ANTIMICROBIAL ACTIVITY OF AQUEOUS EXTRACTS OF POTENTILLA REPTANS L. RHIZOME AND AERIAL PART

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ANTIMIKROBNA AKTIVNOST VODENIH EKSTRAKATA NADZEMNOG DELA I RIZOMA POTENTILLA REPTANS L.

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ABSTRACT

Potentilla reptans is a little studied plant of the genus Potentilla, the family Rosaceae. The aim of this study is to determine antimicrobial effects of aqueous extracts of P. reptans aerial part and rhizome against standardized bacterial strains.

The antimicrobial activity of aqueous extracts of P. reptans aerial part and rhizome was tested against one fungus, Candida albicans, and two standard bacterial strains, Staphylococcus aureus and Escherichia coli, using an agar diffusion method.

Both examined extracts showed a significant antimicrobial activity against Escherichia coli and Staphylococcus aureus at the concentrations of 10 to 150 mg/ml. The rhizome extract showed stronger antimicrobial effect against the tested strains of bacteria than the aerial part extract.

The obtained results represent preliminary results of antimicrobial activity of this plant and suggest that in future, the studies should examine antimicrobial activity against other bacterial strains and minimum inhibitory concentration.

Keywords: *Potentilla reptans; Antimicrobial effect; Agar diffusion method.*

SAŽETAK

Potentilla reptans je malo istraživana biljka roda Potentilla familije Rosaceae. Cilj ove studije je utvrđivanje antimikrobnih efekata vodenih ekstrakata herbe i rizoma P. reptans na standardizovanim bakterijskim sojevima.

Antimikrobna aktivnost vodenih ekstrakata herbe i rizoma Potentilla reptans je ispitivana na jednu gljivicu Candida albicans i dva standardna bakterijska soja Staphylococcus aureus i Escherichia coli korišćenjem agar-difuzione metode.

Oba ispitivana ekstrakta su pokazala da u koncentracijama od 10 – 150 mg/ml ispoljavaju značajnu antimikrobnu aktivnost prema Escherichia coli i Staphylococcus aureus. Ekstrakt rizoma je pokazao jači antimikrobni efekat nego herba kod oba ispitivana bakterijska soja.

Dobijeni rezultati predstavljaju preliminarne rezultate o antimikrobnom delovanju ove biljke i ukazuju da buduća istraživanja mogu da idu u smeru ispitivanja antimikrobnog delovanja na drugim bakterijskim sojevima, kao i utvrđuvanje minimalne inhibitorne koncentracije.

Ključne reči: *Potentilla reptans; Antimikrobno delovanje; agar difuziona metoda.*

ABBREVIATIONS

P.rep-a - Potentilla reptans aerial part **P.rep-r** – Potentilla reptans rhizome

INTRODUCTION

Potentilla reptans L. (*P. reptans*) is one of three hundred *Potentilla* species belonging to the genus *Potentilla*, the family Rosaceae. The genus *Potentilla* is mostly characterized by perennial, rarely biennial or annual herbaceous plants (1). *P. reptans* is a perennial herbaceous plant with an erect rhizome. The stem is herbaceous, thread-like, creeping, up to 100 cm long, and the leaves are palmately five or seven lobed. It is usually found near the shores, in wet and flood meadows (2). The rhizome and aerial part of this plant are used in traditional medicine in the treatment of rheumatism, scabies, diarrhea, viral infections and as a remedy for wound-healing detoxification or internally

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in jaundice and dysentery (1). Studies that have examined the pharmacological characteristics of *P. reptans* aerial part proved its antioxidant and anti-ulcer activities (3, 4). Anti-inflammatory effect of the rhizome and aerial part was evaluated and proven by experimental mouse ear edema model (5). The results proved the presence of following compounds (Chinic acid, Caffeic acid, Protocatechuic acid, Luteolin-7-O-glucoside, Quercetin-3-O-glucoside, Rutin, Quercetin, Kaempferol-3-O-glucoside, Apigenin-7-O-glucoside) in the aerial part of *P. reptans L.* and Catechin as a dominant compound in the rhizome of this plant, as well as the presence of Chinic acid, Gallic acid, Protocatechuic acid, Epicatechin, Quercetin (5).

