

ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY  
OF *POTENTILLA REPTANS* L.MARINA T. TOMOVIC<sup>1\*</sup>, SNEZANA M. CUPARA<sup>1</sup>, MARIJA T. POPOVIC-MILENKOVIC<sup>2</sup>,  
BILJANA T. LJUJIC<sup>3</sup>, MARINA J. KOSTIC<sup>4</sup> and SLOBODAN M. JANKOVIC<sup>4</sup><sup>1</sup>Department of Pharmacy, Faculty of Medical Sciences, University of Kragujevac,  
Svetozara Markovica 69, 34000 Kragujevac, Serbia<sup>2</sup>Community Pharmacy Kragujevac, Kralja Aleksandra I Karadjordjevic 36, 34000 Kragujevac, Serbia<sup>3</sup>Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences,  
University of Kragujevac, Svetozara Markovica 69, 34000 Kragujevac, Serbia<sup>4</sup>Department of Pharmacology, Faculty of Medical Sciences, University of Kragujevac,  
Svetozara Markovica 69, 34000 Kragujevac, Serbia

**Abstract:** *Potentilla* species have been used in traditional medicine in the treatment of different ailment, disease or malady. *Potentilla reptans* (*P. reptans*) has been scarcely studied. The aim of this study was to test antioxidant and anti-inflammatory activity of *P. reptans* aerial part and rhizome. DPPH assay was used to measure antioxidant activity of aqueous plant extracts. Anti-inflammatory effect was evaluated by experimental animal model of phenol-in-acetone induced mice ear edema. DPPH radical-scavenging activity of both tested extracts was concentration dependent with IC<sub>50</sub> values 12.11 µg/mL (aerial part) and 2.57 µg/mL (rhizome). Maximum anti-inflammatory effect (61.37%) was observed after administration of 10 mg/ear of the rhizome extract and it was 89.24% of effect induced by dexamethasone as a standard. In conclusion, *P. reptans* rhizome aqueous extract possesses anti-inflammatory effect and higher antioxidant activity than aerial part.

**Keywords:** *Potentilla reptans*, DPPH, anti-inflammatory effect, total phenols, flavonoids, procyanidins

*Potentilla* species have been used for a long time in traditional medicine of cultures in Europe, Asia and Northern America, such as *Potentilla erecta* which is official in pharmacopoeias of different European countries and was used for the treatment of purulent facial eczema and buccal ulcerations. Plants were mainly prepared in the form of water or alcoholic extract and applied either topically for the treatment of mouth ulcers, throat inflammation, wound-healing or internally in jaundice and dysentery. Traditional medicine sporadically employed *Potentilla* species in the treatment of hepatitis, rheumatism, scabies, diarrhea, viral infections and as a remedy for detoxification (1).

*P. erecta*, *P. anserina*, *P. aurea* and *P. reptans* were included in homeopathic pharmacopoeias for preparation of homeopathic remedies. In spite of a big variety of traditional uses for *Potentilla* species, studying phytochemistry and pharmacology of this genus has not been completed. Clinical studies of *P. erecta* rhizome extracts showed positive results in

the treatments of colitis ulcerosa and viral children diarrhea (2, 3).

*Potentilla reptans* (*P. reptans*) has been the least studied among the members of genus. Nine compounds have been found in aerial parts of *P. reptans*. They are flavonoids (kaempferol, quercetin, quercetin-3'-glucoside, quercetin-3,7-diglucuronide, 2',4',6',4-tetrahydroxychalkon-2'-O-β-D-glucoside), ellagic acid, p-coumaric acid, caffeic acid and ferulic acid (1). Animal models were used to test antiulcerogenic and antioxidant activities of *P. reptans* (4, 5). There are no data about chemical content or pharmacology available for the underground part of *P. reptans*.

*P. reptans* is a perennial plant with thick vertical rhizome, prostrate and elongated stem with a rosette of leaves. Petals are golden yellow, usually twice as long as sepals. The flowers have five petals, rarely four (6).

The aim of this study was to determine principle active compounds in aqueous extracts of *P. rep-*

\* Corresponding author: e-mail: marinapop@gmail.com; phone: +381 34 306 800 ext. 225; fax:+381 34 306 800 ext. 112

*tans* rhizome and aerial part and to evaluate and compare antioxidative potential and anti-inflammatory effects of studied extracts.

## EXPERIMENTAL

### Extract preparation

*P. reptans* was collected from autochthonous sources in Serbia. The voucher specimens were deposited in Department of Biology, Faculty of Natural Sciences, University of Kragujevac and botanical garden of Department of Biology, Faculty of Natural Sciences, University of Belgrade, with number BEOU 16405.

