In Vitro Antimicrobial and Cytotoxicity Activities of Some Medicinal Plant Extracts against Oral Microbial Pathogens

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Abstract

Introduction: Medicinal plants have long been of great interest to scientists in the search for the best treatment of diseases, especially the infectious diseases. In recent years, the use of herbal medicines has become more well-known because of their antimicrobial, anti-fungal, anti-cancer and less side effects.

Aim: The aim of this study was to investigate the antimicrobial and antifungal effects of Urtica dioica, Equisetum arvense, and Punica Granatum peel extracts on two common oral microorganisms, Streptococcus mutans and Candida albicans.

Materials and methods: The study investigated the hydro-alcoholic extract of the plants. The antimicrobial activity of the extracts was evaluated using the method of measuring the inhibition of microorganisms, and the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined using different concentrations of the extracts and also biofilm assay and SEM were determined. Also cell viability was assessed by MTT assay on human gingival fibroblast cells.

Results: The lowest MIC against S. mutans and C. albicans was related to the hydro-alcoholic extract of U. dioica. There was a significant reduction in the microbial biofilms by all three extracts. Among them, U. dioica could decrease the biofilms of S. mutans and C. albicans more than other extracts. In addition, the best results for growth inhibition zone were the hydro-alcoholic extracts of E. arvense and U. dioica with 35 and 30 mm growth zone, respectively. The results of SEM showed that P. granatum peel, U. dioica and E. arvense could destroy microbial biofilms without exerting any cytotoxic effects on HGF cell.

Conclusions: The results of the study suggest that U. dioica, E. arvense, and P. Granatum peel extracts can be used as mouthwash with the least significant difference with routine mouthwashes. Also, the plant-based mouthwashes may be more suitable substitutes for chemical types in the future.

Keywords

chlorhexidine, Equisetum arvense, fluoride, nystatin, Punica Granatum peel, Urtica dioica

INTRODUCTION

One of the effective ways to reduce the number of bacteria in the oral cavity is using disinfectant solutions that are used as a mouthwash.¹ Daily use of mouthwash with regular toothbrushes and dental floss effectively reduces the bacterial population of the mouth and ultimately prevents gum disease and tooth decay and promotes wound healing.
Among the types of mouthwash that are used in dentistry, chlorhexidine, fluoride, and nystatin have a special place in the variety of mouthwashes used in dentistry, which is the main cause of this large effect on Gram-positive and negative, non-toxic, its effect is durable. But they also have side effects, including discoloration of the teeth, sensitivity and burning of the oral mucosa, changes in the sense of taste and sometimes swelling of the parotid glands. Some of the most important properties of a good disinfectant can be 1) the antimicrobial effect, 2) the disinfectant property, 3) reasonable price, 4) color and grace acceptable, 5) easy access, 6) its non-toxicity. From the past, the use of medicinal plants has been of great interest in the treatment of diseases, especially infectious diseases. In recent years, the use of herbal medicines has become commonplace due to the antimicrobial, antifungal, anti-cancer, and fewer side effects of oral hygiene. Plant-based mouthwashes are more appropriate than chemical mouthwashes due to their antimicrobial, antifungal, anti-cancer properties, and fewer side effects of oral hygiene. Plant-based mouthwashes are more appropriate than chemical mouthwashes due to their antimicrobial, antifungal, anti-cancer properties, and fewer side effects of oral hygiene. However, the use of herbal mouthwash, the complications listed for chemical mouthwashes (such as the effects of chlorhexidine) can be reduced.

*Streptococcus mutans*, Gram-positive cocci shaped bacteria are part of the natural flora of the human mouth, also known as the most important cause of tooth decay. Growth and metabolism of *S. mutans* alter the environmental conditions of the oral flora and this can rapidly colonize the organisms and cause dental plaque.

