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Antimicrobial activity of Cornelian cherry (*Cornus mas* L.)

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PRZECIWDROBNOUSTROJOWE DZIAŁANIE DERENIA

SUMMARY

A lot of Cornaceae family plants are used in a traditional medicine. Few studies described its antimicrobial activity, but only bark and fruit extracts were investigated so far. In our study ethanol or methanol extracts from different part of cornelian cherry: bark, fruits, leaves and seeds were used to evaluate their activity against 4 species of bacteria: Gram-positive – Staphylococcus aureus and Streptococcus pyogenes, Gram-negative – Escherichia coli and Pseudomonas aeruginosa and 3 species of fungi – Candida albicans, Aspergillus fumigatus, Trichophyton mentagrophytes. It was found that the highest antimicrobial activity obtained with disc-diffusion method revealed leaves and seeds extracts against S. aureus and C. albicans with diameters of inhibition zones between 10-15 mm. S. pyogenes and T. mentagrophytes were resistant to all extracts. In case of P. aeruginosa and E. coli the most effective was ethanol seed extracts. Our results show cornelian cherry as one from small know plants with interesting properties. This effect can be useful as new potential method of food protection before biological damage and also as new type of antibacterial and antifungal drugs.

KEY WORDS: CORNELIAN CHERRY – METHANOL AND ETHANOL EXTRACTS – ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

Introduction

Men by whole life meet from bacteria and fungi. One of them can be useful for peoples, another are unimportant and some of them are dangerous. They spoil food and may cause human and animal diseases. Therefore continually are searched new biological products which can brake or destroy their development, being simultaneously harmless for men. In the recent years have increased interest in research of antimicrobial activity of medicinal plants (1). But there are only a few studies on antimicrobial activity of cornelian cherry (*Cornus mas* L.) one from *Cornaceae* family plant focused only on bark and fruits of this plant (2, 3).

Cornelian cherry is a shrub or small tree of height from 3 to 8 m; it blooms very early – from February to March, fruits usually are ripened in September. The fruit is of reasonable size, up to 25 mm long, with a single large seed (*endocarp*). Fruit are used fresh, dried or in preserves. They are juicy, with a nice acid flavour. Roasted seeds are ground into a powder and used as coffee substitute. They are also suitable for oil extraction. The leaves are a good source of tannin. Cornelian cherry is widely used in Chinese and American Indian traditional medicine. The bark and fruits are used as astringent, febrifuge and nutritive. Fruits are good for bowel complaints and fevers, some authors pointed out their curative effect to cholera. The flowers are used against diar-

rhoea (4). Fruit extracts are also used for cosmetic purposes, replacing synthetic astringent substances, so they are claimed to exert a favorable action on the human complexion (5).

The food is the second target/place for antimicrobial substance. In the present moment with regard on demand on food with prolonged durability complies different methods of protection before growing of bacteria or fungi. Often applied synthetic agents are harmful for human especially they cause allergies (7, 8). From this reason seeks different additions to food which can eradicate bacteria and fungi being simultaneously harmless for men. Maybe substances isolated from cornelian cherry can be applied to food protection in future.

The aim of this study was to evaluate the antimicrobial potential of cornelian cherry.

Material and Methods

Biological materials and extracts preparation

All plant materials: fruits, seeds (September – October 2008), leaves (June 2008) and bark (February 2009) used in this experiment were obtained from experimental orchard in Garlica Murowana near the Cracow, Poland. This orchard is a property of Agriculture University of Cracow. Fruits were collected at the end of September and frozen at -20°C. Pulp was separated from seeds by manual crushing of defrosted fruits. After this, pulp was shaken with methanol or ethanol for 24 h, then centrifuged for 15 min at 5000 rpm and freeze drying until dry mass.

Seeds, leaves and bark were frozen with fluid nitrogen and powdered. Powder was shaken with methanol or ethanol for 24 h, then centrifuged for 15 min at 5000 rpm and freeze drying until dry mass.

Microorganisms and media

In this study four bacteria species were used, 2 Gram-positive and 2 Gram-negative: *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* and *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 respectively and 3 species of fungi: yeast – *Candida albicans* ATCC 90028, mould – *Aspergillus fumigatus* and dermatophyte – *Trichophyton mentagrophytes* ATCC 18748.