Aerial part and rhizome were collected in May to August 2010 and separated. Sources of plant material were three different locations in Serbia: Sumarice nearby city of Kragujevac, Oplanić and Dobroselica on the mountain Zlatibor. The collected material was dried under the shade. Aqueous extracts were prepared separately extracting aerial part and rhizome of *P. reptans*. Forty grams of dried, milled parts of the plant were extracted by 600 mL of hot distilled water (7). Dry extracts were obtained by evaporation under reduced pressure (RV05 basic IKA, Germany).

### Determination of the total phenols

Determination of the total phenols was performed according to the standard method of Singleton et al. (8), customized for 96-well microplates (9). We used Folin-Ciocalteu's reagent (FC) (Fisher Scientific, UK), anhydrous  $\text{Na}_2\text{CO}_3$  (Analytika, Czech Republic) and gallic acid (Sigma Aldrich, Germany) as standard. The following concentrations of extracts were prepared: 0.5, 0.25, 0.125 and 0.063 mg/mL. Gallic acid (100–0.063  $\mu\text{g/mL}$ ), was used as a standard for plotting a calibration curve. Thirty microliters of each extract or standard solution was added to 150  $\mu\text{L}$  of 0.1 mol/L FC reagent and mixed with 120  $\mu\text{L}$  of sodium carbonate (7.5%) after 6 min. Absorbance at 760 nm was measured after 120 min. The content of total phenol compounds in examined extracts was expressed as mg of gallic acid equivalents (GA) per gram of dry extract weight.

### Analysis of individual phenol compounds (HPLC)

Prepared extracts were diluted in mobile phase (0.05%  $\text{HCOOH}$  :  $\text{MeOH}$ , 1 : 1, v/v) to final concentrations of 20 mg/mL and 2 mg/mL and analyzed by HPLC with LC-MS/MS detection. A series of dilutions of 45 reference standards mixture was pre-

pared in 1.5 ng/mL to 25  $\mu\text{g/mL}$  range, to perform quantification. Separation was achieved using Series liquid chromatograph (Agilent Technologies 1200) coupled with Triple Quad mass selective detector with electrospray ion source (Agilent Technologies 6410A). Five microliters of extract/standard was injected and compounds were resolved on a Zorbax Eclipse XDB-C18 (50  $\times$  4.6 mm, 1.8  $\mu\text{m}$ ) column, set at 50°C. Mobile phase consisting of 0.05 %  $\text{HCOOH}$  and  $\text{MeOH}$ , was delivered in gradient mode (0 min 30% B, 6 min 70%, 9–12 min 100%, post time 3 min), at 1 mL/min flow. Eluted compounds were detected in dynamic SRM mode. Obtained results were analyzed using MassHunter Workstation Software – Qualitative Analysis (B.03.01). A calibration curve (MRM peak area vs. concentration) was plotted for each compound.

### Determination of procyanidins

The content of procyanidins was calculated using the method described in European Pharmacopoeia 6.0 (10) and expressed as equivalent of cyanidin chloride. We used butanol ( $\text{BuOH}$ ) (POCH, Poland) and cyanidin-chloride (Carl Roth, Germany). The investigated extract was hydrolyzed under reflux by an  $\text{EtOH/HCl}$  mixture. Procyanidins were separated with  $\text{BuOH}$  from the aqueous layer. The absorbance was measured at 550 nm by spectrophotometer (Cecil CE 2021) (11).

### Determination of flavonoids

The content of flavonoids was calculated by aluminum chloride colorimetric method (12) adapted for 96-well microplates (9). We used  $\text{AlCl}_3 \times 6\text{H}_2\text{O}$ ,  $\text{CH}_3\text{COONa} \times 3\text{H}_2\text{O}$  (Centrohem, Serbia), quercetin (Sigma-Aldrich, Germany), methanol ( $\text{MeOH}$ ) (J.T. Baker, USA). Test samples were prepared in concentrations of 10.0, 5.0, 2.5, 1.25 and 0.625 mg/mL. As standard we used quercetin. Thirty microliters of extract or standard was diluted by 90  $\mu\text{L}$  of methanol and 6  $\mu\text{L}$  of 10% aluminum chloride, 6  $\mu\text{L}$  of 1 mol/L sodium acetate and 170  $\mu\text{L}$  of distilled water were added. Absorbance at 415 nm was measured after 30 min. All samples were made in triplicate and mean values for flavonoid content were expressed as milligrams of quercetin equivalents per gram of dry extract weight calculated according to the standard calibration curve.