Candidiasis is the most common oral fungal infection and is caused by *Candida* fungi. In terms of mycology, 10 types of *Candida* are important, which *Candida albicans* is found to be normal flora in the mouth and is also one of the most important causes of oral infectious diseases. Using the disinfectant solution as a mouthwash is one of the effective ways to reduce the number of microorganisms in the oral cavity. Daily use of mouthwash with regular toothbrushes and dental floss reduces effectively the microbial population of the mouth and ultimately prevent the periodontal disease, dental caries, and promote wound healing. Among the types of mouthwash that are used in dentistry, chlorhexidine, fluoride, and nystatin have a special place due to its effect on Gram-negative and Gram-positive bacteria, non-toxicity to the host cells and its stability. But they also have side effects, including discoloration of the teeth, sensitivity and burning of the oral mucosa, changes in the sense of taste and sometimes swelling of the parotid glands. The most important properties of a good disinfectant can be an antimicrobial effect, reasonable price, acceptable odor and grace, easy access, and non-toxicity.

From the past, the use of medicinal plants in the treatment of diseases, especially microbial infections, has been highly regarded. As well as, recently, the use of herbal medicines has become commonplace due to the antimicrobial, antifungal, anti-cancer properties, and fewer side effects of oral hygiene. Plant-based mouthwashes are more appropriate than chemical mouthwashes due to their natural ingredients for compatibility with body physiology and lower probability of poisoning.

Based on the literature, antimicrobial effects of the *Urtica dioica* L. leaf have been performed and proven. The *U. dioica* has long been known as the herbal remedy, especially in Asia and South America. Oral gargle with herbs is a necessary factor to relieve swelling and gum injuries. In previous studies, the effects of this plant have been proven on some bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*. The *Equisetum arvense* is a medicinal plant that has been used in Europe and China since ancient times. Studies have been conducted on the antibacterial and antifungal effects of the *E. arvense* on *S. aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *C. albicans*, and *Saccharomyces cerevisiae*. *Pomegranate* is a tree fruit called *Punica granatum*. According to the previous studies, the antibacterial and antifungal effects of ethanol, methanol, and Estonia extracts of *P. granatum* have been partially proven. Although traditional medicine uses different herbs as antiseptic, anti-inflammatory, and rheumatoid or ulcerative medicines, there is no investigation about the use of these plants and comparing them with traditional irrigation solutions in dentistry.

**AIM**

Since *U. dioica*, *E. arvense*, and *P. granatum* have many biological properties such as antimicrobial and anti-inflammatory effects, this current study aimed to investigate the effects of their hydro-alcoholic extract as mouthwash on *S. mutans* and *C. albicans* compared with chlorhexidine, fluoride, and nystatin as standard mouthwashes. It is hypothesized that the medicinal plant extracts can have antimicrobial properties similar to conventional mouthwashes.

**MATERIALS AND METHODS**

**Preparation of plant extracts**

**Preparation of P. granatum peel extract**

The plants tested in this study were prepared and approved by the Herbarium Department of the Faculty of Pharmacy, University of Tehran. In order to prepare the aqueous extract, fresh fruits of *P. granatum* peel were handpicked, washed, and peeled. The peeled fruits were boiled in distilled water for 1 hour to obtain a uniform and concentrated solution which was then filtered using Whatman paper.

**Preparation of U. dioica and E. arvense extracts**

The hydro-alcoholic extract of *U. dioica* and *E. arvense* were
prepared based on the previous study. Briefly, the plants were washed, wiped, and dried; their required parts including leaves and stems were powdered by electric grinding. Then 50 gr of the plant powder was poured into the decanter tank and 70% ethanol was added to it, which was 3-4 cm higher than the powder inside the decanter. After 72 hours, the separatory funnel tap was opened and the extracts were removed. These steps were repeated after 48 and 24 hours.

All extracts were transferred to a rotary evaporator apparatus (Heidolph, Germany) to evaporate the solvent and concentrate. and the resulting materials were then converted to dry powder at 40°C. 100 mg of each extract was dissolved in 1 ml of sterile distilled water and was filtered under sterile conditions with a 0.22 µm pore filter under the hood. Eventually, the extract storage solution (100 mg/mL) was stored in a sterile and dark glass container at 4°C prior to its use.

