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Michal Tomczyk, Adrian Wiater, Malgorzata Pleszczyńska. In vitro Anticariogenic Effects of Aerial Parts of Potentilla recta and Its Phytochemical Profile. *Phytotherapy Research*, 2010, 10.1002/ptr.3262 . hal-00599833

HAL Id: hal-00599833

<https://hal.science/hal-00599833v1>

Submitted on 11 Jun 2011

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***In vitro* Anticariogenic Effects of Aerial Parts of *Potentilla recta* and Its Phytochemical Profile**

Journal:	<i>Phytotherapy Research</i>
Manuscript ID:	PTR-10-0100.R2
Wiley - Manuscript type:	Full Paper
Date Submitted by the Author:	02-Jun-2010
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Keyword:	Potentilla recta, extracts, polyphenolic compounds, anticariogenic activity



In vitro Anticariogenic Effects of Aerial Parts of *Potentilla recta* and Its Phytochemical Profile

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Abstract

In this study, for the first time, we investigated *in vitro* inhibitory effects of *Potentilla recta* extracts and subfractions obtained with solvents of different polarity (aqueous, 50% ethanolic, diethyl ether, ethyl acetate and *n*-butanolic) against cariogenic *Streptococcus* spp. strains. It was found that the tested samples inhibited the growth of oral streptococci. Furthermore, all five *P. recta* preparations exhibited an inhibitory effects on water-insoluble α -(1→3)-, α -(1→6)-linked glucan (mutan) and artificial dental plaque formation. The ethyl acetate fraction showed the highest anti-biofilm activities especially against *S. sobrinus* GCM 20381, with a minimum mutan and biofilm inhibition concentrations of 6.25 and 25 μ g/mL, respectively. The phytochemical profile of active constituents in the investigated samples were analyzed. The high polyphenolics (total phenol, phenolic acids, tannins, proanthocyanidins, flavonoids) content were found. The ethyl acetate fraction showed the

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3 highest concentration of total polyphenol content which may correlate with high cariogenic activity
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5 of this subfraction. The results demonstrate that *P. recta* extracts and subfractions could become a
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7 useful supplements for pharmaceutical products as a new anticariogenic agent in a wide range of
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9 oral care products. Further studies are necessary to clarify the precise bioactive constituents of *P.*
10
11 *recta* responsible for anticariogenic properties.
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17 *Key words:* *Potentilla recta*; extracts; polyphenolic compounds; anticariogenic activity
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22 **TITLE RUNNING HEAD:** Anticariogenic Effects and Phytochemical Profile of *Potentilla recta*
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25 26 27 **INTRODUCTION**

28
29 Dental caries is an infectious disease which is widely distributed throughout the world and is the
30
31 most prevalent chronic oral disease in humans. The formation of dental caries is caused by the
32
33 colonization and accumulation of oral pathogens and extracellular polysaccharides (dextrans and
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35 mutans) that are synthesized from sucrose by glucosyltransferases of mutans streptococci
36
37 (*Pleszczyńska et al.*, 2003). Numerous synthetic substances and antibiotics have been used in the
38
39 control of dental plaque. However, these compounds cause many unexpected side-effects (*Sato et*
40
41 *al.*, 2002; *Park et al.*, 2003). Recently, the use of medicinal plant and natural products, incorporated
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43 in food and beverages, has been claimed to offer potential candidates for anticariogenic agents.
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45 Among these phytoconstituents, several polyphenolic compounds like tannins, flavonoids and also
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47 alkaloids seem to be the most promising biomolecules (*Eley*, 1999; *Smullen et al.*, 2007; *Petti and*
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49 *Scully*, 2009).
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55 The genus *Potentilla* L. (Rosaceae) has been known since ancient times for its curative
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57 properties. Extracts of the aerial and/or underground parts have been applied in traditional medicine
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59 for the treatment of inflammations, wounds, certain forms of cancer, infections, diarrhoea, diabetes
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3 mellitus and other ailments. Most of the pharmacological effects can be explained by the high
4 amount of polyphenolic compounds (hydrolyzable and condensed tannins, flavonoids) and also
5 triterpenoids (Tomczyk and Latté, 2009; Tomczyk *et al.*, 2009).
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10 Sulphur cinquefoil (*Potentilla recta* L.) is a long-lived invasive perennial plant from Eurasia
11 that has become one of the most serious invaders of natural area grasslands in North America and
12 Canada. *P. recta* is a perennial herb which widely inhabits well-drained soil in full sun to part
13 shade, and prefers calcareous soils. It is found in a wide range of natural and agricultural habitats
14 (Ball *et al.*, 1968; Delgado *et al.*, 2000; Lesica and Martin, 2003; Perkins *et al.*, 2006). *P. recta* has
15 been reported to contain 2-pyrone-4,6-dicarboxylic acid, which seems to be a chemotaxonomic
16 marker for the *Potentilla* species (Wilkes and Glasl, 2001). Ranges of seasonal changes in the
17 content of chlorophyll, carotenoids, free organic, ascorbic and triterpenic acids, neutral triterpenoids
18 and fat oils in some organs and overground phytomass of *P. recta* in the conditions of Belarus have
19 also been determined (Rupasova *et al.*, 2001). Pharmacological studies of the plant have so far
20 focused on preliminary antimicrobial activities, as well as the inhibitory action on h-AR, and
21 haematological activity, including antiplatelet aggregating and anti-coagulating activities (Enomoto
22 *et al.*, 2004; Tosun *et al.*, 2006; Tomczyk *et al.*, 2008).
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41 Considering the traditional use of the *Potentilla* L. species, it would be meaningful to
42 investigate its biological properties in the dental field. The purpose of this study was to evaluate the
43 effects of extracts (aqueous, 50% ethanolic) and fractions prepared by the partitioning of aqueous
44 methanolic extract with solvents of different polarities (diethyl ether, ethyl acetate and *n*-butanolic)
45 from the aerial parts of *P. recta* against cariogenic *Streptococcus* spp. strains (*S. mutans* CAPM
46 6067, *S. sobrinus* CAPM 6070, *S. sobrinus* GCM 20381 and *S. sobrinus/downei* CCUG 21020).
47 Furthermore, their inhibitory effects on water-insoluble α -(1→3)-, α -(1→6)-linked glucan (mutan)
48 and artificial dental plaque formation were examined. The phytochemical profile of active
49 constituents in the investigated samples and plant material was also analyzed.
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MATERIALS AND METHODS

