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ANTIMICROBIAL, ANTIBIOFILM-FORMING AND SOME BIOCHEMICAL PROPERTIES OF *Potentilla erecta* RHIZOME EXTRACT

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The purpose of the work was to study the antimicrobial, antibiofilm-forming, antioxidant and some biochemical properties of alcoholic extracts of *Potentilla erecta* L. rhizome. The plants for the study were gathered around the village of Luta, Velyky Berezny rayon, Transcarpathia. From the *Potentilla erecta* L. rhizome, ethyl and methyl alchogol extracts were produced. The aim of the study was their antioxidant activity (by DPPH method), total tannin and flavonoids (by spectrophotometric method), and antimicrobial activity (by diffusion-into-agar method). The clinical isolates were isolated with the use of differentially diagnostic nutrient media. The antibiofilm activities of the extracts were tested in standard 96-well microtitration plates.

Ethyl and methyl extracts of *Potentilla erecta* L. rhizome were shown to reveal high antioxidant activity. Antimicrobial activity of the extracts against *Staphylococcus* genus bacteria and *Candida* genus fungi was established. The study proved high capacity of ethanol extract for bacterial biofilm destruction.

Thus, the study showed the antimicrobial, antioxidant and antibiofilm-forming activity of tormentil ethyl extract against the isolates from the mouth cavities of patients suffering from parodentium inflammatory diseases, which fact contributes to the application prospects of this extract as an active base for mouth cavity hygiene preparations.

Key words: antimicrobial effect, antibiofilm formation, plant extracts, antioxidant activity, flavonoids, tannins.

Studies aimed at the search of natural substances with antimicrobial activity, including those derived from plants. This trend is connected with the diversity of biologically active compounds that have a broad spectrum of pharmacological activity and exhibit antioxidant, anti-inflammatory and even anticancer properties [1]. Substances of plant origin are widely used both in conventional and folk medicine, as well as in food, pharmaceutical and beauty industries. Studies aimed at the search of substances that, apart from their antimicrobial activity, can destroy bacterial biofilm are also of significant importance nowadays. The microorganisms of the biofilm are known to possess a higher level of resistance to antimicrobial preparations, and as such they serve an additional factor of pathogenicity [2, 3]. This problem is especially vital for mouth cavity diseases, where the prevailing majority of agents of inflammatory diseases are part of the biofilm, which complicates treatment of persisting diseases [4]. In our previous works, we showed the high percentage of antibiotic-resistant microorganism strains

within microbial associations of mouth cavity affected by chronic inflammatory process [5, 6]. In that case, it was *Staphylococcus* spp. genus bacteria and *Staphylococcus* spp. + *Candida* spp.; *Staphylococcus* spp. + *Enterobacteriaceae* spp. microorganism associations that were the dominating associates during an inflammatory process, on the background of the most complicated clinical course [7]. In [8] it was shown that the microorganisms being part of the biofilm were characterized by a higher level of resistance to antimicrobial preparations. This is why, the search of the substances with antimicrobial and antibiofilm-forming activities presented a particular interest. In our previous works we also showed the antimicrobial activity of essential oils and cowberry extract against clinical microorganism isolates [9, 10].

The *Potentilla* genus is a member of the *Rosaceae* family, *Rosoideae* subfamily, which is mainly distributed in temperate, arctic and Alpine zones of the Northern hemisphere. Extracts of the aerial and/or underground parts have been applied in traditional medicine for the treatment of inflammations, wounds, certain forms of cancer, infections due to bacteria, fungi and viruses, diarrhoea, diabetes mellitus and other ailments [11].

The substances extracted from rhizomes of *Potentilla* genus plants are known to possess antimicrobial properties, but no data on the effect of the extracts upon antibiotic-resistant clinical isolates and their antibiofilm-forming properties have been available so far.

The purpose of the work was to study the antimicrobial, antibiofilm, antioxidant and some biochemical properties of alcoholic extracts of *Potentilla erecta* L. rhizome.

Materials and Methods

The plant materials were collected in the vicinity of the village of Luta, Velyky Berezny rayon, Trancarpathia, dried at the temperature of 30–35 °C in shadow, then ground and placed in tightly closed containers.