### Evaluation of antioxidative activity

Investigated plant extracts were tested by DPPH assay according to the method of Soler-Rivas et al. (13). It was adapted for 96-well microplates (9, 14). We used the following materials: DPPH ( $\alpha,\alpha$ -

diphenyl- $\beta$ -picrylhydrazyl) (Fluka, Switzerland), butylated hydroxytoluene (BHT) (Alfa Aesar, USA), butylated hydroxyanisole (BHA) (Merck, Germany), quercetin (Sigma-Aldrich, Germany), rutin (Fluka, Switzerland), propyl gallate (PG) (Alfa Aesar, USA), dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Germany). Ten microliters of examined extract solutions, in series of seven concentrations of double dilution in DMSO (5.0–0.078 mg/mL for *P. reptans* aerial part and 0.345–0.005 mg/mL for *P. reptans* rhizome as initial concentration), were added to 100  $\mu$ L of 90  $\mu$ mol/L DPPH solution in methanol and the mixture was diluted with 190  $\mu$ L of methanol. After that, we have obtained final concentration for *P. reptans* aerial part 166.7–2.6  $\mu$ g/mL and for *P. reptans* rhizome 11.49–0.180  $\mu$ g/mL. As a control, the exact amount of extract was substituted with DMSO. Absorption at 515 nm was measured by the microplate reader after 60 min (Multiskan Spectrum, Thermo Corporation). Synthetic antioxidants (BHT, BHA, PG, quercetin and rutin) served as a positive control. The radical-scavenging capacity (RSC) was calculated by the equation:

$$\text{RSC} = 100 - (A_a - A_{\text{corr}}) / A_{\text{control}} \times 100$$

where  $A_a$  = average absorbance of the probes for a given concentration sample level;  $A_{\text{corr}}$  = correction of extract absorbance (with no reagents);  $A_{\text{control}}$  = absorbance of the DPPH radical (with no extract).

The extract concentration which causes 50% of DPPH inhibition ( $IC_{50}$ ), was calculated from the RSC concentration curve.

### Experimental animal edema model

Anti-inflammatory effect of investigated extracts was evaluated by experimental mouse ear edema model. Inflammation was induced by application of an irritant agent on the mouse ear. Treated animals received investigated extracts, while control animals received distilled water or dexamethasone as positive control, 15 min prior to the application of irritant agent. The ear edema was measured and compared among different animal groups. It was expressed as the difference of ear weight, with and without inflammation (15).

We used dexamethasone (Dexason<sup>®</sup>, Galenika, Serbia), acetone in phenol (irritant agent) (Zorkapharma, Serbia).

Female and male BALB/c 5–6 weeks old mice, were used in this study (purchased from the Military Medical Academy, Belgrade, Serbia). They were kept in environmentally controlled conditions (22°C, 12 h light/dark cycle), in cages, with free access to standard pellet diet and water. Prior to the experiment, they fasted for 15 h. Adaptation to the

test environment lasted 2 h before the experiment. The experiment was approved by the Ethics Committee of Medical Faculty, Kragujevac, number 016427/2. All of the animal procedures complied with the National Institutes of Health guidelines for humane treatment of laboratory animals.

Animals were divided into eight groups, 8 animals in each. Six groups were treated by different concentrations of investigated aqueous extracts of *P. reptans* aerial part and rhizome (2.5, 5.0, 10.0 mg/ear). Two groups served as control, receiving distilled water or dexamethasone (0.08 mg/ear). Treated groups received extracts on the right ear, 15 min before the application of the irritant agent (20  $\mu$ L of 10% phenol in acetone). Both irritant agent and investigated extracts were applied on the inner and outer surface of the right ear.

The ear edema measurements were performed 1 h after irritant agent application. Edema was expressed as the difference between left and right ear weight, which appeared due to inflammatory challenge. One hour after induction of inflammation, mice were sacrificed by the overdose of ether anesthesia and both ears were removed. Edema was quantified as the weight difference between the two ears (16).

The anti-inflammatory effect was calculated as a percent of edema inhibition in the treated group of animals relative to the control of animals, using the relation:

Edema /inhibition (%) = 100 [(Rt - Lt)/(Rc - Lc)]  
where Rt = mean weight of right ear of treated animals; Lt = mean weight of left ear of treated animals; Rc = mean weight of right ear of control animals; Lc = mean weight of left ear of control animals.

### Statistical analysis

The results are expressed as the mean  $\pm$  standard error of measurement (SEM). For statistical analysis one-way analysis of variance (one-way ANOVA) was used, followed by Dunnett T3 *post hoc* test, where applicable. The probability of null hypothesis lower than 0.05 ( $p < 0.05$ ) was considered to be an indicator of statistically significant difference among experimental groups. All calculations were made by statistical software SPSS version 18.

## RESULTS

Quantitative phytochemical analysis of major compounds found in the rhizome and aerial part of *P. reptans* is presented in Table 1.

Table 1. Quantitative phytochemical analysis of aqueous extracts of *P. reptans* rhizome (Pr-R) and areal part (Pr-A) (the mean value  $\pm$  SD of three measurements).