Plant Material. Seeds of *P. recta* were requested from the Giardino Botanico Alpino, Cogne, Italy (index seminum: 1549). Plants were cultivated in a common plots at the Medicinal Plants Garden near Medical University of Białystok, Poland. Aerial parts of plants were collected during June-August 2007-2009. Voucher specimens of plants (No. PRE-06019) have been deposited in the Herbarium of the Department of Pharmacognosy, Medical University of Białystok, Poland.

Preparation of Extracts and Subfractions. Different solvent systems (water, 50% ethanol, diethyl ether, ethyl acetate and *n*-butanol) were used to prepared the extracts and subfractions.

Preparation of Extracts. Powdered plant material (2.0 g) was separately extracted with water (2 x 150 mL) – PRE or 50% ethanol (2 x 150 mL) – PRE1 in an ultrasonicator bath (Sonic-5, POLSONIC, Poland) at controlled temperature ($40 \pm 2^\circ\text{C}$) for given certain time 45 min. Supernatans were filtered through a funnel with glass wool, which was washed with 5 mL of solvent and concentrated to dryness under vacuum (Büchi System, Switzerland) at controlled temperature ($40 \pm 2^\circ\text{C}$) and subjected to lyophilization using LABCONCO vacuum concentrator until a constant weight were obtained. Yields: PRE – 82 mg (4.10%); PRE1 – 96 mg (4.81%).

Preparation of Subfractions. Accurately weighed 2.0 g quantities of plant material in powder form were separately extracted with methanol (3 x 50 mL) and once with 50 mL of 80% (v/v) methanol in an ultrasonicator bath (Sonic-5, POLSONIC, Poland) at controlled temperature ($40 \pm 2^\circ\text{C}$) for given certain time 45 min. After solvent evaporation under reduced pressure from each sample, the methanolic extracts were diluted with water and successively partitioned between chloroform and then diethyl ether (PRE2), ethyl acetate (PRE3) and *n*-butanol (PRE4). All

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3 subfractions were concentrated to dryness under vacuum controlled temperature (Büchi System,
4 Switzerland) (temperature: $40 \pm 2^\circ\text{C}$) and subjected to lyophilization using LABCONCO vacuum
5 concentrator until a constant weight were obtained. Yields: PRE2 – 13 mg (0.61%); PRE3 – 46 mg
6 (2.34%); PRE4 – 93 mg (4.67%).
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12 13 14 15 **Phytochemical Profile**

