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Antimicrobial effect of an aqueous extract of *Potentilla erecta* rhizome

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Summary

Potentilla erecta is a therapeutic plant used in folk medicine to treat inflammatory states, wounds and diseases of the alimentary tract. The results of the study reveal the effects of an aqueous extract of *P. erecta* rhizome on certain microorganisms occurring in food. The main components of the extract were catechins. The extract was shown to display an inhibiting effect against Gram-positive bacteria such as *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633, as well as against yeast such as *Candida lipolitica* KKP 322 and *Hansenula anomala* R 26. The extract did inhibit the growth of Gram-negative bacteria, however, no inhibiting effects were observed on moulds in the studied range of concentrations, i.e. 0.13 to 64 mg dry matter/ml.

Key words: *antimicrobial, Potentilla erecta, water extract*

INTRODUCTION

Diseases induced by food- and water-borne bacteria have been considered as a severe global problem [1]. Moreover, many microorganisms cause food spoilage, problematic both to consumers and food industry. Hence, novel natural compounds exhibiting bactericidal effects have drawn increasing interest worldwide. Extracts from seasoning plants and herbs exhibiting such properties are used traditionally and commercially to extend the shelf life of food products and to improve food safety [2]. Many authors attribute the anti-bacterial properties of plant extracts to components such as phenolic compounds, tannins, catechins and aldehydes [3]. A natural combination of these compounds displays greater antimicrobial effects as compared to its individual components, which are attributed to synergistic interactions [1].

P. erecta is a therapeutic plant that belongs to the *Rosaceae* family. Plants of this family occur in Northern hemisphere in the zones of moderate and arctic climate as well as in Alpine areas. *P. erecta* is a very common plant, occurring especially on meadows, in bushes and in forests. Hence, for therapeutic purposes, it is acquired from natural habitats [4,5]. In folk medicine, extracts from its roots are applied to treat inflammatories, wounds and diseases of alimentary tract. It is also used as an antiseptic agent especially in the therapies of oral cavity and throat ailments [6]. Results of recent clinical surveys have shown that extracts from *P. erecta* rhizome may be regarded as a safe bactericidal agent in treating acute toxicity in humans [7].

So far, 43 chemical compounds have been identified in *P. erecta* rhizome. The largest group is represented by tannins, the total content of which varies from 17 to 22%. They include mainly condensed tannins (5–20%) and hydrolyzing tannins occurring in significantly smaller quantities (3.5%) [5, 8]. The rhizome of this plant has also been reported to contain a few precursors of condensed tannins, including (+)-catechins, (-)-epicatechins, and (+)-gallocatechins. Their typical components are also triterpenoids, including tormentoside (rosamultin) [9]. Other components occurring in minor concentrations are flavonoids, including mainly organic and polycarboxylic acids (3,4-dihydroxy-benzoic acid, gallic acid, *p*-coumaric acid, salicylic acid, syringic acid and caffeic acid) [10, 11, 5]. Even early reports have demonstrated the antibacterial effects of extracts from *P. erecta* rhizome, with the effects being attributed mostly to condensed and hydrolyzing tannins. These compounds have been reported to display cytotoxic activity against *Herpes* virus types I and II as well as against *Influenza* virus type A2 and *Cowpox* virus [12, 13, 5, 14]. Moreover, clinical surveys have proved that the extract from *P. erecta* rhizome appeared to be effective in the treatment of diarrhea caused by rotavirus in children [15]. Recent investigations were mainly focused on the analyses of extracts from the aerial parts of plant of the genus *Potentilla* and have demonstrated that the extract is highly efficient in inhibiting the cariogenic activity of the bacteria occurring in the oral cavity of man [16].

Till now, several researchers have been focused on the influence of the extracts of the underground parts of *Potentilla erecta* on the inhibition of the growth of microorganism in food. For this reason, authors decided to examine the antibacterial properties of an aqueous extract from *P. erecta* rhizome against typical microorganisms inducing food spoilage and food poisoning in humans. Further analyses should be carried out to determine the contents of the active compounds, including tannins, polyphenolic compounds and precursors of condensed tannins.

