Antimicrobial Activity of Different Extracts from Rhizome and Root of *Potentilla erecta L.* Raeuschel and *Potentilla alba* L. *Rosaceae*⋆

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The tested plant material of rhizome with roots of *Potentilla erecta* (L.) Raeuschel and *Potentilla alba* L. was collected in 2003. Determination of the total phenolics content, non-tannin phenols was conducted by applying the method of Folin-Ciocalteau reagent and proanthocyanidin content by Porter. The method used for determination of the antibiotic activity was used in accordance with the European Pharmacopoeia (procedure 2.7.2.) on medium A and bacteria *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739 and *Candida albicans* ATCC 10231. The water, ethyl acetate, acetone and ethanol extracts, prepared earlier, were tested. Medium A was used for testing *Staphylococcus aureus* and *Escherichia coli* while Medium F was used for testing Candida *albicans*.
The values obtained after testing phenol compounds (% on dry plant material) are as follows: the value for *Potentilla erecta*: total phenolics 16.90%, Non-tannin phenolics 0.09%, Proanthocyanidins 2.70% while the value for *Potentilla alba*: total phenolics 11.74%, Non-tannin phenolics 0.71%, Proanthocyanidins 2.73%. The ethanol and acetone extracts have the antimicrobial effect on *Escherichia coli*, ethyl acetate extract of rhizome *Potentilla erecta*, while water extracts of both tested species in dissolution 1:20 have the antimicrobial effect on *Staphylococcus aureus*. The tested species have not had any effect on Candida *albicans* fungus.

Key words: Plants, Medicinal; Potentilla; Rosaceae; Anti-Bacterial Agents; Microbial Sensitivity Tests .

Introduction

Following the previous research on the antimicrobial activity of plant sorts *Potentilla*, belonging to the family of Rosaceae, the present study is a continuation of further examination of the antimicrobial activity of those plants (1, 2). The testing of the antimi-

crobial activity of the higher plants is topical both in respect of finding a rational replacement for the existing antibiotics but also due to a resistance of pathogen bacteria to antibiotics after a long usage (2, 3, 4).

Material and methods

The plant material (rhizome and root) was collected in Bosnia and Herzegovina during September 2003 (in the vicinity of small towns of Olovo and Han Pijesak). The picked plants were cleaned, washed and dried in thin layers protected from the direct sun light. The dried plant material was kept in paper containers. Prior to the experiment the material was cut and pulverized.

Extracts preparation

Acetone extract and ethanol extract: The extraction of the fresh pulverized rhizome and roots (*Potentilla erecta* and *Potentilla alba*) was done by using the 70 % acetone or by 70% ethanol in the course of 24 hours at the temperature + 4 °C with periodical mixing. One part of rhizoma and root was extracted with 10 parts of the solvent. After extraction the material was separated from the extract by filtration and rinsed by a double quantity of the solvent. The obtained extract was evaporated to dryness at a lowered pressure and temperature below 35 °C. If necessary, it was kept in the inert atmosphere until usage. Prior to examination the extracts were dissolved in dimethyl sulphoxide p.a. in the same volume as the initial plant material mass. The antimicrobial activity of the dimethyl sulphoxide on the examined bacterial strains was not noticed.

Ethyl acetate extract: One part of the pulverized rhizome and root was poured over by ten parts of water at the room temperature and extracted in the ultrasonic mixer in 30 minutes. The powder was separated from the extract by filtration and rinsed with one part of the water. The obtained water extract was extracted three times by the equal vol-

Figure 1. *Potentilla erecta* (L.) Raeuschel (5)

Figure 2. *Potentilla alba* L. (6).

ume of ethyl acetate. The ethyl acetate extracts were joined while water was removed by filtration over anhydrous sodium sulfate. The ethyl acetate extract was evaporated at a lower pressure in rotavapour at the temperature up to 40 °C. After that, the dry extract was suspended in the same water volume as the initial pulverized plant material mass in order to be deposited on the microbiological base.

Antimicrobial activity examination

A diffusion method according to the European Pharmacopoeia edition 5 (Ph. Eur. ed 5) was used as a method for the examination of antibiotic activities (7). The choice of the method was based on its simplicity and widespread application, but also because it enables us to compare the obtained results with others.

The culture media for examination of Bacillus subtilis ATCC 6632 was of the following composition: Peptone 5 g, Meat extract 2,4 g, Agar 15 g, Purified water up to 1000 g (8).

The culture media for the examination of Staphylococcus aureus ATCC6538, Staphylococcus epidermidis ATCC 122228 and Escherichia coli ATCC 8739 was the culture media A for the examination of antibiotics by a diffusion method (8).

Density of inoculums:
Staphylococcus aureus ATCC6538 T= 80%
Escherichia coli ATCC 8739 T= 60%
Bacillus subtilis ATCC 6633 T=30%
Candida albicans ATCC 10231 T=80%

Spectrophotometric determination of phenolics

The phenolic analysis started with the plant material crushing and its extraction (9). In case when phenolic compounds were difficult to solve, the examined material was treated by hydrolysis during the process of extraction in order to get soluble compounds which were analyzed as derivates.

To examine this group of phenolic compounds the spectrophotometric method was used with Folin- Ciocalteu reagent because of the simplicity and selectivity of reagent to the phenol group (9). The obtained results of the examination with Folin- Ciocalteu can be termed as the tannin index because tannin is a standard in this kind of examination.