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20 **Determination of Total Phenolic Content.** Total phenolic contents in extracts and subfractions
21 (PRE-PRE4) were determined spectrophotometrically at 765 nm (SPECORD 40, Analytik Jena,
22 Germany) after the reaction with Folin-Ciocalteu's phenol reagent as gallic acid equivalents
23 GAE/100g in mg per g of dry weight (dw) according to the manual colorimetric method described
24 by Tawaha *et al.* (2007). A 50 μL sample aliquot of extract and subfraction were dissolved in 450
25 μL of distilled water and 2.5 mL of 0.2 N Folin-Ciocalteu's reagent. After 5 min 2 mL of saturated
26 sodium carbonate Na_2CO_3 solution (75 g/L) were added. Samples were vortexed and incubating in
27 the dark at room temperature for 2 h. Quantitative measurements were performed, based on a
28 standard calibration curve of different concentrations of gallic acid (20-500 mg/L). All
29 measurements were performed in triplicate. The results are given in Table I.
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46 **Determination of Total Phenolic Acids Content.** Total phenolic acids content in plant material
47 was determined by use the spectrophotometric method with Arnov's reagent according to the
48 procedure described in Polish Pharmacopoeia VI (2002). Stock solution was prepared from 1.0 g of
49 the powdered sample mixed with 25 mL of water (two times) and shaken for 30 min each, then
50 filtered. Phenolic acids were determined from 1 mL aliquot stock solution mixed with 5 mL of
51 water, 1 mL hydrochloric acid (18g/L), 1 mL of Arnov's reagent and 1 mL of sodium hydroxide
52 solution (40g/L) and diluted with water to 10 mL. Phenolic acids were measured
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3 spectrophotometrically at 490 nm The percentage of phenolic acids, expressed as caffeic acid
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5 equivalent on dry weight, is calculated from the expression:
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$$A \times 1.7544/m,$$

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10 where A is the absorbance of the test solution at 490 nm and m mass of the powdered drug, in
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12 grams. The results are given in Table II and are means of experiments conducted in triplicate.
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17 **Determination of Total Flavonoid Content.** The total content of flavonoids was determined by
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19 the spectrophotometric method by Christ and Müller (1960) and followed the procedure described
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21 in Polish Pharmacopoeia VI (2002). Each powdered sample (0.6 g) was mixed with 20 mL of
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23 acetone, 2 mL of 25% hydrochloric acid (281 g/L) and 1 mL of 0.5% urotropine (methenamine)
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25 solution (5 g/L) and heated in a water bath under reflux for 30 min. The obtained extract was
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27 filtered through cotton wool, and the sediment with the cotton wool was heated twice for 10 min.
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29 with 20 mL of acetone. The extracts were mixed and diluted with acetone to 100 mL in a
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31 volumetric flask. Then, 20 mL of this solution was diluted with 20 mL of water, extracted with 15
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33 mL of ethyl acetate and then, three time with 10 mL of ethyl acetate. Organic phases were mixed
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35 and washed twice with 40 mL of water, fitered to volumetric flask and diluted with ethyl acetate to
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37 50 mL. To four volumetric flasks 10 mL of the obtained solution was added. Then, to three flasks 2
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39 mL of aluminium chloride (20 g/L – methanolic solution) was added and all four flask were filled
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41 with methanol - acetic aid glacial (19:1) to 25 mL. After hydrolysis, the flavonoids were measured
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43 spectrophotometrically at 425 nm by creating a complex with aluminium chloride in a methanol–
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45 ethyl acetate–acetic acid medium. The contents of total flavonoids, expressed as quercetin or
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47 hyperoside equivalent on dry weight, were calculated from the expression, respectively:
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$$A \times 0.875/b \text{ and } A \times 1.25/b,$$

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56 where A is the absorbance of the test solution at 425 nm and b the mass of the powdered drug, in
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58 grams. The results are given in Table II.
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3 **Determination of Total Tannin Content.** The total tannin content was determined by the weight
4 method with hide powder according to the DAB 10 (1998). The results are given in Table II.
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10 **Determination of Total Proanthocyanidin Content.** The total proanthocyanidin content was
11 measured according to the European Pharmacopoeia 6th (2007). Accurately weighted 2.5 g of plant
12 material was heated under reflux for 30 min with 30 mL of ethanol (70% v/v). After that extract
13 was filtered and the residue was flashed with 10 mL of ethanol (70% v/v). Then 15 mL of 25%
14 hydrochloric acid was added with 10 mL of water. The solution was heated under reflux for 80 min.
15 After cooling, extract was filtered and filled up with ethanol (70% v/v) to 250 mL. Then 50 mL of
16 solution was evaporated to about 3 mL and transferred to a separating funnel with 15 mL of water.
17 The solution were then extracted with *n*-butanol (3 x 15 mL, each). The organic layers were mixed
18 and transferred to volumetric flasks and filled up with *n*-butanol to 100 mL. The absorbance was
19 measured spectrophotometrically at 545 nm. The contents of proanthocyanidin, expressed as
20 cyanidin chloride equivalent on dry weight, were calculated from the expression:
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$$A \times 500/75 \times m,$$

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38 respectively, where *A* is the absorbance of the test solution at 545 nm and *m* the mass of the
39 powdered drug, in grams. The results are given in Table II.
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45 **Anticariogenic Activity**