MATERIAL AND METHODS

Plant material

The plant material originated from barren lands in Koryciny-Borki village (Podlasie region, Poland), located 122 m above the sea level (N 52°39,412' E 022° 45,987'). The fresh weight of the rhizome accounted to 16.89 g/plant, and after drying, the weight was found to be 6.71 g/plant (mean of 20 plants).

DETERMINATION OF THE TOTAL CONTENT OF TANNINS IN RHIZOMES

The total content of tannins in rhizomes was determined after their extraction using the spectrophotometric method following the procedure described in Polish Pharmacopoeia VI [17]. Two grams of finely-powdered plant material were dissolved in 250 ml of water. Five milliliters of the filtrate (I) were diluted in 25 ml water. Two milliliters of this solution were then transferred to a 25-ml flask, to which 1 ml of 2N Folin-Ciocalteau reagent and 10 ml of water were added. Finally, the sample was filled up with 10.6% solution of sodium carbonate. The absorbance was measured after 30 minutes at a wavelength of 760 nm (A_1). Water was used as the blank. In order to determine non-binding polyphenols (NAP), 10 ml of the filtrate (I) were mixed with 100 mg of hide powder (Merck, Germany) and shaken for 60 minutes. Two milliliters of this solution were then sampled and the concentration of the polyphenols was determined as described above. The results of absorbance measurement at a wavelength of 760 nm were denoted as (A_2). Shortly before the determination, 0.05 g of pyrogallol was dissolved in water and filled up with water to the volume of 100 ml. Then, 5 ml of the resultant solution were filled up with water to the volume of 100 ml. Next, 2 ml of the resultant solution were transferred to a 25-ml flask, to which 1 ml of 2N Folin-Ciocalteau reagent and 10 ml of water were added. Finally, the sample was filled up with 10.6% solution of sodium carbonate. The absorbance was measured after 30 minutes at a wavelength of 760 nm (A_3), using water as a blank. The percentage content of total tannins (TT) was computed per pyrogallol ($C_6H_6O_3$) according to the formula:

$$TT\ (\%) = 62.5 \times (A_1 - A_2) \times m_2/A_3 \times m_1,$$

where m_1 – mass of plant material [g], m_2 – mass of pyrogallol [g].

Determination of total content of polyphenolic acids in rhizomes

The content of total polyphenolic acids, in rhizomes after their extraction was determined with use of spectrophotometric method using the Arnov's reagent, according to the procedure described in Polish Pharmacopoeia [17]. One gram of powdered rhizomes of *P. erecta* was mixed with 25 ml of water, shaken for 30 minutes and filtrated. The filtrate was collected in a 50-ml measuring flask. The pre-extracted sample was poured again with the same volume of shaken and filtrated water. The volume of the resultant extract was made up to 50 ml with distilled water and then mixed. Then, a mixture was prepared of 1 ml of the investigated extract, 5 ml of water, 1 ml of hydrochloric acid (18 g/l), 1 ml of Arnov reagent, 1 ml of sodium hydroxide solution (40 g/l), and 10 ml of distilled water. Phenolic acids were assayed spectrophotometrically at $\lambda = 490$ nm. Their percentage content expressed per caffeic acid in dry matter was computed using the following formula:

$A \times 1.7544/m$,

where A – absorbance of the analyzed solution, m – mass of a powdered sample of plant rhizome [g].

Three replicate analyses were carried out.

Determination of content of catechins in rhizomes

One gram of rhizome dry matter was extracted with 100 ml of ethanol in Büchi B-811 Extraction System (Switzerland) in 25 cycles. After the evaporation of the solvent, the sample was dissolved in 10 ml of methanol and filtrated through a Supelco IsoDisc PTFE filter (25 mm x 0.45 μm). The resultant filtrate was analyzed for the content of catechins using a Shimadzu HPLC system (Shimadzu Corp., Japan) composed of: an LC–10AD pump, an SPD-M 10A VP diode detector and an SIL-20A autosampler. The separation was conducted using a Luna C18(2) column (250 x 4.6, 5 μm) (Phenomenex, USA). Gradient elution of 10% acetonitrile (ACN) (mobile phase A) and 55% ACN (mobile phase B) (LabScan, Poland) in water was performed at pH 3.0, flow rate 1 ml/min and temperature 30°C. Peaks were identified by comparison of retention time and spectral data with those of standards purchased from ChromaDex (USA). The signal from the diode detector was recorded in the form of a series of chromatograms in a wavelength range of 190–900 nm. Results were integrated at the wavelength corresponding to the maximum absorbance of the individual compounds using CLASS VP 7.3 software (Shimadzu Corp., Japan).