A large number of *Potentilla* species showed moderate to high antimicrobial activity. Antimicrobial activity was demonstrated against *Streptococcus mutans* and *Streptococcus sobrinus,* while moderate antibacterial activity was observed against *Staphylococcus aureus* and *Bacillus substilis,* and there is no such activity or it is very weak against *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (1). A study showed antimicrobial activity of *P. reptans* aerial part and rhizome against gram-positive *Staphylococcus aureus* and *Bacillus subtilis* and gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* bacterial strains (6).

The aim of our study is to justify the use of this plant as antimicrobial agent in traditional medicine.

METHODS

Plant material

The aerial parts of *P. reptans* were collected from May to August 2010, and rhizome in October of the same year. Plant material was dried for two weeks (in a windy, shady place). Before preparation of the extract, the material was kept at the temperature of 6-8^{0}C. Immediately prior to extraction, the plant material was powdered. The voucher specimens were deposited in herbarium of botanical garden of Department of Biology, Faculty of Natural Sciences, University of Belgrade, Serbia, no. BEOU 16405.

Preparation of dry extracts

Aerial part and rhizome extracts were obtained by the infusion method (7). For extraction, 20g of dried and powdered aerial part (*P.rep-a*), and rhizome (*P.rep-r*) and 200 ml of boiling distilled water were used. The resultant extract was filtered and evaporated using a rotary vacuum evaporator at 40^{0}C, (RV05 basic IKA, Germany).

Testing of antimicrobial activity

In order to determine possible antimicrobial activity, *P.rep-a* and *P.rep-r* aqueous extracts were tested in vitro against one fungus and two standard ATCC bacterial strains using the agar diffusion method (8). Standard bacterial strains used in the test were: *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, as well as the fungus *Candida albicans* ATCC 10231.

The testing started at concentrations ranging from 10 μg/ml to 10 mg/ml, wherein the positive results were observed at concentrations above 10 mg/ml. The experiment was further carried out at concentrations: 10, 50, 75, 100 and 150 mg/ml which were obtained by diluting the appropriate amount of extract in distilled water.

Müeller Hinton agar medium was used for testing the sensitivity of bacteria to the obtained extracts (HiMedia Laboratories, India, LOT 0000099844), and Sabouraud agar plate was used for the fungus *Candida albicans* (Institute of Immunology and Virology "Torlak", Belgrade).

The concentration of bacterial broth was diluted (10^2 organisms/ml) and volume of 0,1 ml of each broth was applied to the surface of the agar plate. Then the appropriate strain substrate was poured into sterile 90 mm diameter petri dishes, so that the thickness of the solidified agar was 4 mm. The reservoirs of diameter 12 mm were made in agar and in each well was introduced appropriate concentration of extracts in volume – of 150 μl.

Thus prepared agar plates were incubated at 37^{0}C during before 24 h. Inhibition zone diameters of plant extracts and standard substances were determined. Inhibition zones were determined by measuring the diameters in millimeters (12 mm diameter of the reservoir was subtracted from the displayed values of the inhibition zone diameter), and in cases when the inhibition zone diameter was smaller or equal to 12 mm, the tested sample was considered to be inactive (8).

Statistics

Statistical analysis was performed using SPSS software. Inhibition zone values for each disk were shown in a scatter diagram, and linear regression lines were calculated using the least squares method. Diffusion zone values for each concentration of the extracts were plotted on scatter diagrams, and regression lines were calculated by the least squares method. The significance of the difference in the activity of the extracts against selected microorganisms was calculated using Mann – Whitney test ($p < 0,05$).

RESULTS

Agar diffusion test results showed that the aqueous extracts of *P. reptans* aerial parts and rhizome at the concentrations of 10 – 150 mg/ml had significant antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Maximum antimicrobial activity was displayed at the highest concentrations applied. Aerial part extract at the concentration of 150 mg/ml showed 95,24% of ceftriaxone activity (30 μg/disk) against *Escherichia coli,* and 68,75% against *Staphylococcus aureus*. Rhizome extract at the concentration of 150 mg/ml showed 133,3% activity of the standard against *Escherichia coli*, and 87,5% of ceftriaxone activity (30 μg/disk) against *Staphylococcus aureus*. The strain of *Candida albicans* showed moderate sensitivity to *P.reptans* rhizome and the highest applied concentration exhibited 50% activity in comparison to that of the refer-

ence substance - nystatin (25 µg/disk). The results of antimicrobial activity are shown in Table 1.