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50 **Bacterial strain, media and growth conditions.** The cariogenic streptococci used in this
51 study included *Streptococcus mutans* CAPM 6067, *S. sobrinus* CAPM 6070 (The Collection of
52 Animal Pathogenic Microorganisms, Brno, Czech Republic), *S. sobrinus* GCM 20381 (formerly *S.*
53 *mutans* 20381) (The German Collection of Microorganisms, Braunschweig, Germany) and *S.*
54 *sobrinus/downei* CCUG 21020 (formerly *S. mutans* OMZ 176) (The Culture Collection, University
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3 of Göteborg, Sweden). The strains were stored as glycerol stocks at -20°C. Bacteria were grown at
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6 37°C in minimum medium for *S. mutans* (Fujiwara *et al.*, 1978) containing (g/L) glucose (10), L-
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8 glutamic acid (2), L-cysteine-HCl (0.2), L-leucine (0.9), NH₄Cl (1), K₂HPO₄ (3.5), KH₂PO₄ (1.5),
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10 NaHCO₃ (4.2), MgSO₄·7H₂O (1.2), MnCl₄·4H₂O (0.02), FeSO₄·7H₂O (0.02), sodium pyruvate
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12 (0.6), and (mg/L) riboflavin (1), thiamine-HCl (0.5), biotin (0.1), nicotinic acid (1), p-aminobenzoic
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14 acid (0.1), calcium pantothenate (0.5), pyridoxal-HCl (1), tris-maleate buffer (pH 7.4) to 1 L. The
15
16 final pH of the medium was 7.1. After 24 h incubation bacterial cultures were centrifuged (17000 x
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18 g for 30 min), and the supernatants were used as cell-free glucosyltransferases.
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25 **Agar-well diffusion method.** For the investigation of the antibacterial activity the agar-well
26
27 diffusion method was used. Samples for the tests were dissolved in dimethylsulfoxide (DMSO).
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29 Wells (5 mm diameters) were cut in the inoculated agar plates. The solutions of tested extracts
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31 (50 µL) were spotted onto such prepared plates (5 mg of preparations per well) and diameter of
32
33 inhibition zone was measured after 24 h incubation at 37°C. Antibacterial activity was expressed as
34
35 the mean of inhibition diameters (mm) produced by the plant extract.
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41 **Inhibition of mutan synthesis.** The inhibitory effect of the *P. recta* extracts and subfractions on
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43 mutan synthesis was examined. A two-fold serial dilution of each extract (concentration ranging
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45 from 6.25 to 800 µg/mL) in 1% DMSO (100 µL final volume) was prepared in Eppendorf tubes.
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47 All tubes were filled with 1.0 mL of 24-h-old post-culture supernatant of the tested bacterium and
48
49 100 µL of sucrose solution (final concentration of 3%, w/v) in 0.1 M potassium phosphate buffer
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51 (pH 6.0) in the presence of 0.05% sodium azide as a preservative. Ellagic acid (Sigma-Aldrich,
52
53 Germany), in form of sodium ellagate, from 6.25 to 1.87 µg/mL, was used as the positive control,
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55 the medium without extracts was used as the non-treated control. After incubation for 24 h at 37°C,
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57 the formed water-insoluble polysaccharide (mutan) was collected by centrifugation at 16099 x g for
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10 min, washed thoroughly with deionized water and determined, utilizing phenol-sulfuric acid method with glucose as a standard (Dubois *et al.*, 1956).

Inhibition of biofilm formation. The effect of *P. recta* crude extracts and subfractions on cariogenic streptococci biofilm formation was examined. A two-fold serial dilution of each extract in 1% DMSO (final volume 300 μ L) was prepared in glass tubes (1.2 \times 9.5 cm). All tubes were filled up with 1.2 mL of culture medium with sucrose (final concentration of 3%, w/v) and then inoculated of 20 μ L of overnight broth culture of the tested bacterium. Final concentrations of extracts and subfractions ranged from 6.25 to 800 μ g/mL. Ellagic acid (in form of sodium ellagate, from 1.87 to 50 μ g/mL) was used as the positive control, the medium without extracts was used as the non-treated control. After incubation for 24 h at 37°C, media from each tube were decanted and planktonic cells were removed by washing with distilled water. The adhered biofilm was stained with 1.5 mL of 1% erythrosin B for 5 min, rinsed thoroughly with water, and dried at room temperature. The bound dye was then removed from biofilm with 3 mL of 1M NaOH. Tubes were set on a shaker for 5 min to allow full release of the dye. Biofilm formation was quantified by measuring optical density at 525 nm.

Data analysis. Statistical analysis was performed on all the three replicates from each treatment. Standard deviations and analysis of variance (ANOVA) were determined using and analysis of variance (ANOVA). Significance was evaluated at $p < 0.05$ for all tests. Mean separation was accomplished using least significant difference test.