Active constituents	Pr-A	Pr-R
Total phenols (mg of GA <sup>a</sup> /g)	116.0 $\pm$ 16	468.6 $\pm$ 60
Flavonoid content (mg of Q <sup>b</sup> /g)	10.1 $\pm$ 1	3.9 $\pm$ 2
Procyanidin content (mg of C <sup>c</sup> /g)	1.11 $\pm$ 0.30	98.4 $\pm$ 4.3

<sup>a</sup>GA = gallic acid, <sup>b</sup>Q = quercetin, <sup>c</sup>C = cyanidin chloride

Table 2. Quantitative and qualitative analysis of individual compounds found in rhizome (Pr-R) and aerial part (Pr-A) of *P. reptans*.

Compound name <sup>a</sup>	t <sub>r</sub> <sup>b</sup> (min)	t <sub>r</sub> <sup>c</sup> (min)	t <sub>r</sub> <sup>d</sup> (min)	Quantification (mg/kg)	
				Pr-R	Pr-A
Chinic acid	0.48	0.48	0.48	87.2	78.7
Gallic acid	0.57	0.55		65.9	
Catechin	0.74	0.67		20103	
Protocatechuic acid	0.76	0.73	0.72	34.3	16.2
Epicatechin	0.97	0.93		39.7	
Esculetin	1.08		1.03		9.05
Caffeic acid	1.13		1.08		164
p-Coumaric acid	1.64		1.59		3.14
Luteolin-7-O-glucoside	2.12	2.06	2.06	6.50	13.50
Quercetin-3-O-glucoside	2.22		2.16		38.3
Rutin	2.17		2.12		49.1
Quercetin	2.75	2.70	2.70	150	47.5
Kaempferol-3-O-glucoside	2.80		2.74		27.5
Apigenin-7-O-glucoside	2.67		2.60		21.6
Kaempferol	4.49		4.42		3.72

<sup>a</sup> The numbers refer to compounds signed on the HPLC spectrum <sup>b</sup> Retention times of the standards <sup>c</sup> Retention times of the compounds identified in the extract *P. reptans* rhizome. <sup>d</sup> Retention times of the compounds identified in the extract *P. reptans* aerial part.

Table 3. DPPH free radical scavenging activity of the *P. reptans* extracts aerial part (Pr-A) and rhizome (Pr-R).

Sample	IC <sub>50</sub> <sup>a</sup> (μg/mL)
Pr-A	12.11 $\pm$ 0.216
Pr-R	2.57 $\pm$ 0.340
BHT	11.65 $\pm$ 1.144
BHA	1.57 $\pm$ 0.093
Quercetin	0.41 $\pm$ 0.169
Rutin	1.42 $\pm$ 0.173
PG	0.62 $\pm$ 0.030

<sup>a</sup> The mean value  $\pm$  SD of three measurements.

Quantitative and qualitative analyses of individual compounds found in rhizome and aerial part of *P. reptans* are presented in Table 2, Figures 1 and 2

Both investigated extracts of *P. reptans* possessed DPPH free-radical-scavenging activity. Rhizome showed slightly better antioxidant effect. When the extract of *P. reptans* aerial part was applied in the concentration range of 166.7-2.6 μg/mL, its DPPH free-radical-scavenging activity varied from 98.9 to 12.3% (IC<sub>50</sub> value 12.11  $\pm$  0.216 μg/mL). Application of *P. reptans* rhizome extract in the concentration range of 11.49-0.180 μg/mL possessed DPPH free-radical-scavenging activity 91.2-

1.1% ( $IC_{50}$  value  $2.57 \pm 0.340 \mu\text{g/mL}$ ). Results of DPPH assay performed on *P. reptans* extracts are presented in Table 3.

*P. reptans* aqueous extract of rhizome reduced edema in experimental mouse ear model in a dose-dependent manner. Maximum edema reduction of 61.37% was observed for the concentration 10 mg/ear of rhizome extract. In the same conditions of experiment, dexamethasone (used in concentration of 0.08 mg/ear) achieved 68.77% of edema inhibition. It should be noted that other investigated concentrations of *P. reptans* aerial part and rhizome have shown some edema reduction effect, only 10 mg/ear of rhizome extract and 0.08 mg/ear dexamethasone as positive control have produced statistically significant reduction (Table 4).

## DISCUSSION AND CONCLUSION

Natural compounds with antioxidant properties play an important role as mediators in reactions where reactive oxygen species are being formed. Diminishing harmful potential of these reactive structures, natural antioxidants such as different types of phenol compounds, may prevent development of chronic and/or debilitating diseases such as cancer, cardiovascular diseases, etc. (17, 18).

