folia medica

Folia Medica 63(6):932-40
DOI: 10.3897/folmed.63.e56840

Original Article

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

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Received: 23 July 2020 ♦ **Accepted:** 4 Sep 2020 ♦ **Published:** 31 Dec 2021

Citation: Amirinia F, Rad HS, Pourhajibagher M. In vitro antimicrobial and cytotoxicity activities of some medicinal plant extracts against oral microbial pathogens. Folia Med (Plovdiv) 2021;63(6):932-40. doi: 10.3897/folmed.63.e56840.

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, antifungal, 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. mutants* 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.[2] 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.[3-5] Some of the most important properties of a good disinfectant can be 1) the antimicrobial effect, 2) the disinfectant property, 3) reasonable price, 4) odor and grace acceptable, 5) easy access, 6) its non-toxicity.[6-8] From the past, the use of medicinal plants has been of great interest in the treatment of diseases, especially infectious disease.[9-11] 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.[9-12] Plant-based mouthwashes are more appropriate than chemical mouthwashes due to their natural ingredients for compatibility with body physiology and lower probability of poisoning.[13] Therefore, with 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.[14]

Using the disinfectant solution as a mouthwash is one of the effective ways to reduce the number of microorganisms in the oral cavity.[1] Daily use of mouthwash with regular toothbrushes and dental floss reduce 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.[2] 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.[3-5] The most important properties of a good disinfectant can be an antimicrobial effect, reasonable price, acceptable odor and grace, easy access, and non-toxicity.[6-8]

From the past, the use of medicinal plants in the treatment of diseases, especially microbial infections, has been highly regarded.[9-11] 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.[9,10,12] Plant-based mouthwashes are more appropriate than chemical mouthwashes due to their natural ingredients for compatibility with body physiology and lower probability of poisoning.[13]

Based on the literature, antimicrobial effects of the *Urtica dioica* L leaf have been performed and proven.[15] 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.[16] In previous studies, the effects of this plant have been proven on some bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli.*[15-17]

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.*[18-21]

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.[6,14-16,22] 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.[23]

Preparation of U. dioica and E. arvense extracts

The hydro-alcoholic extract of *U. dioica* and *E. arvense* were

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prepared based on the previous study.[24] 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.[24]

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.[24,25]

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 vibrated 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% CO_2. 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 Turnidge's method was used to determine the MIC of alcoholic and aqueous extracts of the studied plants.[26] For this purpose, 100 µL of BHI broth medium was poured into 96 well bottom 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.[10] 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%.[10]

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 the growth of each microorganism. Ampicillin was used as a positive control and *S. mutans* and *C. albicans* 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. Ethanol 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 spectrophotometer (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. albicans* were evaluated by SEM based on a previous study.[28] Briefly, 100 µL of each of the microorganisms with a final concentration of 1.5×10^8 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) supplemented with 10% fetal bovine serum, 0.1 mg/mL streptomycin, and 100 U/mL penicillin, and then incubated at 37°C in a 5% CO_2 incubator. It was incubated at 37°C with 5% CO_2 and 95% humidity. The culture medium was changed every three days. In the third subculture, a seeding density of $3\text{-}4\times10^5$ cells/well was placed in a flat-bottomed 96-well cell culture microplate and allowed to adhere for 24 hours at 37°C in a CO_2 incubator. After 24 hours of incubation, HGF cells were then treated with different extracts at MBC/MFC doses.

Finally, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium 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 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. mutants* and *C. albicans* was related to the hydro-alcoholic extract of *U. dioica*, which had better results than other extracts.

Table 1. The MIC, MBC, and MFC of different extracts against *S. mutans* and *C. albicans*

Microorganism	Extracts	MIC (mg/mL)	MBC/MFC (mg/mL)
C. albicans	*P. granatum peel*	7.5	15.0
	U. dioica	1.0	2.0
	E. arvense	120.0	120.0 <
S. mutans	*P. granatum peel*	60.0	120.0
	U. dioica	30.0	60.0
	E. arvense	120.0	120.0 <

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, fluoride mouthwash had no inhibitory effect on any microorganism ($p>0.05$).