RESULTS AND DISSCUSION

Over the past years many studies on the anticariogenic effect of polyphenols extracted from different types of plants (such as tea, apple or hop) have been reported (Ito *et al.*, 1984; Tagashira *et al.*, 1997; Sakanaka *et al.*, 1989; Yanagida *et al.*, 2000). These earlier investigations proved that plant polyphenolics have a wide range of pharmacological properties, such as anticariogenic activity in many pharmacological models such as *in vitro*, *in vivo* and studies on humans (Petti and Scully, 2009). The development of therapeutic agents aimed at disrupting both colonization of the teeth by dental pathogens and the subsequent formation of dental plaque is one of the prime strategies to reduce the incidence of tooth decay (Bowen, 1999). Among the different targets, glucosyltransferases (GTFs) produced by cariogenic streptococci such as *S. mutans* and *S. sobrinus* play a crucial role as one of the most important virulence factors since they are involved in both the formation of the plaque matrix and colonization of oral bacteria. Mutans streptococci produce at least three different GTFs: GTF B, which synthesizes mostly insoluble α -(1→3)-linked glucan; GTF C, which synthesizes a mixture of insoluble and soluble α -(1→6)-linked glucan, and GTF D, which synthesizes soluble glucan. Of these, GTFs B and C appear to be the most strongly associated with dental caries (Duarte *et al.*, 2003). The GTFs synthesize up to 30-40% (dry weight) of the polysaccharide fraction of the dental plaque matrix (Shani *et al.*, 1998). These glucans (especially water-insoluble mutan) can enhance the pathogenic potential of dental plaque by promoting the accumulation of oral bacteria on human teeth. Therefore, one of the strategies to control plaque formation is to inhibit the oral pathogens' growth and the activity of glucosyltransferases.

The antimicrobial activities of *P. recta* extracts against mutans streptococci were assessed by the presence or absence of inhibition zones and zone diameter (Table 3). The results showed that extracts and subfractions, except PRE against *S. mutans* 6067, inhibited the growth of all the tested