Antibacterial activity

Preparation of the bacterial and fungal strains

S. mutans strain ATCC 35688 and C. albicans ATCC 10231 were purchased from Pasteur Institute of Tehran. C. albicans was incubated in Sabouraud dextrose broth (SDB) (QUELAB LABORATORIES INC, Canada) for 24 hours at 37°C in aerobic conditions and then yeast cells were grown on a Sabouraud dextrose agar (SDA) (QUELAB LABORATORIES INC, Canada) plate for 48 hours at 37°C. An inoculum suspension was prepared by selecting 5 colonies of C. albicans and suspending them in 5 mL of SDB. After incubation for 24 hours at 37°C, the suspension was retracted and the cell density was adjusted with a spectrophotometer by adding sufficient sterile SDB to increase the transmittance to 0.5 McFarland's standard at 660 nm. The inoculum suspension was diluted until an optical density of 0.08-0.013 was obtained, resulting in a suspension of 10^8 cells/mL of yeast cells. S. mutans were cultured overnight in 5 mL of Braun Heart Infusion broth (BHI) (QUELAB LABORATORIES INC, Canada) at 37°C and 5% CO₂. Ultimately, the bacterial suspension was adjusted to 0.5 McFarland's standard similar to the C. albicans.

Determination of the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and the minimum fungicidal concentration (MFC)

MIC

Jorgensen and Turndige's method was used to determine the MIC of alcoholic and aqueous extracts of the studied plants. For this purpose, 100 µL of BHI broth medium was poured into 96 well microplate wells. Then, 100 µL of the stock of each extract was inoculated separately in the first well of each row of the microplate. After several times of pipetting, 100 µL of the contents of the first well was inserted into the second well and again after several times of pipetting, 100 µL of the contents of the second well was transferred to the third well and continued to 10 wells. In the final well, after several times of pipetting, 100 µL of the well was discarded. In this case, each extract was diluted 1:2 (in all wells). Then 100 µL of microbial suspensions of S. mutans and C. albicans was added to each well with a final concentration of 1.5×10^6 CFU/mL (dilution of 10/10 microorganisms). The microplates were then incubated for 24 hours at 37°C under the conditions required for the growth of each microorganism. After 24 hours, the minimum concentration of each extract that was capable of inhibiting microbial growth was considered as MIC.

MBC and MFC

To determine the minimum lethal concentration of the extracts, microplate wells in which microbial growth was not observed were performed using the spot culture method. For this purpose, 2 µL of the well contents that showed no microbial growth was poured into Müller Hinton (MH) agar (Merck, Germany). After incubation for 24 hours at 37°C under the conditions required for the growth of each microorganism, the cultured plates were monitored for microbial growth. MBC and MFC were considered as the minimum concentration of each extract that was capable of killing S. mutans and C. albicans, respectively up to 99.9%.

Disk agar diffusion (DAD) test

The antimicrobial and antifungal effects of alcoholic extracts released from disks on agar medium were determined by disk agar diffusion (DAD) assay. In this test, each of the microorganisms was prepared with 200 µL of 0.5 McFarland's microbial suspension (1.5×10^8 CFU/mL) in MH broth (Merck, Germany) and cultured on the MH agar using sterile swabs. Then, 20 µL of each extract with MIC, and 2-8× MIC doses obtained from the previous section, chlorhexidine 0.2% (Behsa hydro-alcoholic Pharmaceutical Company, Iran), 0.2% fluoride (Behsa Pharmaceutical Company, Iran), and nystatin 100,000 (Emad Pars Therapy) were inoculated on blank disks as the control group. After drying, the disks were placed at 2 cm intervals on a plate surface. The plates were incubated at 37°C for 24 hours under appropriate conditions based on the type of microorganism. After incubation, the growth inhibition zone around the disk was measured.

Determination of the anti-biofilm activity of plant extracts

First, 100 µL of the extracts at MBC/MFC doses was added to each of the 96 well microplate wells. Then, 100 µL of each of the microorganisms was added to wells with a final concentration of 1.5×10^8 CFU/mL. The microplates were incubated for 24 hours at 37°C under the conditions required for microbial growth. Microbial growth was poured into Müller Hinton (MH) agar (Merck, Germany) and cultured on the MH agar. After incubation for 24 hours at 37°C under the conditions required for the growth of each microorganism, the cultured plates were monitored for microbial growth. MBC and MFC were considered as the minimum concentration of each extract that was capable of inhibiting microbial growth was considered as MIC.
required for the growth of each microorganism. Ampicillin was used as a positive control and S. mutans and C. albi-
cans as the negative controls. After 24 hours of incubation, all wells were slowly evacuated and 200 µL of pure ethanol was added to the wells to fix the microbial biofilm and the microplate was kept at room temperature for 10 min. Etha-
hol was then removed, and 200 µL of 1% crystal violet was added to the wells and the microbial biofilms were kept at room temperature for 20 min. After dyeing and washing with distilled water, 33% acetic acid was added to the wells and absorbance of all wells was read with a spectropho-
tometer (BioTek Elx 808, USA) at 550 nm. Each test was repeated three times.27