In the case of (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and epigallocatechin (-)-gallate the wavelength corresponded to 206 nm, whereas in ellagic acid the wavelengths corresponded to 254 nm. Contents of the individual biologically active compounds were expressed in mg per 100 g d.m. of rhizomes.

Preparation of concentrated extract from rhizomes of *Potentilla erecta*

Extracts from *P. erecta* rhizomes were prepared using the method of continuous exhaustive extraction in the Büchi B-811 Extraction System (Switzerland). The plant material was extracted with water in 15 cycles, and the boiling point of the solvent was maintained. The resultants crude extracts were filtered through a blotter paper filter and evaporated in a Rotovaporator R-205 Büchi (Switzerland). The following temperatures were applied: heating bath 60°C, condensate 40°C and cooling water 20°C. The subatmospheric pressure reached 72 mbar (7.2 kPa). The extract was concentrated to the weight equal to 0.56 g d.m./ml. The results were expressed in mg per 100g d.m. of the extract.

Microorganisms strains

The following reference standard strains were used in the study: Gram-positive bacteria - *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923; Gram-negative bacteria - *Escherichia coli* ATCC 25922, *Salmonella* Enteritidis ATCC 13076; as well as fungi - *Penicilium expansum* ATCC 7861 and *Aspergillus niger* ATCC 9142, obtained from the American Type Culture Collection. Other strains used in the study included *Proteus mirabilis* 14a obtained from the collection preserved at the National Institute of Public Health and National Institute of Hygiene (Warsaw, Poland), *Candida lipolytica* KKP 322 and *Hansenula anomala* R 26 were obtained from the collection preserved at the Institute of Agricultural and Food Biotechnology (Warsaw, Poland). The bacterial strains were cultured on nutrient agar medium, and fungi on Sabouraud agar medium. All strains were stored at 4°C.

Determination of antimicrobial properties

The antimicrobial activity of the extract from *P. erecta* rhizome was assayed using the macrodilutions method [18, 19, 20]. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) or minimal fungicidal concentration (MFC) were also determined.

Double series of extract dilutions (0.13 to 64 mg d.m./ml) was prepared in Mueller-Hinton broth (Merck, Poland) for bacteria and on Sabouraud broth (BTL,

Poland) for fungi. In addition, a negative control (culture medium and inoculum) was additionally prepared for each experimental series. Finally, each test tube contained 2 ml of the culture medium with appropriate concentration of the analyzed extract and 0.1 ml of inoculum. The bacterial inoculum introduced to each test tube originated from 18–20-h cultures (10^7 cfu/ml), whereas the inoculum of yeast originated from 48-h cultures (10^7 cfu/ml). The inoculum of moulds was a suspension of spores prepared in physiological saline (10^7 spores/ml) from 21-day culture. Bacteria were incubated at a temperature of 37°C ± 1°C for 18–20 h, whereas fungi at a temperature of 28 ± 1°C for 48 h. The growth of microorganisms in each test tube was observed and compared with negative control. It is interpreted that the absence of the growth of a strain is attributed to the bactericidal or fungicidal activity of the extract. The minimal bacteriostatic concentration (MIC) was determined as the lowest concentration of the tested extract that inhibited the visible growth of microorganisms. The minimal bactericidal concentration (MBC) or minimal fungicidal concentration (MFC) was determined by inoculating 100 μl from each test tube that showed no visible growth of strains and from one test tube that showed visible growth of strains on Mueller-Hinton agar (BTL, Poland) in the case of bacteria and on Sabouraud agar (BTL, Poland) in yeast and moulds. MBCs and MFCs were defined in terms of minimal amount of extract that reduced microorganisms' growth down to 50 CFU corresponding to a 99.9% reduction of microorganisms.