Each microorganism prior to the antimicrobial testing was cultured on suitable media: Blood Agar for bacteria and Sabouraud Glucose Agar for fungi. The disk diffusion test was made on Mueller Hinton Agar (Biocorp) supplied with 2% glucose and Mueller Hinton Agar supplied with 2% glucose and 5% sheep blood for *S. pyogenes*.

Agar disk diffusion method

Blank paper disks (6 mm in diameter, EMPOL, Poland) were impregnated with 20 μl of individual extracts in three concentrations of each extract: 10 mg/ml, 1 mg/ml and 0.1 mg/ml to obtain 200 μg, 20 μg and 2 μg of extract respectively per disk. The disks were allowed to dry at room temperature. The strain to be tested was initially suspended in a sterile distilled water to a turbidity matching 0.5 McFarland standard with densitometer DEN-1 (BioSan). The prepared inocula were swabbed onto the surfaces of Mueller Hinton Agar plates with the sterile swab, and left to dry at room temperature. Disks with extracts were placed manually onto inoculated agar plate. The plates were incubated in an inverted position under conditions appropriate for the organisms tested. The plates with bacteria were incubated at 37°C for 24 h, while for *Candida, Aspergillus* and *Trichophyton* at 27°C for 48 h, and in case of moulds for the moment in which fungal growth could be observed with naked eye: 5 and 7 days, respectively. The inhibition zones were recorded.

Results

Among examined microorganisms the most susceptible was *Staphylococcus aureus* strain. The six from eight investigated extracts were effective against it. Activity have shown, both methanol and ethanol extracts of seeds and leaves (zones 12 mm) and ethanol extract of bark (zone 9) and methanol extract of fruits (zone 8 mm). Activity against *P. aeruginosa* have shown five extracts (ethanol extract of seeds, leaves and bark – zone 10, 9, 7 mm respectively, and both methanol and ethanol extract of fruits – zones 7 and 8 mm respectively). The half of investigated extracts were active against *E. coli* and *C. albicans*. In the case of *E. coli* were ethanol extracts of seeds and leaves (zones 10, 7 mm respectively) and methanol and ethanol extracts of fruits (both 8 mm zones) and in the case of *C. albicans* effective were both methanol and ethanol extracts of seeds and leaves (zones 12, 10, 15, 12 mm respectively). *A. fumigatus* was susceptible only to methanol extracts of seeds. Resistant to all extracts were *S. pyogenes* and *T. mentagrophytes*.

We found that the broadest antimicrobial spectrum have shown ethanol extracts of seeds and leaves. They had activity against *S. aureus*, *E. coli*, *P aeruginosa* and *C. albicans*. In opposite, the methanol extracts

Table 1. Inhibition zone for bacteria (in mm, includes disk diameters – 6 mm).

Extracts (200 μg/disk)		*S. aureus*	*E. coli*	*P. aeruginosa*	*S. pyogenes*
Seeds	methanol	12	0	0	0
	ethanol	12	10	10	0
Leaves	methanol	12	0	0	0
	ethanol	12	7	9	0
Bark	methanol	0	0	0	0
	ethanol	9	0	7	0
Fruits	methanol	8	8	7	0
	ethanol	0	8	8	0
Control		Cefotaxime 30 μg	Amoxicillin with clavulanic acid 30 μg	Piperacillin 100 μg	Penicillin 10 μg
		15	22	32	35

Table 2. Inhibition zone for fungi (in mm, includes disk diameters – 6 mm).

Extracts (200 μg/disk)		*C. albicans*	*A. fumigatus*	*T. mentagrophytes*
Seeds	methanol	12	12*	0
	ethanol	10	0	0
Leaves	methanol	15	0	0
	ethanol	12	0	0
Bark	methanol	0	0	0
	ethanol	0	0	0
Fruits	methanol	0	0	0
	ethanol	0	0	0
Nystatin (control) 200 μg		24	12	16

*Microcolonies inside the growth inhibition zone.

of these cornelian's parts had only activity against *S. aureus* and *C. albicans*.

Extracts of bark and fruits have no antifungal properties also methanol extracts of bark have any activity to all investigated microorganisms.

Detailed data are shown in table 1 and 2.