Biofilm assay using scanning electron microscopy (SEM)

Antibiofilm effects of extracts on S. mutans and C. albi-
cans were evaluated by SEM based on a previous study.28
Briefly, 100 µL of each of the microorganisms with a final
concentration of 1.5x10^8 CFU/mL was cultured on dental
slabs. To form the microbial biofilm, the dental slabs were
incubated at 37°C for 1 week. Then, 100 µL of each extract
at MBC doses was added to the microbial biofilm and the
dental slabs were incubated for 24 hours at 37°C. After
dehydration of solution on the slabs using a graded series
of ethanol (60%, 70%, 80%, 90%, and three times in 100%)
for 10 min each, it was dried and the slide was observed
under the SEM.

Cell viability assay

The extracts were diluted with sterile distilled water at a
rate of 0.12 mg/mL and were filtered using a syringe filter
with a 0.22-µm pore size. Human gingival fibroblast cells
(HGF, CELL NO. IBRC C10459) were obtained from the
National Center for Biological Genetic Resources of Iran.
The cell line then was cultured in Dulbecco’s Modified Eagle
Medium (DMEM) medium (Gibco, Germany) supple-
mented with 10% fetal bovine serum, 0.1 mg/mL strep-
tomycin, and 100 U/mL penicillin, and then incubated
at 37°C in a 5% CO2 incubator. After 24 hours of incuba-
tion, HGF cells were then treated with different extracts at
MBC/MFC doses.

Finally, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-
tetrazolium bromide (MTT) colorimetric assay was used
to determine the cytotoxic effects of extracts on HGF cells.
After 24 hours, the supernatant was removed from the cells
and 10 µL of MTT (stock solution; sib) was added. The
microplate was then incubated at 37°C for 4 hours and
then resuspended in 100 µL of dimethyl sulfoxide (DMSO).
Finally, cellular uptake was measured at 570 nm using a
spectrophotometric microplate reader (BioTek Elx 808,
USA). Cytotoxicity was calculated by the following formula:
% of cytotoxicity rate = 1− (OD drug exposure / OD con-
control)×100

Statistical analysis

All experiments were repeated three times. The results
were analyzed using SPSS 18 software. One-way ANOVA
was used for statistical analysis. P-values less than 0.05 were
considered statistically significant.

RESULTS

Determination of the MIC, MBC, and
MFC against S. mutans and C. albicans

The results of the MIC test to evaluate the antibacterial and
antifungal activity of hydro-alcoholic extracts of U. dioica
and E. arvense, as well as the aqueous extract of P. granatum
peel against S. mutans and C. albicans are shown in Table
1. The lowest MIC against S. mutans and C. albicans was
related to the hydro-alcoholic extract of U. dioica, which
had better results than other extracts.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Extracts</th>
<th>MIC (mg/mL)</th>
<th>MBC/MFC (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. granatum peel</td>
<td>7.5</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td>U. dioica</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>E. arvense</td>
<td>120.0</td>
<td>120.0 &lt;</td>
<td></td>
</tr>
<tr>
<td>S. mutans</td>
<td>P. granatum peel</td>
<td>60.0</td>
<td>120.0</td>
</tr>
<tr>
<td></td>
<td>U. dioica</td>
<td>30.0</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>E. arvense</td>
<td>120.0</td>
<td>120.0 &lt;</td>
</tr>
</tbody>
</table>

Disk agar diffusion test

According to the results presented in Table 2, among the
extracts investigated, P. granatum peel (8×MIC) was able
to show a higher growth inhibition zone on C. albicans,
whereas E. arvense growth inhibition zone (8×MIC) was
higher against S. mutans compared to other extracts. As
Table 2 showed, the best results for the growth inhibition
zone were the hydro-alcoholic extracts of E. arvense and U.
dioica with 34 and 29 mm growth zone at a 8×MIC dose,
respectively, and CHX had the highest growth inhibition
zone in the other cases. Nystatin mouthwash was also more
effective than extracts against C. albicans. In contrast, fluo-
ride mouthwash had no inhibitory effect on any microor-
ganism (p>0.05).