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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. mutants* 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)**. 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-

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

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

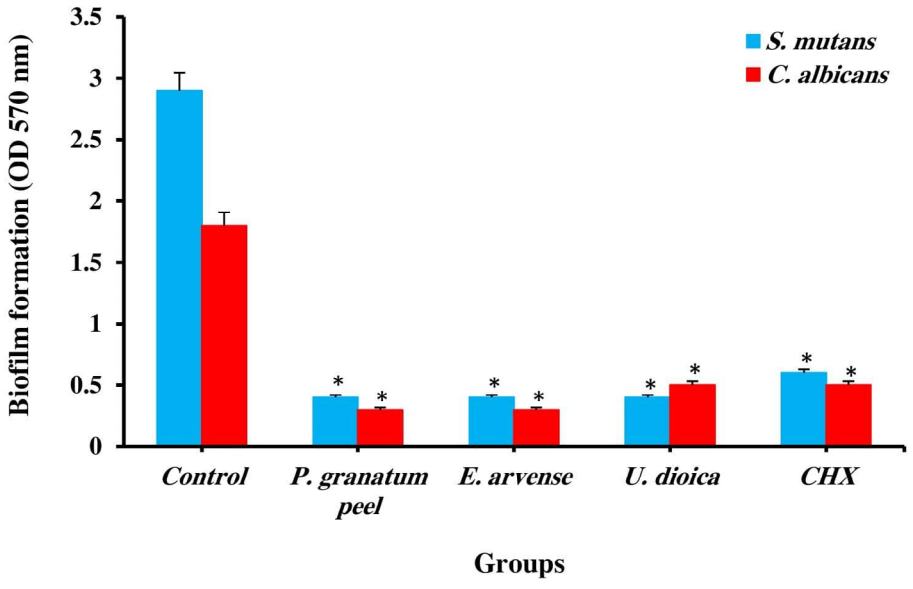

Figure 1. Determination of anti-biofilm effects of different plant extracts against *S. mutans* and *C. albicans*.

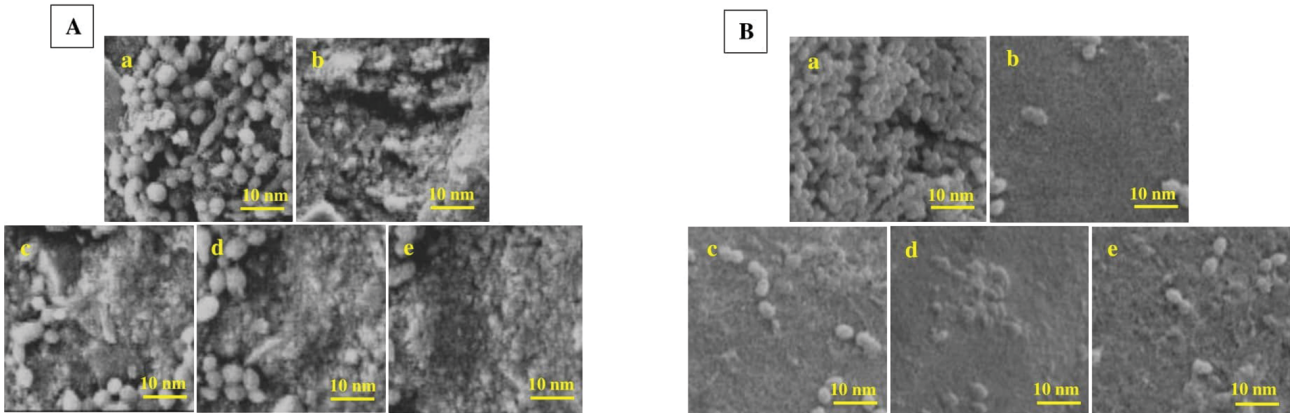

Figure 2. SEM images of *C. albicans* (**A**) and *S. mutans* (**B**) biofilm removal after different treatment modalities at 5000× magnification (scale bar represents 10 μm). **A**: **a**) *C. albicans* (control); **b**) nystatin; **c**) *P. granatum peel*; **d**) *U. dioica*, and **e**) *E. arvense*.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

