratia marcescens*	*Proteus myxofaciens*	*Micrococcus luteus*	*Pseudomonas putida*	*Bacillus subtilis*	*Bifidobacterium* sp.	*Clostridium* sp.	Sig.[z]
Black elder (flower)	E	0.78 ^{a}	0.17 ^{b}	0	0	0	0.17 ^{b}	0.17 ^{b}	0.11 ^{b}	0	0	0	**
	M	0.11 ^{b}	0	0	0.17 ^{b}	0	0.5 ^{b}	0.22 ^{a}	0.22 ^{a}	0	0	0	**
	W	0.94 ^{ab}	0	0	1.44 ^{a}	0.94 ^{ab}	1.44 ^{a}	1.33 ^{a}	1.22 ^{a}	1.17 ^{a}	0.22 ^{c}	0.11 ^{c}	*
Hawthorn (flower)	E	1.5 ^{a}	0	1.33 ^{a}	1.22 ^{a}	0	0.11 ^{b}	0	0.17 ^{b}	0	0	0	**
	M	0.17 ^{b}	0	0	0	0.28 ^{a}	0.28 ^{a}	0	0	0.5 ^{a}	0	0	**
	W	0.67 ^{bc}	0	0	1.5 ^{a}	1.5 ^{a}	1.39 ^{a}	0.89 ^{ab}	1.39 ^{a}	0.89 ^{ab}	0	0.11 ^{c}	*
Locust (flower)	E	0	0	0	0	0	0	0	0	0	0.06 ^{b}	0.11 ^{a}	**
	M	0	0	0	0	0	0.33 ^{a}	0	0.17 ^{a}	0	0.06 ^{b}	0	**
	W	1 ^{ab}	0	0	1.5 ^{a}	1.33 ^{a}	1.28 ^{a}	0.89 ^{ab}	1 ^{ab}	0.89 ^{ab}	0.11 ^{c}	0.44 ^{c}	*
Cowberry (fruit)	E	0	0	0	1.11 ^{a}	0.11 ^{b}	0.28 ^{b}	0	0	0.5 ^{a}	0	0	**
	M	0.39 ^{a}	0	0	0	0	0.5 ^{a}	0	0	0.28 ^{a}	0.06 ^{b}	0	*
	W	0	0	0	1.5 ^{a}	0.22 ^{b}	1.44 ^{a}	0.33 ^{b}	0.67 ^{b}	1.17 ^{a}	0	0.33 ^{b}	**
Mulberry (fruit)	E	0.89 ^{a}	0	0	0.28 ^{b}	0	1 ^{a}	0.78 ^{a}	0	0	0	0	**
	M	0	0	0	0	0	0.5 ^{a}	0.33 ^{a}	0.28 ^{a}	0.33 ^{a}	0	0.06 ^{b}	**
	W	0.67 ^{bc}	0	0	1.67 ^{a}	0	1 ^{ab}	1.5 ^{a}	1.17 ^{a}	1.67 ^{a}	0.11 ^{c}	0.44 ^{bc}	*
Quince common (fruit)	E	0	0	0	0.88 ^{a}	0	0.06 ^{b}	0	0.28 ^{a}	0	0	0	**
	M	0.17 ^{b}	0	0	0	0	0.39 ^{a}	0	0	0.56 ^{a}	0	0	**
	W	1.5 ^{a}	0	0	0.94 ^{ab}	0.56 ^{bc}	1.17 ^{a}	0.5 ^{bc}	1.22 ^{a}	1.39 ^{a}	0.22 ^{c}	0.56 ^{bc}	*
Dogwood (fruit)	E	0.72 ^{a}	0	0	0.67 ^{a}	0	0	0.06 ^{b}	0	0	0	0	**
	M	0.17 ^{a}	0	0	0	0	0.22 ^{a}	0.11 ^{b}	0	0.39 ^{a}	0	0	**
	W	0.67 ^{c}	0	0	1 ^{ab}	0.67 ^{bc}	1.28 ^{a}	1.44 ^{a}	1.44 ^{a}	1.33 ^{a}	0.22 ^{c}	0.11 ^{c}	*
Black elder (fruit)	E	1.22 ^{a}	0	1.83 ^{a}	0.28 ^{b}	0	1.11 ^{a}	1.17 ^{a}	0	0	0	0	**
	M	0.17 ^{b}	0	0	0	0	0.22 b	0.5 a	0	0.44 a	0	0	**
	W	0	0.44 ^{bc}	0	1.28 ^{a}	0.94 ^{ab}	1.28 ^{ab}	1.5 ^{a}	0.78 ^{ab}	0.94 ^{ab}	0.56 ^{bc}	0.22 ^{c}	*
Quince common (leaves)	E	0.06 ^{b}	0	0.28 ^{b}	0	0.83 ^{a}	0.5 ^{a}	0	0	0	0	0	**
	M	0	0	0	0	0	0.5 ^{a}	0	0.39 ^{b}	0	0	0	**
	W	1.33 ^{a}	0.22 ^{c}	0	1.17 ^{ab}	1.11 ^{ab}	1.44 ^{a}	1 ^{ab}	1.22 ^{a}	1.22 ^{a}	0.56 ^{bc}	0	*