Our results suggested that rhizome extract of *P. reptans* is four times richer in total phenol compounds than *P. reptans* aerial part. Interestingly, flavonoid content of aerial part is higher than flavonoid content of rhizome. We observed that procyanidins are main contributors to the high value

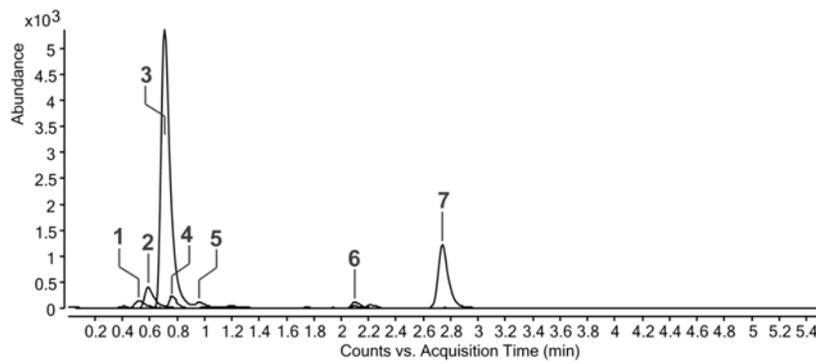


Figure 1. HPLC chromatogram of *Potentilla reptans* rhizome aqueous extract: 1 – chonic acid, 2 – gallic acid, 3 – catechin, 4 – protocatechuic acid, 5 – epicatechin, 6 – luteolin-7-O-glucoside, 7 – quercetin

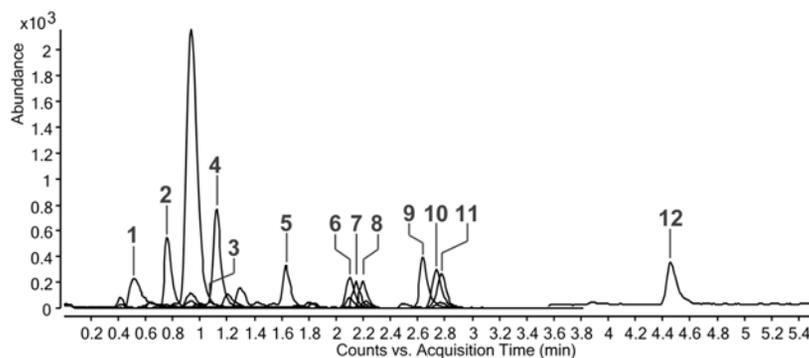


Figure 2. HPLC chromatogram of the aqueous extract of *Potentilla reptans* aerial parts: 1 – chonic acid, 2 – protocatechuic acid, 3 – esculetin, 4 – caffeic acid, 5 – p-coumaric acid, 6 – luteolin-7-O-glucoside, 7 – rutin, 8 – quercetin-3-O-glucoside, 9 – apigenin-7-O-glucoside, 10 – quercetin, 11 – kaempferol-3-O-glucoside, 12 – kaempferol

Table 4. Inhibition of edema by *P. reptans* aerial part (Pr-A) and *P. reptans* rhizome (Pr-R).

Sample	Concentration (mg/ear)	Edema (mg)	Inhibition (%)
Pr-R	2.5	21.61 ± 9.91	14.18
Pr-R	5	17.54 ± 11.78	30.34
Pr-R	10	9.73 ± 4.22 <sup>a</sup>	61.37
Pr-A	2.5	19.89 ± 9.46	21.00
Pr-A	5	26.36 ± 7.74	-4.71
Pr-A	10	18.47 ± 17.75	26.62
Dexamethasone (control)	0.08	7.86 ± 4.74 <sup>a</sup>	68.77
Water (control)	0	25.18 ± 9.24	0

<sup>a</sup>p < 0.05 comparison with control groups.

of total phenols in *P. reptans* rhizome (Table 1). Comparing individual phenol compounds in *P. reptans* aerial part and rhizome we observed that most phenol compounds are present in aerial part in higher content than in rhizome, with exception of gallic acid, catechin, epicatechin, procatechuic acid and quercetin, which are dominantly present in rhizome. Flavonoids are considered as powerful antioxidants among plant-derived phenols and particularly kaempferol, quercetin and rutin were studied (19, 20). Caffeic acid and p-coumaric acid also exhibit noticeable antioxidant effect (21, 22). Although literature data suggest that apigenin, luteolin and kaempferol have been found in rhizome of *P. species* (23-26), we have not identified these compounds in *P. reptans* rhizome. The amount of procyanidins we found in *P. reptans* rhizome (98.4 g/kg) is higher than the one found in *P. alba* root (80 g/kg dry weight) (27). We also compared our results with the content of total phenols, flavonoids and procyanidins found in the comparative study of aerial part of ten different species of *Potentilla*. *P. reptans* aerial part contains 116.0 mg/g of total phenols, which is the second highest value of phenols in comparative analysis, after *P. fruticosa* (116.3 mg/g). *P. reptans* aerial part is richer in total flavonoids (10.1 mg/g) than *P. fruticosa* (7.0 mg/g). Aerial part of *P. reptans* have the smallest quantity of procyanidins 1.1 mg/g among other compared species (28).