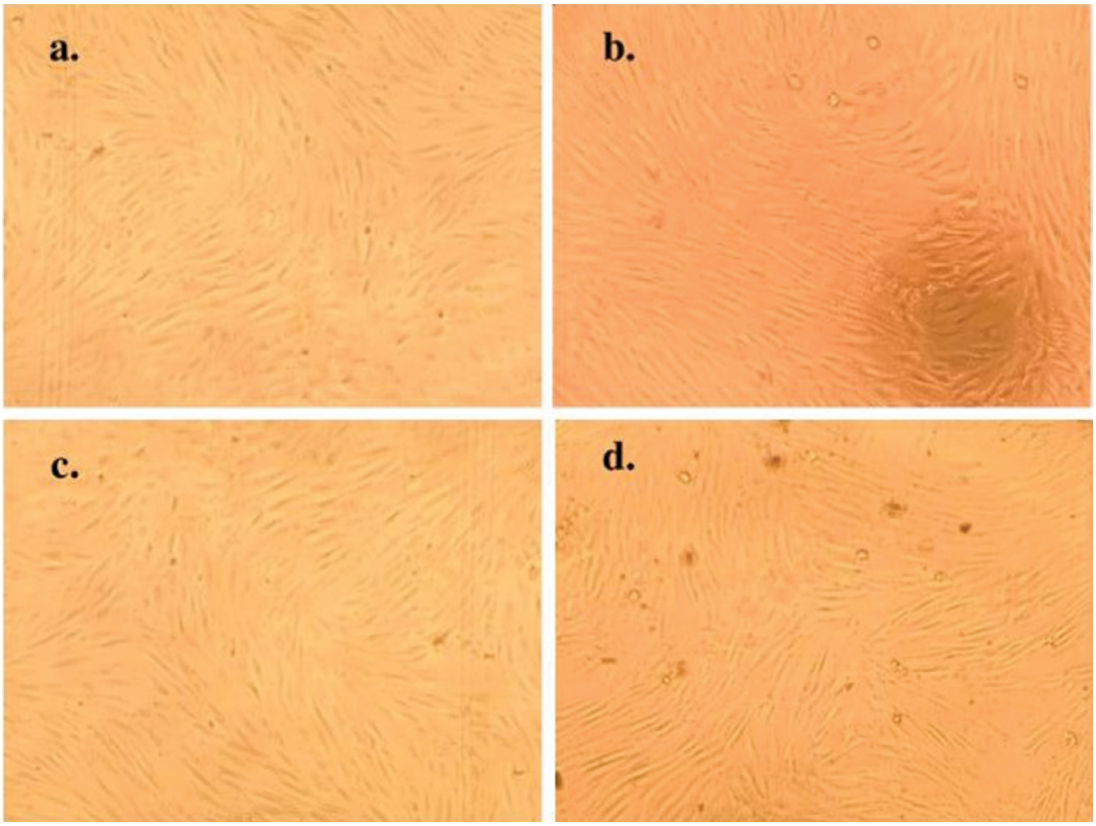

Figure 3. Cell viability of treated HGF cell by MTT assay: **a**) Control (HGF cell without treatment); **b**) *P. granatum peel*; **c**) *U. dioica*, and **d**) *E. arvense*. ×40 magnification.

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, Jyoti 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 standard 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 bacteria 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-candidate 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 mouthwashes.

Acknowledgements

This research was supported by Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences & Health Services grant No. 97-02-70-38871.

REFERENCES

1. Abdelrahman HF, Skaug N, Francis G. In vitro antimicrobial effects of crude Miswak extracts on oral pathogens. In Vitro 2002; 10(14):15–21.