RESULTS

The antibacterial activity of plant extracts depends mostly on their chemical composition which, in turn, is affected by multiple factors including among other parts of the plant used for extract preparation, method and conditions of plant cultivation as well as extraction method. The rhizome of *P. erecta* analyzed in our study was found to be characterized by high contents of tannins (21.3%), polyphenolic acids (3.84%) and of a few precursors of condensed tannins (tab. 1). In the aqueous extract obtained from the rhizome, contents of those compounds were several times higher, with the highest concentrations observed for catechin and epicatechin, i.e. 5.32 and 5.87 g/100g, respectively. The extract also contained significantly higher quantities of epigallocatechin gallate, epigallocatechin and epicatechin gallate compared with the rhizome of *P. erecta*.

Table 2 presents results of the analysis of the antimicrobial activity of the aqueous extract from *P. erecta* rhizome against test strains representing selected foodborne pathogens and saprophytes contaminating food. The extract showed a high bacteriostatic and bactericidal activity against Gram-positive bacteria i.e., *S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633. The extract's MIC value against *S. aureus* ATCC 25923 appeared to be the lowest and reached 1 mg/ ml. A two-fold higher value of MIC was noted against *B. subtilis* ATCC 6633, i.e. 2 mg/ml. In addition,

the analyzed extract was observed to exhibit bactericidal activity against those bacteria. However the MBC values were significantly higher than the MIC values and ranged from 8 to 32 mg/ml, respectively. The growth of the Gram-negative bacteria, i.e. *E. coli* ATCC 25922, *S.* Enteritidis ATCC 13076 and *P. mirabilis* 14a, was not inhibited by the aqueous extract from *P. erecta* rhizome in the investigated range of its concentrations.

Table 1.

Contents of identified active components in rhizome of *P. erecta* and in the aqueous extract obtained from the rhizome

Components	Rhizome	Aqueous extract of rhizome
	[g/100 g]	
Catechins	21.3	
Polyphenolic acids	3.84	
Ellagic acid	0.31	–
(+)-Catechin	1.18	5.32
(-)-Epicatechin	0.70	5.87
(-)-Epicatechin gallate	–	0.06
(-)-Epigallocatechin	0.05	0.20
(-)-Epigallocatechin gallate	0.08	1.36

Table 2.

MICs and MBCs/MFCs of the aqueous extract from *P. erecta* rhizome against the test microorganisms

	Microorganisms	MIC	MBC/MFC
		[mg/ml]	
Bacteria	*Staphylococcus aureus* ATCC 25923	1	8
	Bacillus subtilis ATCC 6633	2	32
	Escherichia coli ATCC 25922	> 64	–
	Salmonella Enteritidis ATCC 13076	> 64	–
	Proteus mirabilis 14a	> 64	–
Fungi	*Candida lipolitica* KKP 322	1	> 64
	Hansenula anomala R 26	16	> 64
	Penicillium expansum ATCC 7861	> 64	–
	Aspergillus niger ATCC 9142	> 64	–

– *not determined*

Out of the fungi examined, the most susceptible to aqueous extract from *P. erecta* rhizome turned out to be the yeast strain *C. lipolitica* KKP 322. The extract's

MIC value was found to be 1 mg/ml. The second most susceptible of the analyzed yeast strains *H. anomala* R 26, was inhibited by significantly higher concentration of the extract, i.e. 16 mg/ ml. In turn, neither inhibiting nor fungicidal effects of the extract were observed against test strains of moulds, i.e.: *P. expansum* ATCC 7861 and *A. niger* ATCC 9142, in the studied range of concentrations.