Extracts manufacturing techniques. We made ethyl and methyl extracts of *Potentilla erecta* L. rhizome. A 10 g batch of dry plant material was pulverized to powdery mass. In an Erlenmeyer flask, 10 g of plant material was blended with 200 ml of or 96° ethyl or methyl alcohol (Sigma, Germany). The opening was closed with a food wrap to avoid evaporation. Following a 30-minute-long incubation in the ultrasonic bath (Kraintek) at 35 °C, the blend was filtered through Whatman No. 1 filter paper. The clear solution was placed in an evaporative device (16–17/32″×34-59/64″G5B, Coated Dry Ice Condenser Rotary Evaporator) to obtain pure alcoholic extract at 50 °C, 82 rpm. Then, extracts were subjected to evaporation under reduced pressure at 40 °C in order to remove or ethyl or methyl. As a result, the following pure extracts were obtained: ethyl extract of 0.50 g; methyl extract of — 1.07 g. For the purpose of study, 0.50 g of extract was chosen.

Antimicrobial assay. As test cultures, the following bacteria and yeasts from the American Type Culture Collection were used: *Candida albicans* ATCC 885-653; *Staphylococcus aureus* ATCC 25923; *Escherichia coli* ATCC 25922; *Enterococcus faecalis* ATCC 29212; *Streptococcus pyogenes* ATCC 19615; reference *S. aureus* CCM 4223 biofilm-forming strain. We also used clinical strains of bacteria and yeasts (*S. aureus, E. coli, S. pyogenes*, *E. faecalis, C. albicans*) isolated from the oral cavities of patients suffering from inflammatory periodontium and pharynx. We chose the clinical strains with multiple resistance at least to two classes of antibiotics. As a positive control were used: gentamicin (10 mg/disk) for Gram-negative bacteria, ampicilin (10 mg/disk) for Gram-positive bacteria, nystatin (100 UI) for *Candida*. As negative control were used DMSO.

The microorganisms from the oral cavities of patients with chronic periodontium inflammatory processes were isolated on the basis of the Dental Polyclinic, Uzhhorod National University; the extracts were manufactured and their antioxidative activity and contents of tannins and flavonoids were determined on the basis of the Department of Pharmacognosy and Botany, University of Veterinary Medicine and Pharmacy in Košice, Slovakia; the antimicrobial activity of plant extracts was studied at the Microbiological Laboratory of the Department of Genetics, Plant Physiology and Microbiology, Uzhhorod National University, and Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice.

Antimicrobial activity of *Potentilla erecta* L. rhizome extracts was determined using agar diffusion test [12]. The bacterium inocula 100 µl in the physiological solution were adjusted to the equivalent of 0.5 McFarland standard, and evenly spread on the surface of Muller-Hinton agar (incubated at 37±2 °C for 24 hours); yeasts — on SDA agar (incubated at 35±2 °C for 48 hours). The extracts 20 µl

were introduced into wells 6 mm in diameter. The diameters of the inhibition zones were measured in millimetres including the diameter of the well. Each antimicrobial assay was performed at least three times.

Determination of antibiofilm activity. The antibiofilm activity of the EO were tested in standard 96-well microtitration plates (Greiner-BioOne, Austria) using a modified staining method according to O' Toole [13].

With the purpose of study of the antibiofilm-forming activity, a 18-hour culture of the reference *S. aureus CCM 4223* biofilm-forming strain grown at 37 °C was used. Into the wells, 180 µl of bacterial suspension, Mc Farland in broth (TSB, Himedia, India) were introduced. The *Potentilla erecta* L. rhizome extracts dissolved to the concentrations of 1%, 5% and 10% in dimetylsulfoxide (DMSO; Sigma-Aldrich, USA) was introduced into the wells in the amount of 20 µl. Following the addition of the bacterial suspension, the concentration of plant extracts in the broth equaled to 0.1%, 0.05% and 0.01%, respectively. The wells with only 180 µl of broth and 20 µl of 10% DMSO served as the control.

Following a 24-hour-long incubation in the thermostat at 37°, the supernatant was withdrawn and washed 3 to 5 times with distilled water. Following a 30-minute-long incubation, it was dyed with 200 µl of 0.1% solution of crystal violet; then the dye was withdrawn, and the supernatant washed 3 to 5 times with distilled water. Into every well, 200 µl of 30% acetic acid were added and incubated for 10 min. The optical density was measured on the Synergy HT (Biotek, USA) spectrophotometer at 550 nm.

More than 50% reduction in absorbance of CV was considered as significant inhibition. Statistical Analysis Values mentioned are the mean with standard deviations, obtained from three different observations. Values in the control and treatment groups for various molecules were compared using Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

Antioxidant activity. Detection of free radical scavenging activity of the samples was measured with 2.2-diphenyl-1-picrylhydrazyl (DPPH) [14]. A sample of 0.1 ml was mixed with 1.9 ml of DPPH solution in methanol (0.06 mmol l^{-1}). The absorbance of the reaction mixture was detected with a spectrophotometer Beckman Coulter DU 530. Following incubation in dark for 30 min, the absorbance of each solution was measured at 515 nm (A). The antioxidant activity was expressed as percentage (%) of the scavenging activity. The percentage of DPPH radical scavenging activity was calculated by using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Abs (control)} - \text{Abs(sample)}}{\text{Abs (control)}} \times 100,$$

where Abs (control): Absorbance of DPPH radical + methanol; Abs (sample): Absorbance of DPPH radical + extract.