2. Leikin JB, Paloucek FP. Chlorhexidine gluconate. In: Leikin JB, Paloucek FP, editors. Poisoning and Toxicology Handbook, 4th ed, New York: Informa; 2008: 183–4.
3. Almas K. The antimicrobial effects of extracts of Azadirachta indica (Neem) and Salvadora persica (Arak) chewing sticks. Indian J Dent Res 1999; 10(1):23–6.
4. Almas K, Skaug N, Ahmad I. An in vitro antimicrobial comparison of miswak extract with commercially available non-alcohol mouthrinses. Int J Dent Hyg 2005; 3(1):18–24.
5. da Costa LFNP, Amaral CdSF, da Silva Barbirato D, et al. Chlorhexidine mouthwash as an adjunct to mechanical therapy in chronic periodontitis: a meta-analysis. J Am Dent Assoc 2017; 148(5):308–18.
6. Barret M. The Dakin-carrel antiseptic solution. Dent Cosmos 1917; 59(44):446–8.
7. Kuruvilla JR, Kamath MP. Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. J Endod 1998; 24(7):472–6.
8. Thomas AM, Chandra S, Chandra S, et al. Elimination of infection in pulpectomized deciduous teeth: a short-term study using iodoform paste. J Endod 1994; 20(5):233–5.
9. Haffajee AD, Yaskell T, Socransky SS. Antimicrobial effectiveness of an herbal mouthrinse compared with an essential oil and a chlorhexidine mouthrinse. J Am Dent Assoc 2008; 139(5):606–11.
10. Haghighati F, Jafari S, Beyt EJ. Comparison of antimicrobial effects of ten Herbal extracts with chlorhexidine on three different oral pathogens; an in vitro study. Hakim Res J 2003; 6(3):71–6.
11. Rajabalian S, Mohammadi M, Mozaffari B. Cytotoxicity evaluation of Persica mouthwash on cultured human and mouse cell lines in the presence and absence of fetal calf serum. Indian J Dent Sci 2009; 20(2):169–73.
12. Mozaffari B, Mansouri S, Rajabalian S, et al. In vitro study between antibacterial and cytotoxic effects of chlorhexidine and persica mouthrinses. Shahid Beheshti University Dental Journal 2005; 23(3):494–509.
13. Clark AM. Natural products as a resource for new drugs. Pharm Res 1996; 13(8):1133–41.
14. Regezi JA, Sciubba J, Jordan RC. Oral pathology: clinical pathologic correlations: Elsevier Health Sciences 7th ed, 2016.
15. Dar SA, Ganai FA, Yousuf AR, et al. Pharmacological and toxicological evaluation of Urtica dioica. Pharm Bio 2013; 51(2):170–80.
16. Bisht S, Bhandari S, Bisht N. Urtica dioica (L): an undervalued, economically important plant. Agric Sci Res J 2012; 2(5):250–2.
17. Proestos C, Boziaris I, Nychas GJ, et al. Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. Food chem 2006; 95(4):664–71.
18. Aneja K, Joshi R. Antimicrobial activity of Amomum subulatum and Elettaria cardamomum against dental caries causing microorganisms. Ethnobotanical Leaflets 2009; 2009(7):3–8.
19. Čanadanović-Brunet JM, Ćetković GS, Djilas SM, et al. Radical scavenging and antimicrobial activity of horsetail (Equisetum arvense L.) extracts. Int J Food Sci Tech 2009; 44(2):269–78.
20. Radulović N, Stojanović G, Palić R. Composition and antimicrobial activity of Equisetum arvense L. essential oil. Phtother Res 2006; 20(1):85–8.
21. Sivananthan M. Antibacterial activity of 50 medicinal plants used in folk medicine. Int J Biosci 2013; 3(4):104–121.
22. Abdollahzadeh S, Mashouf R, Mortazavi H, et al. Antibacterial and antifungal activities of Punica granatum peel extracts against oral pathogens. J Dent (Tehran, Iran) 2011; 8(1):1–7.
23. Wafa BA, Makni M, Ammar S, et al. Antimicrobial effect of the Tunisian Nana variety Punica granatum L. extracts against Salmonella enterica (serovars Kentucky and Enteritidis) isolated from chicken meat and phenolic composition of its peel extract. Int J Food Microbiol 2017; 241:123–31.
24. Oral N, Vatansever L, Güven A, et al. Antibacterial activity of some Turkish plant hydrosols. Kafkas Univ Vet Fak Derg 2008; 14(2):205–9.
25. Bassiri-Jahromi S. In vivo comparative evaluation of the pomegranate (Punica granatum) peel extract as an alternative agent to nystatin against oral candidiasis. Iran J Med Sci 2018; 43(3):296–301.