[Y] According to the Scheffe test. means within columns followed by the same letter are not significantly differen;
[Z] Sig: Significance; * and ** display the significance at 5 % and 1 % respectively.

Table II
Antimicrobial activity of selected antibiotics against analyzed microbial strains (inhibition zones in mm; mean of three replicates)

Antibiotic substance	*Saccharomyces cerevisiae*	*Penicillium chrysogenum*	*Aspergillus niger*	*Escherichia coli*	*Serratia marcescens*	*Proteus myxofaciens*	*Micrococcus luteus*	*Pseudomonas putida*	*Bacillus subtilis*	*Bifidobacterium* sp.	*Clostridium* sp.
Amoxicillin (30 µg)	–	–	–	0.22	0.22	0.88	0.33	0.22	0	1.17	1.28
Vancomycin (20 µg)	–	–	–	1.89	1.22	0.83	0.67	0.67	4.44	9.56	8.78
Amfotericin B (100 µg)	7.61	4.11	3.56	–	–	–	–	–	–	–	–

Table III
Antimicrobial activity of different concentrations of black elder flower ethanol (E), methanol (M) and water (W) extracts against the analyzed microbial strains (inhibition zones in mm; mean of three replicates)

Plant material [mg/ml]			*Saccharomyces cerevisiae*	*Penicillium chrysogenum*	*Aspergillus niger*	*Escherichia coli*	*Serratia marcescens*	*Proteus myxofaciens*	*Micrococcus luteus*	*Pseudomonas putida*	*Bacillus subtilis*	*Bifidobacterium* sp.	*Clostridium* sp.
Black eder (flower)	E	2.0	0.50	–	–	0.17	–	–	0.17	–	–	0.11	–
		3.0	0.83	–	–	0.50	–	–	–	–	–	–	–
		4.0	1.17	–	–	–	–	–	0.17	–	–	–	–
	M	2.0	–	–	–	–	–	0.50	0.17	0.33	–	0.17	0.11
		3.0	–	–	–	–	–	–	–	–	–	0.06	–
		4.0	–	–	–	–	–	0.50	–	0.33	–	0.22	–
	W	2.0	0.50	–	–	–	–	–	–	–	0.50	–	–
		3.0	1.00	–	–	–	–	–	–	–	1.00	–	–
		4.0	1.50	–	–	1.00	1.00	–	–	1.00	1.50	–	–

Table IV
Antimicrobial activity of different concentrations of hawthorn flower ethanol (E), methanol (M) and water (W) extracts against the analyzed microbial strains (inhibition zones in mm; mean of three replicates)

Plant material [mg/ml]			*Saccharomyces cerevisiae*	*Penicillium chrysogenum*	*Aspergillus niger*	*Escherichia coli*	*Serratia marcescens*	*Proteus myxofaciens*	*Micrococcus luteus*	*Pseudomonas putida*	*Bacillus subtilis*	*Bifidobacterium* sp.	*Clostridium* sp.
Hawthorn (flower)	E	2.0	0.83	–	–	0.17	0.17	0.17	0.17	–	–	–	–
		3.0	1.17	–	–	0.50	–	–	–	–	–	0.17	–
		4.0	1.50	–	–	–	0.17	0.17	0.17	–	–	–	–
	M	2.0	0.33	–	–	0.17	–	–	–	0.17	–	0.33	–
		3.0	–	–	–	–	–	–	–	–	–	–	–
		4.0	0.33	–	–	–	–	–	–	0.17	–	0.22	–
	W	2.0	0.50	–	–	–	–	–	–	–	–	–	–
		3.0	1.00	–	–	–	–	–	–	–	–	–	–
		4.0	1.50	–	–	1.00	–	–	–	1.00	–	–	–