The mean value of the diameter of inhibition zones *P.rep-r* versus *Staphylococcus aureus* was 25.2 ± 1.16 mm for *P.rep-a* 15.2 ± 3.93 mm. While the mean value of the diameter of inhibition zones *P.rep-r* versus *Escherichia coli* was 23.4 ± 1.5 mm and for a *P.rep-a* 17.2 ± 0.86 mm. The differences in antimicrobial activity of the extracts against respective bacteria are displayed in Figures 1 and 2.

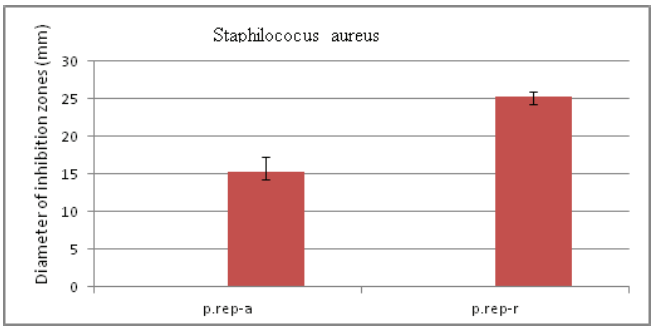

Fig. 1. The difference in activity of extracts of P. reptans aerial parts and rhizome against Staphilococus aureus p > 0,05

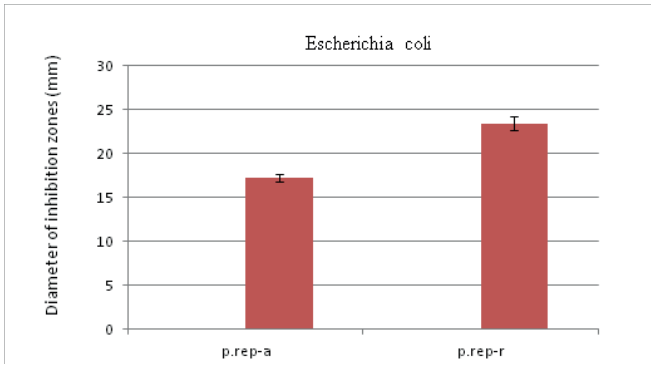

Fig. 2. The difference in activity of extracts of P. reptans aerial parts and rhizome against Escherichia coli p > 0,05

DISCUSSION

Many of the plants used in traditional medicine were studied in order to prove their antimicrobial activity and justify their use in the treatment of various diseases caused by variety of microorganisms. Antimicrobial activity of *P. reptans* rhizome and aerial parts in this study was tested on standardized strains of microorganisms. The extacts of solvents such as ethanol, methanol, acetone, chloroform etc, are richer in active compounds than aqueous solvents and this greatly influences the appearance of antimicrobial effects (9). The tested aqueous extracts were obtained in a manner that is most commonly used in traditional medicine.

The 75% ethanol extract of *P. reptans* rhizome at concentration of 8 µg/mL showed significant antimicrobial effect against S. aureus, as well as the 25% ethanol extract of the rhizome at concentration of 40 µg/mL. The 25% ethanol extract of *P. reptans* leaves showed antimicrobial activity against *B. subtilis* at concentration of 40 µg/mL. Decoctions and 25% ethanol extracts of *P. reptans* root showed the activity against *E. coli* at the concentration of 200 µg/mL (6). Our study results were in accordance with the results that had been shown before but the concentration of our extracts (10 µg/mL) did not show any effect. However, at higher concentrations *P. reptans* aerial part and rhizome showed a significant antimicrobial activity. This difference can be explained by the presence of tannin catechin in large quantities in the rhizome and probably its higher extraction with alcoholic solvents which were used in other study.