Catechin, condensed tannin which is thoroughly researched plant-derived phenol, shows anti-inflammatory effect and protects against oxidative stress (29). Studies on quantification of catechin in different plants, have various results. They differ and imply that some parts/organs of plants like fruits are scarce sources of catechin, while others are con-

sidered as dominant source of catechin (30, 31). We found unusually high quantity of catechin (20.1 g/kg) in rhizome, which is in accordance with data for some *Potentilla* species (1). On the contrary, catechin has not been found in *P. reptans* aerial part. The similar discrepancy between contents of aerial part and rhizome was observed for epicatechin, kaempferol, caffeic acid and p-coumaric acid. Kaempferol, caffeic acid and p-coumaric acid are present dominantly in aerial part, while epicatechin was found only in rhizome. We therefore, suggest that *P. reptans* rhizome represents a considerable source of epicatechin and catechin.

In our study we used DPPH method in order to determine antioxidant effect of *P. reptans* extracts. DPPH method is a useful and reliable tool for estimating free-radical scavenging activity of phenolic compounds (32). Antioxidant activity of *P. reptans* rhizome was observed to be more than five times higher than antioxidant activity of *P. reptans* aerial part (Table 3), though both extracts gave concentration dependent response. In relation to standards, antioxidant activity of rhizome was higher than BHT, lower than quercetin, BHA, PG and rutin.

Previous research of plant material noted a positive correlation between content of total phenols and antioxidant activity (33, 34). In our study, rhizome of *P. reptans* showed free-radical scavenging activity, as have done also other *P. species* extracts made by different solvents and tested by DPPH assay (27, 35, 36). As literature data suggest, catechin act as powerful antioxidant (29). We found catechin to be a major component of *P. reptans* rhizome and it guides to think that free-radical scavenging activity exerted by *P. reptans* rhizome depends in major on abundant presence of catechin. Our

assumption that catechin in rhizome may be a strong contributor to the observed antioxidant activity, is based on the fact that our results for IC<sub>50</sub> finely correlate with results obtained in a similar experiment of aqueous extract of *Bergenia crassifolia* rhizome, (positive relation between catechin content and IC<sub>50</sub>) (34). Therefore, we propose that high content of total phenols, particularly content of catechin in rhizome of *P. reptans*, significantly contributes to strong antioxidant effect performed by aqueous rhizome extract. Considering that substances possessing IC<sub>50</sub> values = 50 µg/mL in the DPPH assay have been categorized as active antioxidants (37, 38), we think that based on our results for IC<sub>50</sub> (2.57 µg/mL rhizome and 12.11 µg/mL aerial part), both extracts may be categorized as active antioxidants. We are aware of synergic action of individual compounds found in plant material as a valid fact, but high amount of catechin present in rhizome seems to be mainly responsible for antioxidant effect exerted by rhizome extract. Analyzing content of individual phenol compounds in aerial part we found that the presence of caffeic acid is the highest. Since caffeic acid is categorized as an active antioxidant (21), we may attribute the effects of antioxidant activity of aerial part of *P. reptans* to its content of caffeic acid.

It is noticeable that the presence of procyanidins, which lately attracted attention of nutrition and medicine, as potent protective antioxidants for human health (39) may also contribute to antioxidant action of rhizome, since considerably higher content of procyanidins was found in rhizome of *P. reptans* than in aerial part.

Studies of *Potentilla* genus reveal that antioxidant effect of some *P. species* (*P. alba* root) is correlated to the content of phenol derivatives (catechin, epicatechin etc.) (28). Our opinion is that this study complements current research of *P. species* (1). *P. reptans* rhizome is richer in total phenols than aerial part and we think that it is why rhizome possessed stronger antioxidant activity than aerial part.

Natural plant-derived antioxidants prevent reactive oxygen species (ROS) to initiate or speed many conditions where inflammatory mediators are implicated (40, 41). Antioxidants lower presence of ROS, which induce release and production of various pro-inflammatory mediators responsible for the inflammation, such as IL-1, IL-6, IL-8, TNF- $\alpha$ , leukotrienes and prostaglandins (42). Experimental model for studying inflammation employees phenol as irritant agent (43). When phenol comes into direct contact with the skin, it damages membranes of keratinocytes. As a result, cytokines are released (IL-1 $\alpha$ , TNF- $\alpha$  and IL-8), which is followed by further

release of other inflammatory mediators (arachidonic acid metabolites) (44, 45). This way an acute local inflammatory response is produced and thus, phenol is a good irritant agent for simulating inflammation (44).