Determination of Total Tannins (TT). The content of tannins was determined using Folin-Ciocalteus method [15]. The absorbance was measured as the absorbance at 750 nm (A), with the use of water as the compensation liquid. The percentage of tannins expressed as pyrogallol was calculated based on the following expression:

$$\text{Tannins (\%)} = \frac{3.125 \times \text{A}}{0.316 \times m},$$

where m — mass of the sample to be examined, in grams; A — absorbance.

The absorbance of the reaction mixture was detected with a spectrophotometer Beckman Coulter DU 530v.

Determination of *Total Flavonoids (TF)*. The flavonoid content was determined by a colorimetric assay as described by aluminium chloride colorimetric method [15]. The absorbance of the test solution was measured at 425 nm with a spectrophotometer Beckman Coulter DU 530.

$$X = \frac{A \times 1.25}{m},$$

where A — absorbance at 425 nm; m — mass of the herbal drug to be examined in grams.

For the results of experiment, we used statistical software Microsoft Office-Excel (2013) with the calculation of averages, error, and standard deviation.

Results and Discussion

The studies have shown that the highest antimicrobial effect of the extracts was registered against *Staphylococcus* genus, *Enterococcus faecalis* bacteria and *Candida* genus microscopic fungi. It was established that the extracts possessed a distinguished antibacterial effect upon MRSA *S. aureus*. Their effect upon *E. coli* was significantly lower. No antibacterial effect of the extracts upon *Streptococcus pyogenes* has been ascertained. The antimicrobial activity of methyl and ethyl extracts would not differ

statistically significantly against bacterial isolates, though ethyl extracts showed a more distinguished antimycotic activity.

The study of the biochemical and antioxidant properties of the extracts has shown a high antioxidant level of tormentil rhizome ethyl and methyl extracts (Table 2).

The results of the present study suggested that the ethanol extract from *P. erecta* rhizome is characterized by high concentrations of tannins and flavonoids (Table 2). The study of the antibiofilm-forming ability of the extracts showed a high antibiofilm-forming effect of ethyl extracts from *Potentilla erecta* L. (Fig. 1). Thus, 0.1% ethyl extracts reduced the biofilm-forming activity of *S. aureus* CCM 4223 by 91.72% as compared with the control (ethyl alcohol). The reduction of extract concentration insignificantly affected the antibiofilm-forming properties of the extract. Say, 0.05% extract caused reduction of the antibiofilm-forming properties of staphylococci by 86.2%, and 0.01% extract — by 83.4%.

The study of antibiofilm-forming properties of methyl extract from *Potentilla erecta* L. rhizome showed that the use of 0.1% extract caused a 71.3% biofilm destruction; the use of 0.05% extract resulted in a 66.6% reduction of biofilm formation; the application of 0.01% extract led to a 50% reduction (Fig. 2).

Thereby, high antibiofilm-forming activity of ethyl and methyl extracts was recorded, however the antibiofilm-forming activity of ethyl extract was more expressive and did not reduce significantly with the reduction of extract concentrations.

The antimicrobial properties of tormentil have been shown in the works by other scholars. Say, tormentil rhizome extract was shown to have an effect against Gram-positive microorganisms that provoke food infections. 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

Table 1. **Antimicrobial activities of the *Potentilla erecta rhizome* extract against typical and clinic opportunistic infectious agents, mm ($n = 3$, $x \pm SD$)**

Test culture	Ethyl extract	Methyl extract
S. aureus ATCC 25923	17.67±0.58*	18.17±0.29*
S. aureus CCM 4223 (biofilm formation)	16.5±0.50*	17.67±0.58*
S. aureus MRSA (clinic), isolate from mouth cavity	16.0±0.50*	17.50±0.50*
Streptococcus pyogenes ATCC 19615	7.33±0.58*	7.50±0.80*
Streptococcus pyogenes (isolate from mouth cavity)	-	-
Escherichia coli ATCC 25922	11.17±0.29*	11.33±0.58*
Escherichia coli (isolate from mouth cavity)	8.17±0.29*	8.67±0.58*
Enterococcus faecalis ATCC 29212	15.67±0.58*	15.00±0.50*
*Enterococcus faecalis (*isolate from mouth cavity)	14.67±0.33*	14.67±0.33*
Candida albicans ATCC 885-653	20.33±0.58*	17.5±0.29*
*Candida albicans (*isolate from mouth cavity)	17.67±0.58*	12.33±0.58*

An extraction solvent (ethanol or methanol) were used as the control:
control of ethanol — no inhibition; control of methanol — no inhibition;
* the data were statistically significant as compared with the control ($P < 0.05$).