26. Jorgensen JH, Turnidge JD. Susceptibility test methods: dilution and disk diffusion methods. Manual of Clinical Microbiology, 11th ed.: American Society of Microbiology 2015; 1253–73.
27. Fujiwara T, Hoshino T, Ooshima T, et al. Differential and quantitative analyses of mRNA expression of glucosyltransferases from Streptococcus mutans MT8148. J Dent Res 2002; 81(2):109–13.
28. Sato Y, Oketani H, Singyouchi K, et al. Extraction and purification of effective antimicrobial constituents of Terminalia chebula RETS against methicillin-resistant Staphylococcus aureus. Biol Pharm Bull 1997; 20(4):401–4.
29. Zouari Bouassida K, Bardaa S, Khimiri M, et al. Exploring the Urtica dioica leaves hemostatic and wound-healing potential. Biomed Res Int 2017; 2017:1–5.
30. Jyoti K, Baunthiyal M, Singh A. Characterization of silver nanoparticles synthesized using Urtica dioica Linn. leaves and their synergistic effects with antibiotics. J Radiat Res Appl Sci 2016; 9(3):217–27.
31. Akhlaghi N, Sadeghi M, Fazeli F, et al. The antibacterial effects of coffee extract, chlorhexidine, and fluoride against Streptococcus mutans and Lactobacillus plantarum: an in vitro study. Dent Res J 2019; 16(5):346–51.
32. de Queiroz GM, Politi FA, Rodrigues ER, et al. Phytochemical characterization, antimicrobial activity, and antioxidant potential of Equisetum hyemale L. (Equisetaceae) extracts. J Med Food 2015; 18(7):830–4.
33. Ferrazzano GF, Roberto L, Catania MR, et al. Screening and scoring of antimicrobial and biological activities of Italian vulnerary plants against major oral pathogenic bacteria. Evid Based Complementary Altern Med 2013; (3):1–10.
34. Mishra MP, Rath S, Swain SS, et al. In vitro antibacterial activity of crude extracts of 9 selected medicinal plants against UTI causing MDR bacteria. J King Saud Univ Sci 2017; 29(1):84–95.
35. Pagliarulo C, De Vito V, Picariello G, et al. Inhibitory effect of pomegranate (Punica granatum L.) polyphenol extracts on the bacterial growth and survival of clinical isolates of pathogenic Staphylococcus aureus and Escherichia coli. Food chem 2016; 190:824–31.
36. Cesur A, Soyer Y. Determining the effects of the stinging nettle (Urtica dioica) extract on biofilm formation of Salmonella enterica serovars. Scripta Sci Pharm 2017; 4(1):1–6.
37. Das G, Patra JK, Choi J, et al. Anticandidal effect of endophytic bacteria isolated from Equisetum arvense L. against Candida albicans and Candida glabrata. Braz Arch Biol Technol 2017; 60:1–7.
38. de Oliveira JR, Vilela PdGF, de Oliveira FE, et al. Antifungal effect of plant extracts on Candida albicans biofilm on acrylic resin. Braz Dent Sci 2013; 16(3):77–83.
39. Menezes SM, Cordeiro LN, Viana GS. Punica granatum (pomegranate) extract is active against dental plaque. J Herb Pharmacother 2006; 6(2):79–92.
40. Dazal M, Gonzales E, Sabino A, et al. Controlling biofilm formation

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by inhibiting the quorum-sensing activity of Pseudomonas aeruginosa using the ethanolic extracts of Piper nigrum (Piperaceae) fruit, Punica granatum (Lythraceae) Pericarp, and Pisum sativum (Fabaceae) seed. Int J Pharm Sci Drug Res 2015; 7(4):349–53.

41. Wojnicz D, Kucharska AZ, Sokół-Łętowska A, et al. Medicinal plants extracts affect virulence factors expression and biofilm formation by the uropathogenic Escherichia coli. Urol Res 2012; 40(6):683–97.

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

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Дата получения: 23 июля 2020 ♦ **Дата приемки:** 4 сентября 2020 ♦ **Дата публикации:** 31 декабря 2021

Образец цитирования: Amirinia F, Rad HS, Pourhajibagher M. In vitro antimicrobial and cytotoxicity activities of some medicinal plant extracts against oral microbial pathogens. Folia Med (Plovdiv) 2021;63(6):932-40. doi: 10.3897/folmed.63.e56840.

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 фибробластов человека.

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 разрушать 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*