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Determination of the anti-biofilm potential of the extracts

The activity of plant extracts at MBC/MFC doses against the total biomass of S. mutans and C. albicans are shown in Fig. 1. Based on the results, all three extracts were able to significantly reduce the microbial biofilm of S. mutans and C. albicans. Among the extracts, U. dioica reduced S. mutans and C. albicans biofilms more than E. arvense and P. granatum peel.

The results showed that there was no considerable difference between plant extracts and CHX (p>0.05). In addition, nystatin and fluoride could not exhibit a good inhibitory effect on the planktonic phase of the two microorganisms studied. According to the results, the U. dioica showed the best inhibitory effect on both of microorganisms.

Biofilm assay using SEM

SEM images of microbial biofilms indicate the attachment of S. mutans and C. albicans on dental slabs (Fig. 2).

Table 2. The growth inhibition zone of S. mutans and C. albicans via different groups

<table>
<thead>
<tr>
<th>Micriorganism</th>
<th>Extracts</th>
<th>8×MIC</th>
<th>4×MIC</th>
<th>2×MIC</th>
<th>1×MIC</th>
<th>CHX</th>
<th>Fluoride 0.2%</th>
<th>Nystatin 100,000 IU</th>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. mutans</td>
<td>P. granatum peel</td>
<td>15</td>
<td>13</td>
<td>10</td>
<td>8</td>
<td>24</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>U. dioica</td>
<td>9</td>
<td>8.5</td>
<td>-</td>
<td>-</td>
<td>19</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>E. arvense</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>22</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>C. albicans</td>
<td>P. granatum peel</td>
<td>20</td>
<td>15</td>
<td>11</td>
<td>-</td>
<td>28</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>U. dioica</td>
<td>29</td>
<td>26</td>
<td>-</td>
<td>-</td>
<td>28</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>E. arvense</td>
<td>34</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>-</td>
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</tr>
</tbody>
</table>

As shown in the figure, P. granatum peel, U. dioica and E. arvense could destroy microbial biofilms.

Cell viability assay result

The cytotoxicity of HGF cells non-targeted and HGF cells-targeting U. dioica, E. arvense and P. granatum peel was evaluated. The results of MTT assay showed non-significant cytotoxicity against HGF cells, with more than 65.8%, 80.75%, and 99.5% cell viability for E. arvense, U. dioica, and P. granatum peel, respectively, which suggested that these extracts had the least toxicity to the eukaryotic cells (Fig. 3).

DISCUSSION

The use of mouthwashes is nowadays expanding due to the increasing incidence of dental caries and periodontal diseases. From a dental perspective, a good mouthwash should have properties such as discoloration of teeth and muco-
Antimicrobial Activities of Three Plant Extracts

Figure 2. SEM images of C. albicans (A) and S. mutans (B) biofilm removal after different treatment modalities at 5000× magnification (scale bar represents 10 μm). A: a) C. albicans (control); b) nystatin; c) P. granatum peel; d) U. dioica, and e) E. arvense. B: a) S. mutans (control); b) CHX; c) P. granatum peel; d) U. dioica, and e) E. arvense.

Figure 3. Cell viability of treated HGF cell by MTT assay: a) Control (HGF cell without treatment); b) P. granatum peel; c) U. dioica, and d) E. arvense. ×40 magnification.

sa, minimal toxic effects, and appropriate taste. However, a mouthwash that has all of these properties has not yet entered the market, and researchers are continuing to provide a mouthwash that has the maximum benefits.6–8 In recent years, due to the natural properties and lower side effects of herbal remedies, the tendency to use these medicines for treatment and prevention of diseases worldwide has increased dramatically, especially in Iran.9,10,12 In the present study, the effect of hydro-alcoholic extracts of U. dioica and E. arvense and aqueous extract of P. granatum peel on S. mutans, one of the most important and most pathogenic caries microorganisms, plays a major role in the onset of caries, as well as C. albicans, the most important infectious agents of oral diseases were studied.