Table 2. **Level of tannins, flavonoids and antioxidant activity in ethyl and methyl extracts of P*otentilla erecta* L. rhizome**

Ethyl extract		Methyl extract	
Absorbance (nm)	%	Absorbance (nm)	%
tannins			
0.81*	8.04*	0.78*	7.74*
flavonoids			
0,112*	0.114*	0.11*	0.14*
antioxidant activity			
0.06*	88.44*	0.05*	91.08*

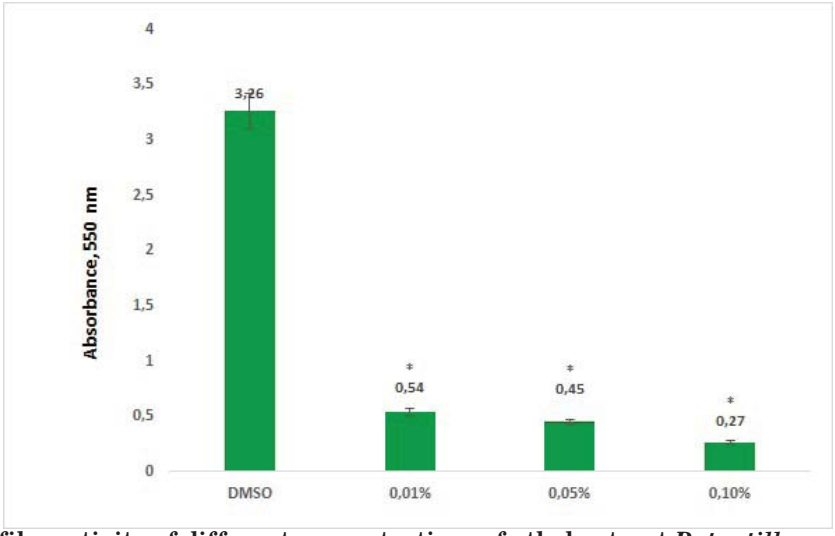

Fig. 1. **Antibiofilm activity of different concentrations of ethyl extract *Potentilla erecta* L. rhizome on biofilm-forming *S. aureus***
* the data were statistically significant as compared with the control ($P < 0.05$)

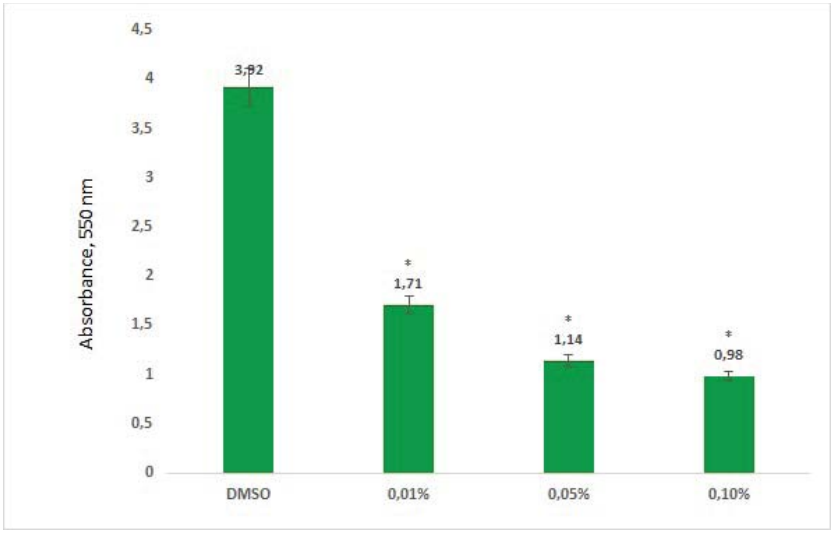

Fig. 2. **Antibiofilm activity of different concentrations of methyl extract *Potentilla erecta* L. rhizome on biofilm-forming *S. aureus***
* the data were statistically significant as compared with the control ($P < 0.05$)

inhibit the growth of Gram-negative bacteria [16]. Another work showed tha antibacterial and antimycotic activity of aqueous extracts.