In the present study we used the method of total phenolics determination in the plant material, phenolics determination in detanninized extract after removing tannin with polyvinyl polypyrrolidone (PVPP). This method was accepted by FAO organization in 2000 as a standard method for tannin determination (7, 9).

Sample preparation: Extraction solvent: acetone diluted with purified water to 70% (v/v) concentration. After grinding, the plant material (IKA Universal mule M 20) was removed into a flask and poured over by the solvent with periodical stirring. The material was left to stay at the temperature of 4 °C for 24 hours.

Afterward, further material processing for the purpose of analysis was carried out.

This method for tannin determination can be used with the insoluble matrix, polyvinyl polypyrrolidone (PVPP, binding tannin) (9). The obtained values can be expressed as a tannin equivalent. The nature of commercial tannin differs from sample to sample. In our examination we used Acidum tannicum p.a. Kemika, Zagreb.

Determination of the proanthocyanidin content

Determination of the proanthocyanidin content was done according to Porter (by vanillin method) in the following way: 1 ml of the water extract (one part of the plant material and 20 parts of purified water) was mixed with 2 ml of the freshly prepared vanillin

solution (1 g vanillin/ 100 ml 70% H_2SO_4) and kept for 15 minutes at the temperature of 20 °C (10). Absorption was measured at 500 nm (10).

Sample preparation: The powdered rhizoma and root (0.500 g) was poured over by 10 ml of water and extracted in 30 minutes in the ultrasonic mixer. After extraction, the plant material was separated from the extract and water was added up to 10.00 ml. The quantity of the 0.20 ml water extract was diluted to 1.00 ml with water. This was made in order to enable the measurement reading of absorption.

Standard preparation: 2, 50 mg of the catechin is dissolved in water and water is added up to 10 ml.

Results

The results of the spectrophotometric determination of total phenolics (Table 1) and proanthocyanidins (Table 2) have shown the similarity in respect of contents.

The results of the antimicrobial activity are presented in Tables 3, 4 and 5.

Table 1. Total phenolics content in the examined *Potentilla* calculated on dry material

Rhizome and root	Total phenolics (%) acetone extraction	Total phenolics (%) ethanol extraction	Phenolics after tannin removal (%)
Potentilla erecta	17.56	16.90	0.09
Potentilla alba	14.10	11.74	0.71

Table 2. Proanthocyanidin content in the examined Potentilla species

Rhizome and root of	% Catechin
Potentilla erecta	2.70
Potentilla alba	2.73

Table 3. Results of antimicrobial activity examination of water extracts

Plant material	Concentration of water root and rhizome extract	Zone of inhibition in mm		
		Staphylococcus aureus ATCC 6538	*Bacillus subtilis* ATCC 6633	*Candida albicans* ATCC 10231
Potentilla erecta L.	1:10	10.7	0	0
Potentilla erecta L.	1:20	8.3	0	0
Potentilla erecta L.	1:30	0	0	0
Potentilla alba L.	1:10	11.0	0	0
Potentilla alba L.	1:20	8.6	0	0
Potentilla alba L.	1:30	0	0	0
Neomycin sulfate	20µg/ml	20	20	0

Legend: 0 = no activity

Table 4. Results of antimicrobial activity examination of ethyl acetate extracts.

Plant material	Concentration of water extract	Zone of inhibition in mm	
		Staphylococcus aureus ATCC 6538	*Bacillus subtilis* ATCC 6633
Potentilla erecta	1:1 ethyl acetate extract	12.6	0
Potentilla alba	1:1 ethyl acetate extract	0	0
Acidum tannicum	2% solution	12.6	0

Legend: 0 = no activity

Table 5. Results of antimicrobial activity examination of ethanol and acetone extracts

Plant material	Concentration of extract	Zone of inhibition in mm	
		Staphylococcus aureus ATCC 6538	*Escherichia coli* ATCC 8739
Potentilla erecta	1:1 acetone extract	*	18.3
Potentilla erecta	1:1 ethanol extract	*	17.7
Potentilla alba	1:1 acetone extract	13.4	15.9
Potentilla alba	1:1 ethanol extract	*	14.7
Acidum tannicum	2% solution	12.6	18.9

Legend: * = no examination performed

Discussion

The obtained results show that total phenolics content is different in the examined species (11). The antimicrobial activity examined on agar by applying a diffusion method has shown that acetone and ethanol extracts differ with regard to the strength of the microbiological response while the extraction of phenolic compounds is better (more quantitative) if the 70% concentration acetone is used instead of ethanol of the same concentration. This fact has been confirmed by the results obtained in other researches on the extraction of phenolics in different plant materials (12, 13, 14, 15 and 16).

The obtained results of those examinations have contributed to the knowledge of the analytics of the *Potentilla* plant sorts, as well as to our knowledge of their antimicrobial activity.

Conclusions

The antimicrobial activity of the examined samples of the plant sorts *Potentilla erecta* and *Potentilla alba* is similar. The choice of solvents such as the 70% acetone has confirmed the predictions in respect of quantitative extraction of the active constituents (phenolics) and a slightly stronger antimicrobial activity in relation to ethanol extract. The methods used have proven to be suitable and they have given reproducible results.

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