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3 oral streptococci. Maximal inhibition zones, in the range of 18 to 24 mm, were obtained for the
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5 PRE2 fraction. All five *P. recta* preparations exhibited an inhibitory effect on mutan and oral
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7 streptococci biofilm formation in a concentration-dependent manner (Figures 1, 2 and Tables 4 and
8
9 5). The action of extracts on water-insoluble mutan synthesis by cell-free glucosyltransferase
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11 preparations from each tested bacteria was expressed as the relative amount of mutan produced in
12
13 the presence of fractions from *P. recta* compared to the amount produced in the absence of any
14
15 extracts. The extracts showed the following order of potency: PRE3>PRE4>PRE1>PRE2>PRE.
16
17 The most effective was ethyl acetate subfraction (PRE3). The minimum mutan inhibition
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19 concentrations (MMIC₅₀) of preparations ranged from 6.25 to 50 µg/mL for all tested strains (Table
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21 4). The data obtained in this study showed that glucosyltransferases of *S. sobrinus* 20381 were more
22
23 resistant to the influence of extracts than other GTF preparations. In the case of biofilm formation,
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25 the MBIC₅₀ values of agents ranged from 25 to 400 µg/mL (Table 5), and the order of potency was
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27 PRE3>PRE1>PRE4>PRE>PRE2. *S. sobrinus/downei* 21020 biofilm demonstrated the lowest
28
29 susceptibility for all extracts and subfractions compared with biofilms formed by other strains.
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31 Similar, the same ethyl acetate subfraction PRE3 significantly reduced both mutan synthesis and
32
33 biofilm formation at the concentration of 12.5 and 100 µg/mL, respectively ($p < 0.05$). That
34
35 suggests a high concentrations of active polyphenolic constituents with anticariogenic activity in the
36
37 investigated subfractions PRE3 (Table 1). The inhibitory effects of all analysed *Potentilla* extracts
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39 were significantly greater than those previously observed in other natural products, for instance
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41 *Nidus Vespaee* extracts (MBIC₅₀ from 8 to 64 mg/mL) (Xiao *et al.*, 2007).
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51 The precise bioactive molecules from *P. recta* extracts and fractions that mediate the
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53 inhibitory effects against the viability of oral pathogenic bacteria are still unknown. Based on the
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55 preliminary phytochemical profile in the present study, the activity of these extracts and fractions
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57 might be related to the presence in plant material of complex polyphenolic compounds like phenolic
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59 acids, tannins (hydrolyzable tannins and proanthocyanidins), as well as flavonoids. But in many
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3 other cases these effects can not be attributed solely to polyphenolics, because plant extracts contain
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5 several other phytochemicals.
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8 The amount of total polyphenolic compounds (phenolic acids, tannins, flavonoids,
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10 proanthocyanidins) in investigated plant extracts and subfractions in most cases correlated with
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12 their anticariogenic activity. The results obtained in the present study shows that all the analyzed
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14 fractions and extracts have relatively high concentrations of polyphenolic compounds. However, all
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16 the fractions and extracts had different total polyphenol content ($p < 0.05$). The ethyl acetate
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18 fraction showed the highest values and diethyl ether fractions showed the lowest one (Table 1). The
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20 content of total polyphenols ranged from 20.9 ± 0.5 mg GAE/g dw for PRE2 to 33.6 ± 1.3 mg
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22 GAE/g dw for PRE3. All these differences in the values of total phenolic content in all the analyzed
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24 fractions and extracts can be attributed to the differences in the composition of these fractions and
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26 extracts. It was obvious that the total phenolic compounds determined by Folin-Ciocalteu's method
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28 had not given a full characterisation of the quality and quantity of the various groups of
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30 polyphenolic compounds. Chromatographic analysis of the ethyl acetate fraction (PRE3) indicated
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32 the presence of, in particular, hydrolysed (ellagitannins or gallotannins) and condensed tannins
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34 (proanthocyanidins), as well as glycosylated derivatives of flavonoid compounds (2D-TLC analysis,
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36 data not shown). In contrast, the chromatographic analysis of diethyl ether fractions proved the
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38 highest concentrations of phenolic acids, flavonoid aglycones and acylated flavonoids (2D-TLC
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40 analysis, data not shown). The presence of these groups of compounds has encouraged us to
41
42 determine them in plant material by weight and spectrophotometrical methods. The results (Table 2)
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44 obtained showed that aerial parts of *P. recta* had relatively very high concentrations of tannins
45
46 (101.0 ± 1.7 mg/g dw) proanthocyanidins (3.7 ± 0.6 mg/g dw) and phenolic acids (5.4 ± 0.3 mg/g
47
48 dw), as well as flavonoids (2.7 ± 0.2 mg/g dw) and (3.9 ± 0.4 mg/g dw), calculated as quercetin,
49
50 flavonol type compound and parallel as a glycoside hyperoside, respectively.
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3 Summarizing, the data presented here imply that the anticariogenic activity of *P. recta*
4 extracts and fractions was much stronger than that of those reported for other *Potentilla* spp.
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6 Recently, Wiater *et al.*, (1998) reported that ethanol extract of rhizomes of *P. erecta* inhibited both
7
8 total GTF activity and mutan synthesis in the range of 500 - 2000 µg extract/mL. From these
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10 comparative data, it can be concluded that different extracts of *P. recta* could be useful for the
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12 development of new anticariogenic agents in a wide range of oral care products such as toothpastes,
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14 mouthwashes and chewing gum. Most certainly, the polyphenolic compounds are responsible for
15
16 this kind of pharmacological property. Regardless of the obtained results, there is a need for further
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18 investigation directed towards the isolation and identification of active compounds in the extracts
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20 and subfractions of the aerial parts of *P. recta* as well as determination of their influence on the
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22 examined activities.
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32 Acknowledgements: This study is financially supported by the Polish Ministry of Science and
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34 Higher Education (Grant No. N N405 621638)
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3 List of figures
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7 Figure 1. The inhibitory effects of all investigated *P. recta* preparations (PRE-PRE4) on mutan
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9 synthesis in concentration-dependent manner. Sodium ellagate as a control. Values are the average
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11 of triplicate.
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16 Figure 2. The inhibitory effects of all investigated *P. recta* preparations (PRE-PRE4) on mutans
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18 streptococci biofilm formation in concentration-dependent manner. Sodium ellagate as a control.
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21 Values are the average of triplicate.
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Table 1. Total phenolic content in the obtained extracts and subfractions from *P. recta*

Extracts	Total phenolic content (mg GAE/g dry weight) ^a
PRE	23.8 ± 0.7
PRE1	27.4 ± 0.6
PRE2	20.9 ± 0.5
PRE3	33.6 ± 1.3
PRE4	25.4 ± 0.9

^aAll data are shown as means ± SD of at least three independent experiments, each performed in duplicated samples ($p < 0.05$).

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60Table 2. Content of polyphenolic compounds in *P. recta*

Polyphenols content (mg/g dry weight) ^a				
Phenolic acids	Flavonoids		Tannins	Proanthocyanidins
	Quercetin	Hyperoside		
5.4 ± 0.3	2.7 ± 0.2	3.9 ± 0.4	101.0 ± 1.7	3.7 ± 0.6

^a Results are means ± SD of three different experiments.