The results of this study showed that the lowest MIC among the extracts was related to the hydro-alcoholic extract of U. dioica, which showed better inhibition than C. albicans and S. mutans. The results are consistent with
a review article published by Zouari Bouassida, which focuses on the effect of *U. dioica* on about 30 organisms. They explained that *U. dioica* can be used as an appropriate antibacterial material.\(^{29}\) Also, Iyoti et al. reported the antibacterial effect of *U. dioica* with silver nanoparticles on a group of Gram-positive and Gram-negative bacteria such as *B. subtilis*, *B. cereus*, *S. epidermidis*, *S. aureus*, *E. coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Serratia marcescens*.\(^{30}\) Also, Proestos et al. showed that *U. dioica* can have very strong antibacterial activity against *S. aureus*, *Listeria monocytogenes*, and *B. cereus* while it has weak antimicrobial effect against *P. putida* and *E. coli* O157:H7.\(^{17}\) In the study by Vatansever, it was demonstrated that nettle slightly reduced *P. fluorescens* but didn't show antibacterial activity against other tested bacteria including *E. coli* and *P. aeruginosa*.\(^{24}\)

Also, according to the results of the DAD test, the highest inhibition zone belonged to *E. arvense* extract on *S. mutans* followed by the hydro-alcoholic extract of *U. dioica* and *P. granatum* peel. None of the fluoride and nystatin mouthwashes investigated in the study prevented the growth of *S. mutans*. That was not unexpected for nystatin due to its antifungal mechanism and results, but for fluoride, the results were consistent with those reported by Akhlaghi et al.\(^{31}\) In their study, fluoride had no inhibitory effect on *S. mutans*.\(^{31}\)

In 2015, de Queiroz et al. investigated the ethanolic and methanolic extract of *Equisetum hyemale* from *E. arvense* plant. In this study, the researchers investigated the effects of this plant on oral microorganisms including *S. aureus*, *S. salivarius*, *S. sanguis*, *S. mitis*, *S. mutans*, *S. sobrinus*, and *Lactobacillus casei* and found that the plant had a good antibacterial effect on oral bacteria.\(^{32}\) In another study, Ferrazzano et al. assessed the antibacterial properties of a group of plants. Their study referred to *Equisetum hyemale*, a species of horse-tail plant, which has been shown to have a good antibacterial effect. In particular, it has an inhibitory effect on *S. mutans*, which is in agreement with the findings of the present study.\(^{33}\) In the study by Canadanovic-Brunet et al., the antioxidant and antibacterial effects of aqueous extracts, N-butanol, and ethyl acetate on *E. arvense* were determined against *E. coli*, *P. aeruginosa*, *B. cereus*, and *S. aureus*. They believed that although the antibacterial effects of this plant were milder than those of herbal antibiotics, it could prevent the growth of the bacteria they studied.\(^{19}\)

In the present study, *Pomegranate peel* showed less strong effect than CHX and nystatin mouthwashes.\(^{23,34,35}\)

Concerning tests of the effects of the extracts investigated on the microbial biofilm, based on the results of the present study, CHX showed the best inhibitory effect on both microorganisms and since the microbial biofilm was much stronger than the planktonic phase, CHX alone was used as standard antibacterial to investigate the effects of the tested extracts on the microbial biofilm of *S. mutans* and *C. albicans*. The present study was in agreement with the results of the experiments of Cesur et al. that investigated the effect of nettle extract on Salmonella biofilm.\(^{36}\) In another study, Das et al. studied the effects of *E. arvense* on the biofilm of *C. albicans* and *C. glabrata*. They concluded that *E. arvense* could be a valuable source for natural anti-candida agents.\(^{37}\) The results of a study by de Oliveira et al. about the effects of *E. arvense* and *P. granatum* peel extracts on *C. albicans* biofilm were also quite consistent with the results of the inoculation study.\(^{38}\) In a study, Menezes et al. evaluated the efficacy of hydroalcoholic *Pomegranate* extract on orthodontic dental plaques in 60 patients and compared to CHX as a standard. In their study, two-stage orthodontic plates were tested: once before mouthwash and once after. Compared to CHX, *Pomegranate* extract showed better antibacterial results.\(^{39}\) In addition, Dazal et al. confirmed that ethanolic extracts of *P. granatum* and *Pisum sativum* prevented the formation of *Pseudomonas* biofilm.\(^{40}\) Wojnicz et al. also investigated the effects of some herbal extracts, including *E. arvense* and *U. dioica* on *E. coli*. Although the other herbal extracts had better inhibitory effects than those of *E. arvense* and *U. dioica*, they concluded in their study that these extracts had good antibacterial effects, especially in the biofilm inhibition of *E. coli*.\(^{41}\)