Most of the biological effects of *Potentilla* species can be explained by the high amount of condensed and hydrolysable tannins present in the aerial and the underground parts, e.g. the antiviral and antimicrobial activities, immunomodulating effects, hepatoprotective and anti-inflammatory effects. Tannins have been known to be important constituents of *Potentilla* species and their extracts, respectively, and the cause for the astringent effects. Therefore thorough phytochemical studies on *Potentilla* species starting especially in the 1960s were primarily focussed on tannins [19].

Thus, our studies have demonstrated the antimicrobial activity of ethyl and methyl extracts of *Potentilla erecta* L. rhizome against *Staphylococcus* genus bacteria and *Candida* genus microscopic fungi. These trends were shown both on typical and clinical strains, the latter being isolated from the mouth cavities of patients suffering from chronic mouth cavity diseases and characterized by a high resistance to antibiotics. Ethyl extract of *Potentilla erecta* L. was shown to display a high antibiofilm-forming activity. A significant antioxidant activity of the reviewed extracts was also demonstrated. The obtained results indicated to good prospects for further research in order to create tormentil-based preparations as mouth cavity care and hygienic products, as far as they —

as contrasted with chemical preparations — may be used for a long period of time as part of mouth cavity care and preventive products; they as a rule have no side effects but possess an anastaltic effect and antioxidant properties. *Potentilla erecta* L. is a specially valuable plant product, for it has long since been used in folk pharmaceutics and medicine of concrete localities.

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АНТИМІКРОБНІ, АНТИБІОПЛІВКОУТВОРЮВАЛЬНІ ТА ДЕЯКІ БІОХІМІЧНІ ВЛАСТИВОСТІ ЕКСТРАКТУ КОРЕНЕВИЩА *Potentilla erecta*

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Метою роботи було дослідити антимікробні, антибіоплівкоутворювальні, антиоксидантні та деякі біохімічні властивості спиртових екстрактів кореневища *Potentilla erecta* L. Згідно з метою досліджень визначали антиоксидантну активність (DPPH методом), загальні таніни та флавоноїди (спектрометрично), антимікробну активність (дискодифузійним методом). Клінічні ізоляти виділяли з використанням диференційно діагностичних середовищ. Антибіоплівкоутворювальну здатність визначали у стандартних 96-лункових планшетах.

Показано високу антиоксидантну активність етилового та метилового екстрактів кореневища *Potentilla erecta* L. Встановлено антимікробну активність екстрактів стосовно бактерій роду *Staphylococcus* і мікроскопічних грибів роду *Candida* та високу здатність етилового екстракту до деструкції бактеріальної біоплівки.

Отже, дослідження показали антимікробну, антиоксидантну та антибіоплівкоутворювальну активність етилового екстракту калгану щодо ізолятів ротової порожнини людей із запальними захворюваннями пародонта, що зумовлює перспективність використання цього екстракту як активної основи препаратів для гігієни ротової порожнини.

Ключові слова: антимікробний ефект, утворення антибіоплівки, рослинні екстракти, антиоксидантна активність, флавоноїди, дубильні речовини.

АНТИМИКРОБНЫЕ, АНТИБИОПЛЕНКООБРАЗУЮЩИЕ И НЕКОТОРЫЕ БИОХИМИЧЕСКИЕ СВОЙСТВА ЭКСТРАКТА КОРНЕВИЩА *Potentilla erecta*

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Целью работы было исследовать антимикробные, антибиопленкообразующие, антиоксидантные и некоторые биохимические свойства спиртовых экстрактов корневища *Potentilla erecta* L. Согласно цели исследования определяли антиоксидантную активность (DPPH методом), общие таннины и флавоноиды (спектрометрически), антимикробную активность (дискодиффузионным методом). Клинические изоляты были выделены с использованием дифференциально диагностических сред. Антибиопленкообразующую способность определяли в стандартных 96-луночных планшетах.

Показана высокая антиоксидантная активность этилового и метилового экстрактов корневища *Potentilla erecta* L. Установлена антимикробная активность экстрактов относительно бактерий рода *Staphylococcus* и микроскопических грибов рода *Candida* высокая способность этилового экстракта к деструкции бактериальной биопленки.

Итак, исследования показали антимикробную, антиоксидантную и антибиопленкообразующую активность этилового экстракта калгана в отношении изолятов ротовой полости людей с воспалительными заболеваниями пародонта, что обусловливает перспективность использования данного экстракта в качестве активной основы препаратов для гигиены полости рта.

Ключевые слова: антимикробный эффект, образование антибиопленки, растительные экстракты, антиоксидантная активность, флавоноиды, дубильные вещества.