Table V
Antimicrobial activity of different concentrations of black locust flower ethanol (E), methanol (M) and water (W) extracts against the analyzed microbial strains (inhibition zones in mm; mean of three replicates)

Plant material [mg/ml]			*Saccharomyces cerevisiae*	*Penicillium chrysogenum*	*Aspergillus niger*	*Escherichia coli*	*Serratia marcescens*	*Proteus myxofaciens*	*Micrococcus luteus*	*Pseudomonas putida*	*Bacillus subtilis*	*Bifidobacterium* sp.	*Clostridium* sp.
Black locust (flower)	E	2.0	0.50	–	–	0.50	–	–	–	–	–	0.22	–
		3.0	0.83	–	–	1.00	–	–	–	0.50	–	–	–
		4.0	1.00	–	–	0.17	–	–	–	0.17	–	0.11	–
	M	2.0	0.83	–	–	–	–	–	0.17	0.50	–	0.22	0.22
		3.0	0.67	–	–	–	–	–	–	–	0.33	–	–
		4.0	0.83	–	–	–	–	–	–	0.50	0.17	0.11	–
	W	2.0	–	–	–	–	–	–	–	–	–	–	0.33
		3.0	–	–	–	1.00	–	–	1.00	–	–	–	0.11
		4.0	–	–	–	1.00	1.00	–	1.00	–	1.50	–	0.44

Table VI
Antimicrobial activity of different concentration of cowberry fruit ethanol (E), methanol (M) and water (W) extracts against the analyzed microbial strains (inhibition zones in mm; mean of three replicates)

Plant material [mg/ml]			*Saccharomyces cerevisiae*	*Penicillium chrysogenum*	*Aspergillus niger*	*Escherichia coli*	*Serratia marcescens*	*Proteus myxofaciens*	*Micrococcus luteus*	*Pseudomonas putida*	*Bacillus subtilis*	*Bifidobacterium* sp.	*Clostridium* sp.
Cowberry (fruit)	E	2.0	0.83	–	0.50	–	–	0.33	–	–	–	–	0.11
		3.0	0.83	–	–	–	0.50	0.33	–	–	0.33	0.17	–
		4.0	0.67	–	0.17	–	0.50	0.67	–	–	–	0.06	–
	M	2.0	0.17	–	0.17	0.17	–	–	0.17	–	–	–	–
		3.0	0.17	–	0.67	–	–	–	–	–	0.67	0.11	–
		4.0	0.33	–	0.83	–	–	–	–	–	0.33	0.11	–
	W	2.0	–	–	–	–	–	–	–	–	–	–	–
		3.0	–	–	–	–	–	–	–	–	–	–	–
		4.0	–	–	–	1.00	–	1.00	–	1.00	1.00	–	0.22

individual bacteria and fungi strains. The results indicate the diverse response of the tested microorganisms to ethanol, methanol and aqueous plant extracts. The highest similarity with respect to the relative sensitivity to the analysed ethanol extracts was shown by 4 groups of microorganisms (Fig. 1). The first included anaerobic microorganisms (*Bifidobacterium* sp., *Clostridium* sp.) and filamentous fungus *P. chrysogenum*. The second group was aerobic bacteria (*P. putida*, *B. subtilis*, *S. marcescens*, *P. myxofaciens*, *M. luteus*), which were characterized by low sensitivity to the analysed plant ethanol extracts. The third group – *S. cerevisiae* and *A. niger*. *E. coli* formed a separate group with a significant Euclidean distance from other microorganisms.

The results of the cluster analysis of methanol plant extracts is shown in Fig. 2. In this case, clusters were determined bacterial microorganisms. The first of these include *S. marcescens* and anaerobic *Clostridium* sp. and *Bifidobacterium* sp. bacteria. *S. cerevisiae* formed a separate group with a significant Euclidean distance from other microorganisms. Aqueous extracts (Fig. 3) were the most homogeneous group with respect to observe the highest similarity of analysed microorganisms. *P. chrysogenum* and *A. niger* strains, as well as anaerobic *Clostridium* sp. and *Bifidobacterium* sp. bacteria proved to be the least sensitive to action of water plant extracts. Other microorganisms have shown inhibition relative to the tested aqueous extracts.