Numerous investigations have shown that phenol compounds found in plants, in particular flavonoids and phenolcarboxylic acids, may act as anti-inflammatory agents (45). In our experiment, *P. reptans* rhizome aqueous extract showed anti-inflammatory effect. *P. reptans* aerial part achieved no significant anti-inflammatory effect. Edema inhibition produced by 10 mg/ear of rhizome extract was 61.27% and is comparable with other results for aqueous plant extracts (57.0% by 5 mg/ear (16), 36.3% by 200 mg/ear (38)). In comparison with effect induced by dexamethasone as standard (15), concentration of 10 mg/ear of *P. reptans* rhizome extract produced maximum edema reduction of 89.24%.

Catechin inhibits release of proinflammatory cytokines (IL-1, TNF- $\alpha$  and prostaglandin E<sub>2</sub>) (46). Quercetin, also found in our investigated extracts, is effective inflammation suppressor (45). The results of this investigation provided evidence that *P. reptans* rhizome extract is topically active in the attenuation of acute dermatitis induced by phenol, in a manner similar to dexamethasone, as the reference anti-inflammatory drug (47). On the basis of our results of DPPH assay and content of phenol compounds in rhizome of *P. reptans*, particularly of catechin present as a major component, we suggest hypothesis that phenol compounds in rhizome may be responsible for anti-inflammatory effect achieved by *P. reptans* rhizome extract.

Our study brings more information about little data available on *P. reptans*. We found that both rhizome and aerial part are good source of total phenols, which are more present in rhizome than in leaves. Rhizome also contains more procyanidins than aerial part. Content of catechin, as a major component is unusually high, catechin being present exclusively in rhizome of *P. reptans*. Aqueous extracts of rhizome and aerial part showed antioxidant activity on DPPH assay, rhizome being more potent antioxidant than aerial part. Both investigated extracts belong to group of active antioxidants. Aqueous extract of *P. reptans* rhizome possesses anti-inflammatory activity.

Since this plant has been scarcely studied up to now, this work may represent a modest contribution to completion of findings on *Potentilla species*. We consider that characterization of aqueous extracts of different parts of *P. species* con-

firms that traditional medicinal uses of this plant should not be disregarded. On the bases of different applications of this plant in ethno-medicine and the fact that it may be considered as an active antioxidant for health preservation, we find appropriate to suggest further pharmacological research of this plant.

#### Acknowledgment

The authors would like to express gratitude to the Medical Faculty, University of Kragujevac for Grant No. JP 28/10 and Ministry of Education and Science of the Republic of Serbia for Grant No. 175014.

#### Conflict of interest

The authors have declared that there is no conflict of interest.