Table **1.** Antimicrobial activity of aqueous extracts of *P. reptans* rhizome and aerial parts

Diameter of inhibition zones (mm)				
Material		*Escherichia coli*	*Staphylococcus aureus*	*Candida albicans*
P.rep-a	10 mg/ml	15	0	0
	50 mg/ml	16	16	0
	75 mg/ml	17	18	0
	100 mg/ml	18	20	0
	150 mg/ml	20	22	0
P.rep-r	10 mg/ml	19	22	0
	50 mg/ml	22	23	0
	75 mg/ml	23	26	0
	100 mg/ml	25	27	16
	150 mg/ml	28	28	17
Ceftriaxone 30 µg/disk		21	32	-
Nystatin 100 IJ/disk		-	-	34

Methanol extract of *P. recta* applied at the concentration of 10 mg/ml caused the inhibition zone (15 mm) against *S. aureus* (10). In this study, aqueous extracts of *P. reptans* rhizome at the concentration of 10 mg/ml also induced the inhibition zone against this bacteria (22 mm), while the aerial part extract at this concentration did not show a significant activity against *S. aureus* and it showed the inhibition zone of 16 mm at the concentration of 50 mg/ml. The inhibition zones after application of 10 mg/ml of *P. reptans* aerial part and rhizome extracts against *E. coli* were 15 mm and 19 mm. These values were significantly larger than the results obtained after application of 10 mg/ml *P. recta* extract (12 mm) (10).

Aerial parts of nine *Potentilla species* (*P. argentea, P. fruticosa, P. recta, P. rupestris, P. erecta, P. anserina, P. nepalensis, P. thuringiaca, P.grandiflora*) showed moderate effect against gram-positive bacteria (*Staphylococcus aureus, Bacillus suptilis*) at minimum inhibitory concentration of 12,5-100 mg/ml, while these extracts did not have any effect against gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*). Moderate antifungal effect against *Candida albicans* was indicated (11). In our study, the rhizome showed moderate effect against *Candida albicans* but *P. reptans* aerial part did not show any effect.

Catechin is a dominant flavonoid from *P. reptans* rhizome and it may be assumed that it is the main carrier of the extract

activity (5,12). The activity of tannins in the plants of *Potentilla genus* against gram-positive, gram-negative bacteria and fungi was studied before when antibacterial and antifungal activity was observed (13). The study performed on three sorts of triticum showed that catechin and its derivates were synthetised in larger quantities within infectious plants rather than healthy ones, and the level of catechin also decreased after the infection ended, so it was considered that the synthesis of catechin was a defence response against pathogen attack (14). As for antimicrobial activity, it was proved that catechin had antimicrobial activity but the mechanism of action has not been explained yet. There is a conjecture that hydroxyl group of catechine molecule as a result of dehydrogenation gets replaced with carboxyl group which can bond with phospolipids within cell membrane, what can induce damages of membrane and physiological functions (15). Microbiological effect of aqueous extracts of *P. reptans* can be induced not only by catechin but larger number of secondary metabolites of different chemical structures which are present in extracts at the same time (16).

Large number of Potentilla species showed moderate effect not only against gram-positive bacteria but also against fungus *C. albicans* while there was not any effect against gram-negative bacteria or the effect was very weak (11). Although it was showed that *Potentilla* plants had moderate antifungal activity, for example tested *Potentilla* species had MIC range of 25-100 mg/ml against *Candida albicans* (7), while *P. recta* at concentration of 10 mg/ml induced the inhibition zone of 21 mm, in our study none of the extracts at this concentration showed clear inhibition zone. Only at concentrations higher than 100 mg/ml the rhizome extract showed the inhibition zone of 16mm.

Antimicrobial activity of aqueous extracts of *P. reptans* aerial part and rhizome showed antimicrobial properties at concentrations of 10 to 150 mg/ml. Aqueous extract of *P. reptans* rhizome at concentrations of 100 to 150 mg/ml showed antifungal activity against *C. albicans* while the aerial part did not show any effect against this fungus. There was a significant difference in the activity of aerial part and rhizome extract against both bacterial strains. The rhizome showed statistically significantly stronger antimicrobial activity against examined bacterial strains than aerial part extract.

The results are in accordance with the results of other *Potentilla species* of this genus, as well as with the results of this species, significantly weaker though. Such weak results can be justified by the method of extract preparation. Obtained results confirmed the use of this plant in traditional medicine for the treatment of diarrhea and other conditions caused by various bacterial strains.

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