Discussion

The experiments included the antimicrobial activity of ethanolic, methanolic and aqueous extracts against selected bacterial and fungal cultures as well as evaluation of minimum inhibitory concentration of plant extracts. Analysis of antifungal and antibacterial activity was carried out by disc diffusion using paper discs. In the experiment 11 species of microorganisms were used – 8 bacterial and 3 fungal strains.

Literature data reported that extracts of black elder flowers as well fruits inhibited Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*) and Gram-negative bacteria (*Salmonella poona*, *Pseudomonas aeruginosa*). Zone of growth inhibition hesitate from 5 to 14 mm. Flavonons, flavonols dihydroflavonols present in many flowers may be responsible for their antimicrobial properties. Furthermore, they can contain lecithin, peptides and oligosaccharides which are inhibitors of transcription and metabolism of the bacterial cells (Hearst *et al.*, 2010).

Experiments of Oliveira *et al.* (2007) showed that methanol extracts of black elder did not inhibit the growth of *B. subtilis*, *P. aeruginosa*, *Aeromonas hydrophila* and *S. aureus*. However, during studies conducted

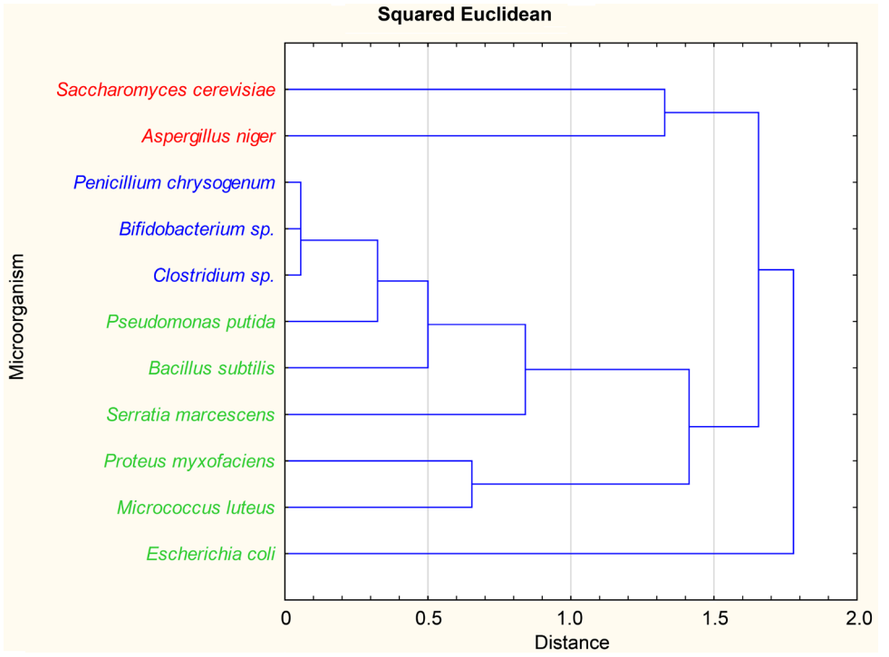

Fig. 1. Diagram of similarities of tested microorganisms with respect to ethanol plant extracts made by dendritic method.