#### REFERENCES

- Tomczyk M., Latté K.P.: *J. Ethnopharmacol.* 122, 184 (2009).
- Huber R., Ditfurth A.V., Amann F., Guthlin C., Rostock M., Trittler R., Kummerer K., Merfort I.: *J. Clin. Gastroenterol.* 41, 834 (2007).
- Subbotina M.D., Timchenko V.N., Vorobyov M.M., Konunova Y.S., Aleksandrovih Y.S., Shushunov S.: *Pediatr. Infect. Dis. J.* 22, 706 (2003).
- Gurbuz I., Ozkan A.M., Yesilada E., Kutsal O.: *J. Ethnopharmacol.* 101, 313 (2005).
- Avci G., Kupeli E., Eryavuz A., Yesilada E., Kucukkurt I.: *J. Ethnopharmacol.* 107, 418 (2006).
- Chaoluan L., Ikeda H., Ohba H.: *Flora of China* 9, 291 (2003).
- Tomczyk M., Leszczyńska K., Jakoniuk P.: *Fitoterapia* 79, 592 (2008).
- Singleton V.L., Orthofer R., Lamuela-Raventos R.M.: *Methods Enzymol.* 299, 152 (1999).
- Beara N.I., Lesjak M.M., Jovin Đ.E., Balog J.K., Anačkov T.G., Orčić Z.T., Mimica-Dukić M.N.: *J. Agric. Food Chem.* 57, 9268 (2009).
- European Pharmacopoeia 6.0, 5th edn., p. 1712, Council of Europe: Strasbourg, France 2008.
- Porter L.J., Hristich L.N., Chan B.G.: *Phytochemistry* 25, 223 (1986).
- Chang C.C., Yang M.H., Wen H.M., Chern J.C.: *J. Food Drug Anal.* 10, 178 (2002).
- Soler-Rivas C., Espin J.C., Wichers H.J.: *Phytochem. Anal.* 11, 330 (2000).
- Orčić Z.D., Mimica-Dukić M.N., Francišković M.M., Petrović S.S., Jovin D.E.: *Chem. Cent. J.* 5, 34 (2011).
- Leite G.O., Leite L., Sampaio R.S., Araruna M.K., Menezes I.R., Costa Ł.G., Campos A.R.: *Fitoterapia* 82, 208 (2011).
- Okoli C.O., Akah P.A., Onuoha N.J., Okoye T.C., Nwoye A.C., Nworu C.S.: *BMC Complement. Altern. Med.* 8, 27 (2008).
- Halliwell B.: *Free Radic. Res.* 25, 57 (1996).
- Halliwell B.: *Nutr. Rev.* 57, 104 (1999).
- Havsteen B.H.: *Pharmacol. Ther.* 96, 67 (2002).
- Sun C., Fu J., Chen J., Jiang L., Pan Y.: *J. Sep. Sci.* 33, 1018 (2010).
- Gülçin I.: *Toxicology* 16, 213 (2006).
- Gülçin I., Topal F., Çakmakçı R., Bilsel M., Gören A.C., Erdogan U.: *J. Food Sci.* 76, C585 (2011).
- Xue P., Zhao Y., Wang B., Liang H.: *J. Chromatogr. Sci.* 45, 216 (2007).
- Wollenweber E., Dorr M.: *Biochem. Syst. Ecol.* 36, 481 (2008).
- Xue P.F., Luo G., Zeng W.Z., Zhao Y.Y., Liang H.: *Biochem. Syst. Ecol.* 33, 725 (2005).
- Yang J., Wen X., Jia B., Mao Q., Wanga Q., Laib M.: *Phytochem. Anal.* 22, 6, 547 (2011).
- Oszmianski J., Wojdyło A., Lamer-Zarawska E., Swiader K.: *Food Chem.* 100, 579 (2007).
- Tomczyk M., Pleszczyńska M., Wiater A.: *Molecules* 15, 4639 (2010).
- Katalinic V., Milos M., Modun D., Music I., Boban M.: *Food Chem.* 86, 593 (2004).
- Suzuki T., Someya S., Hu F., Tanokura M.: *Food Chem.* 93, 149 (2005).
- Tsanova-Savova S., Ribarova F., Gerova M.: *J. Food Compos. Anal.* 18, 691 (2005).
- Lebeau J., Furman C., Bernier J.L., Duriez P., Teissier E., Cotellet N.: *Free Radic. Biol. Med.* 29, 900 (2000).
- Ghasemzadeh A., Jaafar H., Rahmat A.: *Molecules* 15, 6231 (2010).
- Ivanov S., Nomura K., Malfanov I.L., Sklyar I.V., Ptitsyn L.R.: *Fitoterapia* 82, 212 (2011).
- Miliauskas G., Venskutonis P.R., Van Beek T.A.: *Food Chem.* 85, 231 (2004).
- Kalia K., Sharma K., Singh H.P., Singh B.: *J. Agric. Food Chem.* 56, 10129 (2008).
- Cheel J., Theoduloz C., RodriÄguez J., Schmeda-Hirschmann G.: *J. Agric. Food Chem.* 53, 2511 (2005).
- Tadic V.M., Dobric S., Markovic G.M., Đordjevic S.M., Arsic I.A., Menkovic N.R., Stevic T.: *J. Agric. Food Chem.* 56, 7700 (2008).
- Santos-Buelga C., Scalbert A.: *J. Sci. Food Agric.* 80, 1094 (2000).

40. Alder V., Yin, Z., Tew K.D., Ronai Z.: *Oncogene* 18, 6104 (1999).
41. Keane M.P., Strieter R.M.: *Respir. Res.* 3, 5 (2002).
42. Luger T.A., Schwarz T.: *J. Invest. Dermatol.* 95, 100S (1990).
43. Wilmer J.L., Burleson F.G., Kayama F., Kauno J., Luster M.I.: *J. Invest. Dermatol.* 102, 915 (1994).
44. Murray A.R., Kisin E., Castranova V., Kommineni C., Gunther M.R., Shvedova A.A.: *Chem. Res. Toxicol* 20, 1769 (2007).
45. Rogerio A.P., Kanashiro A., Fontanari C., Da Silva E.V.G., Lucisano-Valim Y.M., Soares E.G., Faccioli L.H.: *Inflammation Res.* 56, 402 (2007).
46. Tang L.Q., Wei W., Wang X.Y.: *Adv. Ther.* 24, 679 (2007).
47. Utsunomiya I., Nagai S., Oh-ishi S.: *Eur. J. Pharmacol.* 252, 213 (1994).

*Received: 25. 11. 2013*