Table 3. Effects of *P. recta* extracts and subfractions on bacterial viability obtained by agar-well diffusion method

Samples/control	Zone of inhibition (mm) ^a			
	<i>S. sobrinus</i> 20381	<i>S. sobrinus</i> 6070	<i>S. mutans</i> 6067	<i>S. sobrinus/downei</i> 21020
PRE	16**	10	ni	16***
PRE1	12***	15**	12	17***
PRE2	24*	18***	18***	21*
PRE3	14***	14*	16**	21*
PRE4	11***	11	10	33***
Streptomycin 250 µg/ml	21	11	11	24

^a Antibacterial activity is expressed as the diameter of inhibition zone, including the well diameter (5 mm), DMSO was used as negative control. ni - no inhibition

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

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60Table 4. Effects of *P. recta* extracts and subfractions on the mutan synthesis

Strains	MMIC ₅₀ (µg/mL)				
	PRE	PRE1	PRE2	PRE3	PRE4
<i>S. sobrinus</i> 20381	50	25	25	6.25	12.5
<i>S. sobrinus</i> 6070	50	25	50	12.5	25
<i>S. mutans</i> 6067	50	25	50	12.5	25
<i>S. sobrinus/downei</i> 21020	50	50	50	12.5	50

MMIC₅₀ – minimum mutan inhibition concentration, the lowest agent concentration that showed > 50% inhibition on mutan synthesis.

Table 5. Effects of *P. recta* extracts and subfractions on the formation of mutans streptococci biofilm

Strain	MBIC ₅₀ (µg/mL)				
	PRE	PRE1	PRE2	PRE3	PRE4
<i>S. sobrinus</i> 20381	50	50	200	25	50
<i>S. sobrinus</i> 6070	200	100	400	100	100
<i>S. mutans</i> 6067	200	100	200	50	200
<i>S. sobrinus/downei</i> 21020	200	100	200	200	400

MBIC₅₀ – minimum biofilm inhibition concentration, the lowest agent concentration that showed > 50% inhibition on biofilm formation.