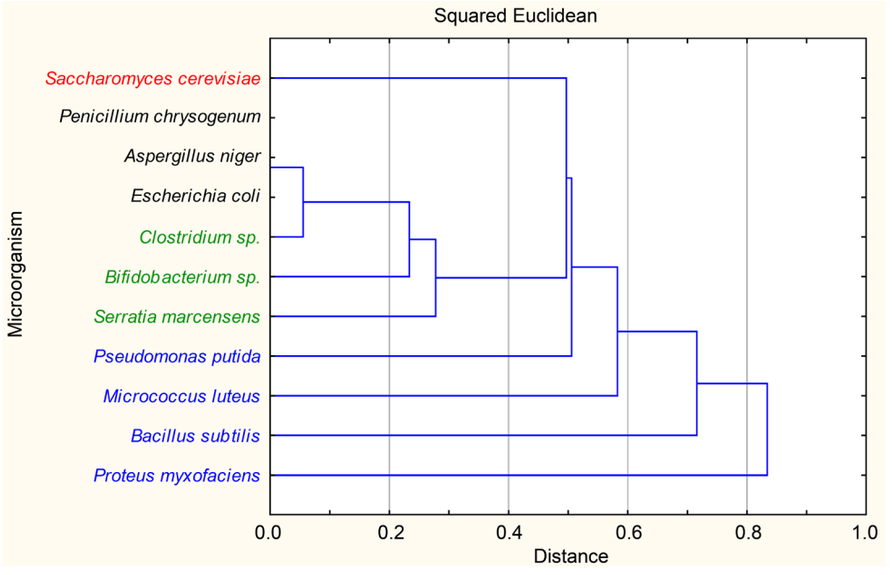

Fig. 2. Diagram of similarities of tested microorganisms with respect to methanol plant extracts made by dendritic method.

by the Bussmann *et al.* (2010) ethanol extract of *S. nigra* slightly inhibited the growth of *S. aureus*, while no inhibition was found in the case of *E. coli*.

The extracts obtained from different organs of the same plant exhibited various action against microorganisms, for example leaves of quince extracts were characterized by better antimicrobial activity than the fruit extracts.

Research conducted by Silva *et al.* (2005) indicates a different polyphenol composition of individual morphological parts of quince. In *Cydonia oblonga* leaves the presence of flavonol glycosides, phenolic acids and derivatives of quercetin were detected, while in fruits concentration of these components was significantly lower. Noteworthy is the fact that the composition of the various parts of the same plant is directly related to

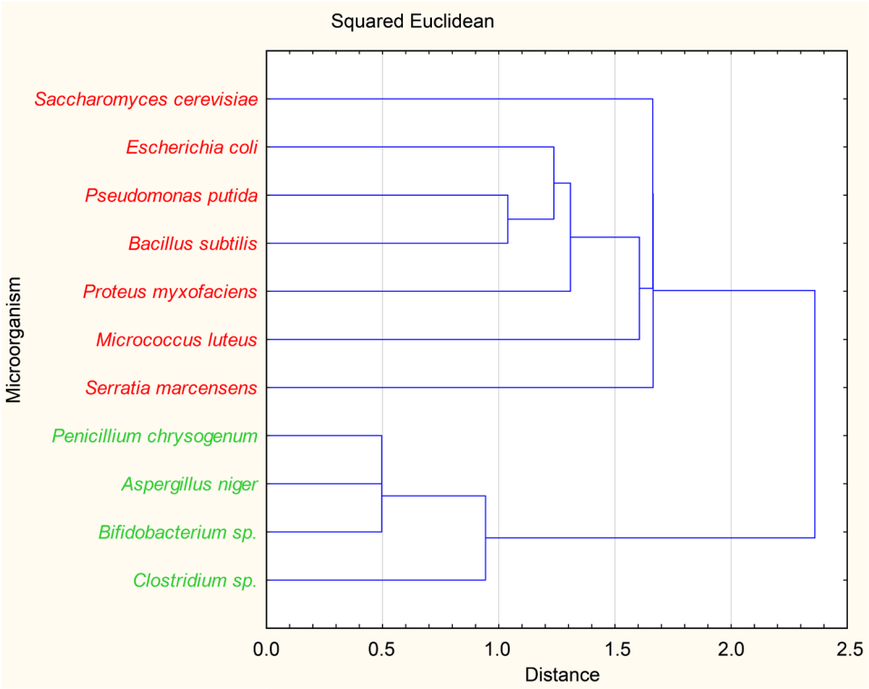

Fig. 3. Diagram of similarities of tested microorganisms with respect to water plant extracts made by dendritic method.

its exposure to environmental conditions and is associated with the degree of its maturity. The content of polyphenolic compounds changes during growth and ripening of quince, which may affect its antimicrobial properties (Wojdyło, 2011).

Aqueous extracts of black elder flowers, in contrast to the fruit, inhibited the growth of *S. cerevisiae* yeast, whereas the latter showed the action against anaerobic bacteria. There were no zones of inhibition around the disks soaked with extracts in the case of *A. niger* and *P. chrysogenum*. Ethanol extracts affected to a greatest extent the growth of *S. cerevisiae* yeasts and *E. coli* bacteria with MIC for these microorganisms being 2 mg/ml. The growth of anaerobic bacteria of the genus *Bifidobacterium* sp. and *Clostridium* sp. was inhibited strongly by aqueous extract of black elder fruit.