Discussion

We have chosen the disk diffusion testing because it is simple, rapid and cost-wise method for susceptibility testing, especially in preliminary study for screening active extracts with unknown composition (9).

Many parts of cornelian cherry plant are applied in folk medicine. Fruits are used in the treatment of gout, anemia, skin diseases, painful joints, and disrupted metabolism. Fruit, leaves, or bark have been employed for gastrointestinal disorders and tuberculosis (10). From the other hand Cornelian cherry has generally been free of disease and pest problems itself, that suggest production by this plant the compounds which protect the plant against microorganisms (11). Despite of those facts there are only few articles about antimicrobial activity of cornelian cherry (2, 3).

In the Dulger and Gonuz study (2), ethanol extracts obtained from bark of cornelian cherry species were found to have inhibition zones aga-

inst *S. aureus*, *P. aeruginosa*, *Proteus vulgaris* and *Micrococcus luteus* of 8-10 mm. However, cornelian cherry was not effective against the other bacteria and the yeast cultures (*E. coli, Klebsiella pneumoniae, Bacillus cereus, Mycobacterium smegmatis, Listeria monocytogenes, C. albicans, Kluyveromyces fragilis, Rhodotorula mucilaginosa* (originally *R. rubra*)) (2). Our study confirmed activity of ethanol extracts of cornelian cherrys' bark against *S. aureus* and *P. aeruginosa*, especially that we used fifty-times lower concentrations per disk than authors mentioned above. We used 200 μg of extract per disk, while Dugler and Gonuz (2) in their study used concentration 10 mg of extract per disk. However, the high amounts of extracts in both studies and small inhibition zones suggest that this activity is not very strong. We also confirm resistance of *C. albicans* against methanol extracts of bark.

Krish et al. (3) investigated spectroscopically inhibition of growth in mixture 100 μl inoculum and 100 μl water or methanol pulp extracts or juice from cornelian cherry. In their study were used four bacterial species: Gram-positive – *Bacillus subtilis* and *B. cereus* and Gram-negative – *E. coli* and *Serratia marcescens*. We are able only compare results for *E. coli.* We obtain small inhibition zones for methanol extract which could be equivalent of growth inhibited to 25% of control growth obtained by Krish et al. (3). These data are difficult to compare, because there are no established methods for comparing the inhibition of growth between different methods of measurement: disk diffusion and inhibition of growth in liquid medium. The Krish et al. (3) studies show also total inhibition of growth in methanol extract for *Bacillus cereus* and *Serratia marcenses*.

In the other studies discussed above only bark and fruits of cornelian cherry plant were used. We found that those parts have lower antimicrobial activity than other used in our study: leaves and seeds.

Neither Dulger and Gonuz (2) study nor Kirsh et al. (3) study didn't investigated any moulds. We used clinically relevant species such *Aspergillus fumigatus* a major etiological agent of aspergillosis and *Trichophyton mentagrophytes* causes of dermatophytosis. We found that *T. mentagrophytes* are resistant to all investigated extracts.

In the case of *Aspergillus fumigatus* we observed inhibition zones for methanol extract of seeds but these zones was not clear (asterisked in table 2). There were few microcolonies in the zone. Although in nystatin control zones were clear. But some findings suggest that this type of growth with microcolonies inside the zone can occur i.e. caspofungin (12). We found that *Aspergillus fumigatus* was susceptible to methanol extract of seeds.

Effectiveness of leaf and seeds extracts against *S. aureus* and *C. albicans* seems to be promising. Especially, that these species in the recent years have increased their resistance to commonly used drugs i.e. methicillin resistant strains of staphylococci (13) and fluconazole resistant *C. albicans* (14).

Our studies show interesting antimicrobial activity of cornelian cherry's stones. For fuller recognition of substance/-s being in extract should execute the recognition of chemical structure of active substance and determine the minimal inhibitory concentration of them via microdilution method, which are planed now.

Conclusions

Our results show cornelian cherry as one from small know plants with interesting properties. This is supported by the fact that alcoholic extract from seeds and leaves have higher antimicrobial activity for *S. aureus, E. coli, P. aeruginosa and C. albicans* than bark and fruits from this plant. This effect can be useful as new potential method of food protection before biological damage and also as new type of antibacterial and antifungal drugs.

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