To the best of our knowledge, this study was the first report of the simultaneous antimicrobial, anti-biofilm, cytotoxicity activities of the crude extracts from *U. dioica*, *E. arvense*, and *P. granatum* peel. The high antimicrobial and anti-biofilm activities of the crude extracts without cytotoxic effects suggest that *U. dioica*, *E. arvense*, and *P. granatum* peel are potential sources of dental care products. However, more in-depth studies are needed to confirm the findings of the present study.

CONCLUSIONS

The results of the study suggest that the extract of the medicinal plants *U. dioica*, *E. arvense*, and *P. granatum* peel not only have antimicrobial activity against *S. mutans* and *C. albicans* in liquid and solid agar media but also possess anti-biofilm activity without cytotoxicity for HGF cells. The present results demonstrate that the medicinal plants could be potentially used as natural additives in the mouthwash.

Acknowledgements

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Противомикробная и цитотоксическая активность экстрактов некоторых лекарственных растений in vitro в отношении микробных патогенов полости рта

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Резюме

Введение: Лекарственные растения издавна интересовали учёных, ищущих лучшее средство от болезней, особенно инфекционных. В последние годы использование медицинских препаратов на основе лекарственных трав стало ещё более популярным из-за их антимикробного, противогрибкового, противоракового действия и меньшего количества побочных эффектов.

Цель: Целью данного исследования было изучить антимикробное и противогрибковое действие экстрактов коры Urtica dioica, Equisetum arvense и Punica Granatum на два распространённых микроорганизма – Streptococcus mutans и Candida albicans.

Материалы и методы: В ходе исследования был изучен водно-спиртовой растительный экстракт. Антимикробную активность экстрактов оценивали путём измерения ингибирования микроорганизмов, а минимальную ингибирующую концентрацию (МИК) и минимальную бактерицидную концентрацию (МБК) определяли с помощью различных концентраций экстрактов, также были выполнены анализ биоплёнок и сканирующая электронная микроскопия (СЭМ). Также жизнеспособность клеток оценивали с помощью МТТ-анализа на клетках фактора роста гепатоцитов (HGF).

Результаты: Самый низкий МИК против Streptococcus mutans и Candida albicans был связан с водно-спиртовым экстрактом Urtica dioica. Было отмечено значительное снижение микробных биоплёнок из всех трёх экстрактов. Среди них Urtica dioica может уменьшить биоплёнки Streptococcus mutans и Candida albicans в большей степени, чем другие экстракты. Кроме того, лучшие результаты по ингибированию зоны роста были у водно-спиртового экстракта Equisetum arvense и Urtica dioica с зоной роста 35 и 30 мм соответственно. Результаты SEM показали, что Punica Granatum peel, Urtica dioica и Equisetum arvense могут разрушить микробные биоплёнки, не оказывая цитотоксического действия на клетки фактора роста гепатоцитов (HGF).

Заключение: Результаты исследования доказывают, что экстракты коры Urtica dioica, Equisetum arvense и Punica Granatum можно использовать в качестве жидкости для полоскания рта с наименьшим отличием от обычной жидкости для полоскания рта. Кроме того, жидкости для полоскания рта на растительной основе могут быть более подходящими заменителями химических веществ в будущем.

Ключевые слова
хлоргексидин, Equisetum arvense, фторид, нистатин, кора Punica Granatum, Urtica dioica