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

--

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

**M. Kryvtsova**

1Uzhhorod National University, Faculty of Biology, Department of Genetics, Plant Physiology and Microbiology, Ukraine

**J. Kosićová**

2University of Veterinary Medicine and Pharmacy in Košice, Department of Microbiology and Immunology, Slovakia

**J. Eftimova**

3University of Veterinary Medicine and Pharmacy in Košice, Department of Pharmacognosy and Botany, Slovakia

**M. J. Spivak**

4Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, Kyiv

E-mail: maryna.krivcova@gmail.com

Received 12.06.2019

Revised 27.08.2019

Accepted 30.10.2019

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 alcohol 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 parodontium 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.
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, ampicillin (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 dimetyl sulfoxide (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°C, 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 530v. The absorbance of the reaction mixture was detected with a spectrophotometer Beckman Coulter DU 530v. The absorbance of the test solution was measured at 425 nm with a spectrophotometer Beckman Coulter DU 530.

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

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 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

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:

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

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:

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

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.

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
Experimental articles

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

The study of antibiotic-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 antibiotic-forming activity of ethyl and methyl extracts was recorded, however the antibiotic-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 ± SD)**

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

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 *Potentilla erecta* L. rhizome**

<table>
<thead>
<tr>
<th></th>
<th>Ethyl extract</th>
<th>Methyl extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance (nm)</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>tannins</td>
<td>0.81*</td>
<td>0.78*</td>
</tr>
<tr>
<td>flavonoids</td>
<td>0.112*</td>
<td>0.11*</td>
</tr>
<tr>
<td>antioxidant activity</td>
<td>0.06*</td>
<td>0.05*</td>
</tr>
</tbody>
</table>

*88.44* | 91.08*
inhibit the growth of Gram-negative bacteria [16]. Another work showed the antibacterial and antmycotic 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 pharmacetics and medicine of concrete localities.

The work was carried out within the framework of the Academic Internship Program of Krivtsova MS. Erasmus +, Kosice University of Veterinary Medicine and Pharmacy, Department of Microbiology and Immunology according to the topic “Use of antimicrobial properties of medicinal herbs and probiotic materials as an alternative means of antibiotic therapy”.

REFERENCES


77
АНТИМІКРОБНІ, АНТИБІОПЛІВКОУТВОРЮВАЛЬНІ ТАДЕЯКІ БІОХІМІЧНІВЛАСТИВОСТІ ЕКСТРАКТУ КОРЕНЕВИЩА Potentilla erecta

М. Кривцова¹, Я. Коцьова², Я. Ефімова³, М. Я. Співак³

¹Ужгородський національний університет, біологічний факультет, кафедра генетики, фізіології рослин і мікробіології, Україна
²Університет ветеринарної медицини та фармації, Кошице, Словаччина
³Інститут мікробіології та вірусології ім. Д. К. Заболотного НАНУ, Київ

E-mail: maryna.krivcova@gmail.com

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 танини та флавоноїди (спектрометрично), антимікробну активність (дискодиффузионним методом). Клінічні ізоляти відділяли з використанням диференційно діагностичних середовищ. Антибіоплівкоутворювальну здатність визначали у стандартних 96-луночних 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 екстрактів кореневища Potentilla erecta L. Загальна танинна та флавоноїдна (спектрометрично), антимікробна активність (дискодиффузионним методом) і деструкція бактеріальної біоплівки.

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

Целью работы было исследовать антимикробные, антибоплёнокообразующие, антиоксидантные и некоторые біохімічні свійства спиртових екстрактів кореневища Potentilla erecta L. Согласно цели исследования определяли антиоксидантную активность (DPPH методом), общие танины и флавоноїди (спектрометрически), антимикробную активность (дискодиффузионным методом). Клинические изоляты были выделены с использованием дифференционально диагностиических сред. Антибоплёнокообразующую способность определяли в стандартных 96-луночных планшетах.

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

Итак, исследования показали антимикробную, антиоксидантную и антибоплёнокообразующую активность этилового экстракта кореневища Potentilla erecta L. Установлена антимикробная активность экстрактов относительно бактерий рода Staphylococcus и микроскопических грибов рода Candida высокая способность этилового экстракта к деструкции бактериальной биоплёнки.

Ключевые слова: антимикробный эффект, образование антибоплёнок, растительные экстракти, антиоксидантная активность, флавоноїди, дубильные вещества.