Because of all parts of the black elder contain sambunigrin, a toxic compound present mainly in the immature fruit and cyanogenic glycosides (prunasin), it is believed that the plant extracts may have antifungal activity (Charlebois *et al.*, 2010). It has been also shown that the antifungal PR 1 proteins which occur in black elder can bind to the protein channels in cell membranes, cause disturbances in the release of calcium ions, thereby have antifungal properties against plant pathogens such as *Uromyces fabae* and *Erysiphe graminins* (Selitrennikoff, 2001).

Hawthorn flowers, like fruits of elderberries contain phenolic acids (chlorogenic acid), quercetin-3-galactoside derivatives, catechins, and a high concentration of flavonoids. In experiments conducted by Proestos *et al.* (2008) was found that plant extracts of hawthorn have little impact against *S. aureus* and *Salmonella enteritidis* and do not inhibit the growth of *E. coli* O157: H7, *B. cereus* and *P. putida* (Aboaba *et al.*, 2008).

The evaluation of antibacterial activity of *Crataegus oxyacantha* showed that the tested bacterial strains of *E. coli*, *P. aeruginosa*, *S. aureus*, *Klebsiella pneumoniae* and *Proteus mirabilis* are sensitive to this plant extracts and the O-glycosides of flavonols were the main components responsible for its antimicrobial properties (Mercincak *et al.*, 2008).

In comparison to the previously discussed extracts, flowers of *Robinia pseudoacacia* showed a higher activity against *P. putida*. Aqueous extracts, in contrast to methanol and ethanol strongly inhibited the growth of *B. subtilis*.

In this study, the higher sensitivity of *E. coli*, *S. cerevisiae* and *P. myxofaciens* on aqueous extracts of this plant compared to methanol was found. Similar results were obtained by Zhang *et al.* (2008), they demonstrated the antibacterial effect of aqueous extracts of black locust against *Staphylococcus* and coli forms as well as antifungal properties against *Plasmopara viticola*.

Ethanol extracts of quince leaves did not act on filamentous fungi, while they influenced stronger than fruit extracts the growth of *S. cerevisiae* yeast and *S. marcescens*, *P. putida* and *M. luteus* bacteria. Coban

and Biyik (2010) obtained similar results in their experiments, their studies showed an effective inhibition of *Proteus* sp., *B. cereus*, *M. luteus* and *Enterococcus faecalis* strains growth (zone of inhibition was 7–13 mm).

The most sensitive for used plant extracts among analysed microorganism was yeast of *S. cerevisiae*. Other studied fungi were generally resistant for used extracts, only lingonberry and quince fruit extracts inhibited the growth of those yeasts. The tested fungal strains proved to be sensitive to amphotericin B. *S. cerevisiae* showed approximately two-fold zone of inhibition with respect to an antibiotic in combination with other microorganisms.

The analysed bacteria were characterized by the resistance to the action of vancomycin and amoxicillin with clavulanic acid. Vancomycin and amoxicillin together with clavulanic acid showed wide spectrum of action on bacterial cultures. We found various activity of these antibiotics against tested strains. Vancomycin was characterized by the highest activity against anaerobic *Bifidobacterium* sp. and *Clostridium* sp. bacteria, which can be connected with the inhibition of peptidoglycan synthesis – the main component of the bacterial cell wall (Wilhelm and Estes, 1999). In the case of amoxicillin with clavulanic acid, the tested microorganisms were not sensitive to the bactericidal activity of this antibiotic.

In this study, a negative control accounted for 80% vol. ethanol and methanol solutions. It has been shown that the type of a solvent had a large effect on the inhibition of the growth of tested microorganisms. Analysing the microbial inhibition zone it was found that ethanol was characterized by a higher effect relative to methanol, which showed lower antimicrobial activity. Based on the obtained results it was found that the alcoholic solvent present in the plant extracts tends to hinder the growth of microorganisms compared to the neutral water.

The results of this study allow concluding that the activity of the analysed species of medicinal plants against tested microorganisms is relatively small. Experiments should be continued enabling plants to increase the efficiency of inhibition of microbial growth including usage of their therapeutic properties in combination with chemotherapeutic agents.

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