DISCUSSION

Plants of the *Potentilla* genus have been in an interest of scientists for years due to vast availability of plant material resulting from the expansive growth of these plants in regions of Northern hemisphere as well as to the abundance of biologically active chemical compounds occurring in their aerial and underground parts [5]. In the European folk medicine, extracts from *P. erecta* rhizome are recommended, among others as a means to alleviate symptoms of food poisonings [6]. The reported study attempted to determine their efficacy in inhibiting the growth of selected foodborne Gram-positive and Gram-negative bacteria as well as selected food-contaminating fungi, likely to induce food poisonings in humans and to cause food spoilage [21]. The results obtained indicate that the aqueous extract from *P. erecta* rhizomes inhibited the growth of selected Gram-positive bacterial strains and yeast strains; however it neither displayed bacteriostatic and bactericidal activity against the Gram-negative bacteria, i.e. *E. coli* ATCC 25922, *S.* Enteritidis ATCC 13076 and *P. mirabilis* 14a, nor fungistatic and fungicidal activity against the analyzed strains of moulds. Similar findings were reported by Tomczyk et al. [22] who investigated the antimicrobial activity of extracts prepared from eleven different species of plants of the *Potentilla* genus. Out of these, the extract prepared from the aerial parts of *P. erecta* exhibited a low antimicrobial activity and even at a concentration of 100 mg/ml it did not inhibiting growth of not only Gram-negative bacteria, i.e. *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, fungi *Candida albicans*, but also of Gram-positive bacteria, i.e. *S. aureus* and *B. subtilis,* which were effectively inhibited by the extract obtained from rhizome that has been analyzed in present study. In turn, a strong inhibiting effect of the extract obtained from the aerial parts of the plant was observed against *Helicobacter pylori* at a concentration of 0.5 mg/ml. A research by Funatogawa et al. [23] demonstrated that *H. pylori* was susceptible especially to epigallocatechin gallate – a component occurring both in the aerial and underground parts of *P. erecta*. Grujić-Vasic et al. [24] investigated the antimicrobial properties of different extract of *Potentilla erecta*. The water extract showed only activity against *Staphylococcus aureus* ATCC 6538, however, did not have antimicrobial properties against *Bacillus subtilis* ATCC 6633 and *Candida albicans*.

A subsequent study, by Tomczyk et al. [16] showed that although the aqueous extracts from the aerial parts of different *Potentilla* species (including *P. erecta*) were found to show intermediate inhibiting activity on the growth of oral

cariogenic streptococci, all the extracts were found to display strong inhibiting effects on biofilm formation by these bacteria on dental plaque as well as on the synthesis of mutan (a water-insoluble glucan). These effects were found to intensify in proportion to polyphenol content in the extracts examined (i.e. contents of tannins, including proanthocyanidins and phenolic acids, and flavonoids). The crude ethanolic extract obtained from *P. erecta* rhizomes was also found to show similar effects, which not only displayed intermediate inhibiting activity on the growth of cariogenic bacteria *Streptococcus sobrinus/downei*, but also strongly suppressed the activity of their glucosyltransferases (GTFs), a virulence factor of these bacteria in oral cavity, and their capability to synthesize mutan as a result of which the bacteria lost their ability to adhere to hard surfaces of teeth [25].

CONCLUSION

The results of present study suggest that the aqueous extract from *P. erecta* rhizome is characterized by high concentrations of tannins, polyphenolic acids and catechins. The extract is capable to prevent the growth of selected microorganisms which induce food poisoning in human and also cause food spoilage. Its actions are attributed to the bacteriostatic and bactericidal activity against Gram-positive bacteria and its inhibiting activity on the growth of selected yeast strains.

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PRZECIWDROBNOUSTROJOWE DZIAŁANIE WODNEGO EKSTRAKTU Z KŁĄCZA *POTENTILLA ERECTA*

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S t r e s z c z e n i e

Potentilla erecta jest rośliną leczniczą wykorzystywaną w medycynie ludowej jako środek do leczenia stanów zapalnych, ran i chorób przewodu pokarmowego. W pracy przedstawiono wyniki dotyczące działania wodnego ekstraktu z kłącza *P. erecta* na wybrane drobnoustroje występujące w żywności. Głównymi czynnymi składnikami ekstraktu były katechiny. Ekstrakt ten wykazywał działanie hamujące w stosunku do bakterii Gram dodatnich: *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 oraz drożdży *Candida lipolitica* KKP 322, *Hansenula anomala* R 26. W badanym zakresie stężeń 0,13 do 64 mg suchej masy/ml ekstrakt nie wykazywał aktywności przeciwko bakteriom Gram ujemnym oraz pleśniom.

Słowa kluczowe: *aktywność przeciwdrobnoustrojowa, Potentilla erecta, ekstrakt wodny*