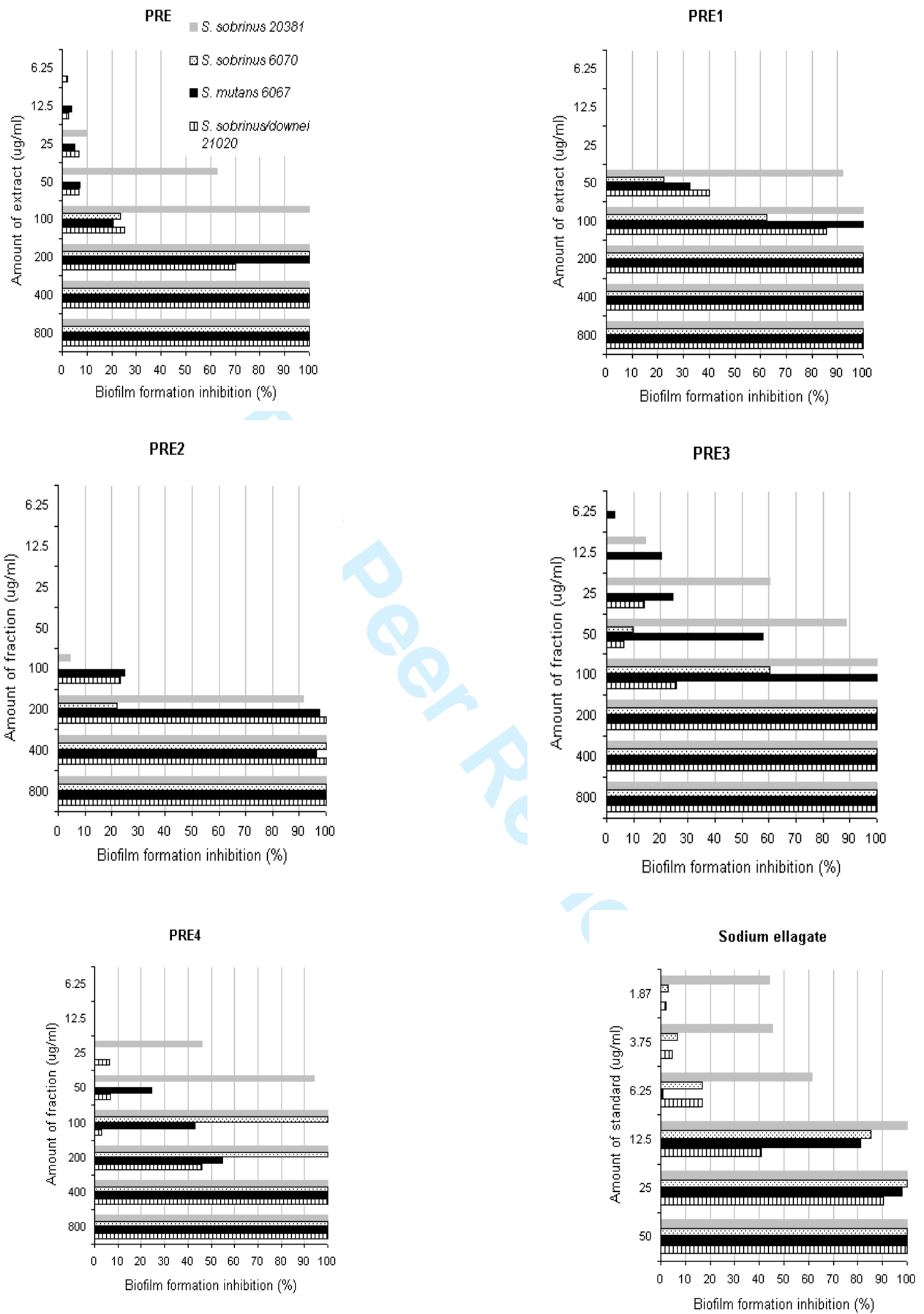


Figure 2

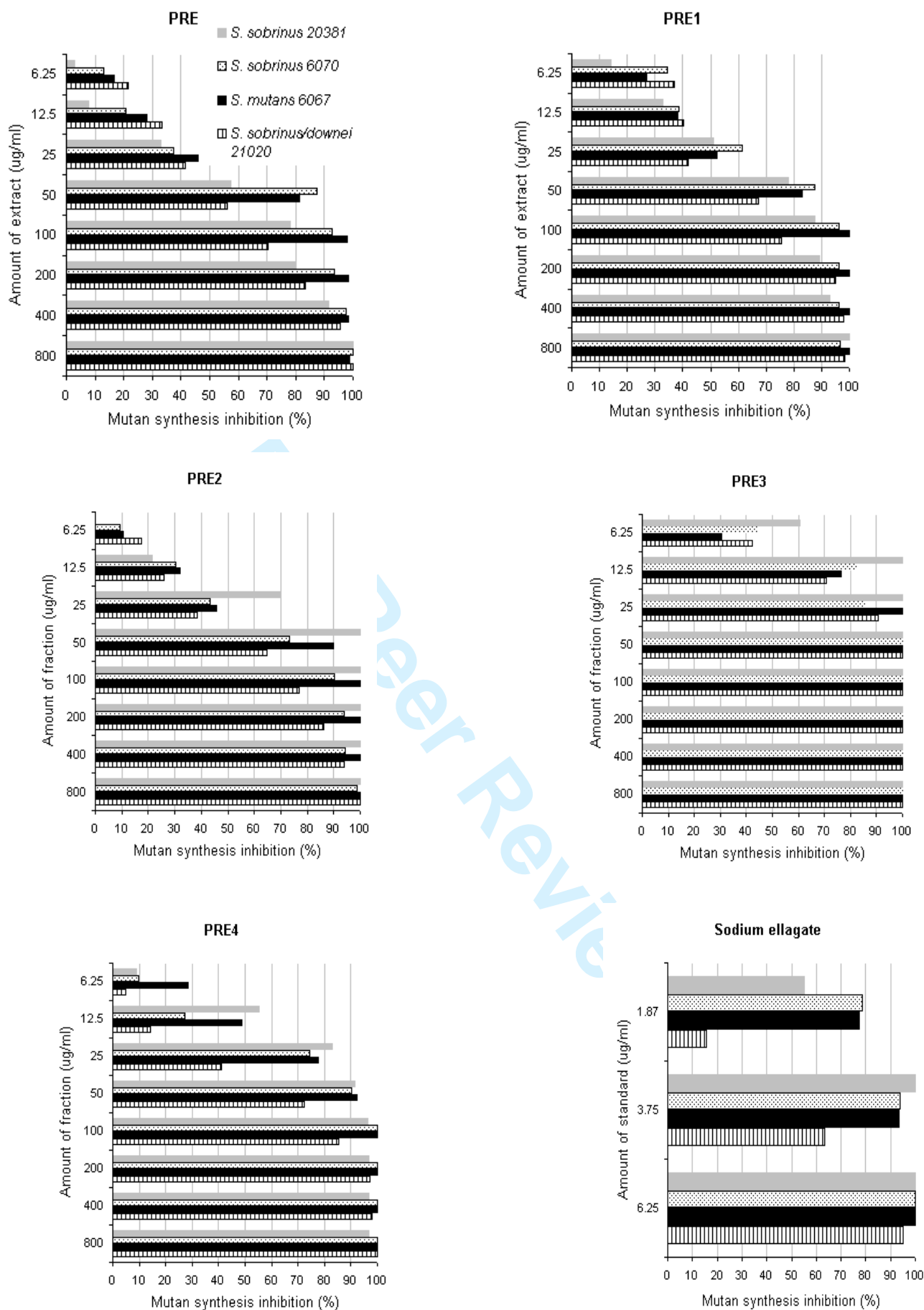


Figure 1