













The Antifungal and Antibacterial Effect of *Citrullus Colocynthis*: In Vitro Study

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Abstract

BACKGROUND: Bacteria almost exclusively cause caries and other oral and dental diseases.

AIM: This study evaluated the inhibitory effect of the alcoholic extract of *Citrullus colocynthis* on bacteria and fungi common in bacterial oral diseases.

METHODS AND MATERIALS: The ethanol extract and essential oil of *C. colocynthis* were obtained through water distillation. After conducting phytochemical tests, including determination of total phenolic and flavonoid compounds of the extracts, GC-MASS test and anti-microbial tests of the extracts were conducted on *S. mutans*, *E. coli*, *S. salivarius*, *L. acidophilus*, *C. albicans*, and *S. aureus*. The cytotoxicity test was assessed by MTT assay.

RESULTS: MIC and MBC values for *S. mutans*, *S. salivarius*, and *L. acidophilus* were 1.56 mg/ml and 3.12 mg/ml, respectively. Moreover, *Citrullus colocynthis* also has an inhibitory effect on bacteria and *C. albicans* at these concentrations, according to the disc agar diffusion (DAD) test. The survival rate of cells at concentrations of 5 mg/ml and 10 mg/ml was <50%, and at concentrations of 2.5 mg/ml and 1.25 mg/ml was <70% after 24 h and 48 hours, respectively.

CONCLUSION: According to the cytotoxicity results of this plant at concentrations of 1.56 mg/ml and 3.125 mg/ml, the ethanolic extract of *C. colocynthis* has inhibitory and lethal effects against pathogenic bacteria and fungi.

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Introduction

Various causes, such as bacterial pathogens, underlying diseases, and genetic factors, can lead to oral and dental diseases, especially dental caries [1], [2]. Bacteria are the most common cause of the development and progression of various caries and other oral and dental diseases; therefore, antibiotic treatments have always played an influential role in improving these problems [3]. According to the latest statistics, the prevalence of dental caries has been reported to be 27–64% among 12-year-old children and 26–83% among adults. Dental caries has been reported up to 68% and at least 12% at different age groups in some developed countries [4]. *Streptococcus mutans*, commonly found in the oral cavity in humans, is a facultatively anaerobic and Gram-positive cocci; it has been associated with caries in several epidemiological studies and now thought to

play a significant role in the onset of caries. The initial development phase of dental caries is associated with this bacterium. *Streptococcus mutans* destroy tooth enamel by fermenting sucrose and producing lactic acid [5]. It also affects tongue and inner cheek causing creamy white lesions. Such bacteria may spread to the palate, gums, and tonsils in immune compromised patients regardless their age [6], [7]. The development of antibiotics is very costly, time-consuming, and risky. The increase in antibiotic-resistant bacteria and the significant decrease in the confirmation of antibacterial agents in recent decades caused widespread concern, and tooth decay has once again been identified as one of the biggest health challenges [8], [9], [10]. Today, drug-resistant microorganisms are emerging due to the widespread uncontrolled use of antibiotics; more than ever, formation of practical and new medical elements is urgently needed [11], [12], [13]. *Citrullus colocynthis* is a plant with tails and ascending stems, having spherical

fruits with green spots. In general, it can be found in the Mediterranean area, especially Izmir in Turkey, Nubia in Africa, and Trieste in Italy. It grows in Southern Iran. Conventionally, *Citrullus colocynthis* used to treat jaundice, bronchitis, asthma, and joint pain [14], [15]. This plant generally contains choline, cucurbitacin glycosides, and glycoside colocynthin; specifically, the plant fruit contains hentriacontane compounds N-octacosanol and sterols, and the roots contain various saponins [16], [17], [18]. Cucurbitacin in this plant is the essential antibiotic and antifungal discovered. Studies indicated that *Citrullus colocynthis* fruits and seeds have various therapeutic effects, including anti-inflammatory, analgesic, root canal treatment, anti-cancer, immune-boosting, antioxidant, and hypoglycemic effect [19], [20], [21], [22]. This study aims to determine the antimicrobial effect of the alcoholic extract of *Citrullus colocynthis* on bacteria and fungi common in oral infections.

Materials and Methods

Essence extraction

Citrullus colocynthis fruit collected (Sistan and Baluchestan Province, Iran) was dried in shade and ground. Then, 330 g of dried fruit was extracted by Clevenger apparatus in three steps each time by distillation with water for 5 h. This plant did not have any special essential oil at this stage, but 10 ml of essence was obtained, dehydrated by sodium sulfate (lacking water), and kept in the dark, closed container away from light and refrigerated at 3°C.

Alcoholic extract

Dried fruits of *Citrullus colocynthis* were crushed by the mill. A total of 400 g of the crushing plant was soaked in 1.2 L of ethanol solution and water at a ratio of 70–30 volumes for 3 days while being stirred twice/day to bring the main compounds into the solution. This process was repeated twice with the chopped plant after 3 days through a filter. As much as 35g of dry extract was obtained after eliminating the ethanol with a rotary evaporator, lyophilizing the extracts, and separating the water.

The total amount of phenols and flavonoids in the alcoholic extract

The total amount of phenolic compounds was determined by diluting Folin-Ciocalteu 2 mg with water and adding 0.5 ml of alcoholic extract, followed by sodium carbonate (2 ml, 75 g/L). A wavelength of 765 nm was used for measurement after centrifugation for 15 s and 30 min at 40°C until staining was complete. Sodium nitrate solution (5%, 0.15 ml) were added to

5 ml of alcoholic extract, then AICL3 solution (10%, 0.15 ml) was added after 6 min, and left it for 6 min to determine the total amount of flavonoid compounds. A final volume of 5 ml of deionized water and sodium hydroxide (4%, 2 ml) is obtained. At 510 nm, the intensity of the pink color was calculated after 15 min.

Determination of MIC and MBC for bacterial strains

Standard strains *S. mutans* (ATCC: 35668), *S. salivarius* (ATCC: 19258), *E. coli* (PTCC: 1269), *S. aureus* (ATCC: 29213), and *L. acidophilus* (ATCC: 4356) bacteria, and standard strains of *C. albicans* were purchased from Iranian Research Organization for Science and Technology and Pasteur Institute of Iran, Tehran, Iran. According to the Clinical and Laboratory Standards Institute, to prepare LHB, horse defibrillated blood was mixed 50/50 with sterile distilled water, and for 6 times, a frozen-defrosted operation was performed on it. then centrifuged at 12,000 g for 20 min, the clear supernatant was separated and mixed with 50% of Mueller Hinton broth in 96-well plates. First, it was poured from the reinforced medium under a completely sterile condition under a class 100 laminar hood. Concentration of 50 mg/ml of ethanolic extract was prepared, and concentration gradient prepared for different wells was 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, 0.09, 0.04, and 0.02 mg/ml, respectively. Then, 100 µl of the alcoholic extract was poured into the first well, and after several up and down, 100 µl was removed from the first well and transferred to the second well. This was conducted up to well number 11, well number 12 was also considered as positive control. Next, 0.5 McFarland solution was prepared from the bacterium. It was diluted with sterile physiological saline at a ratio of 1/100. It was then shaken gently and incubated at 37°C. As much as, 100 µl of 0.5 McFarland was prepared and poured into all wells except well number 11. Well number 11 was considered as the negative control. The plates were embedded with parafilm and incubated at 37°C incubator in anaerobic jar with candles for 24 h. The results were read after 24 h in the incubator according to the growth of the bacteria. To calculate the MBC, a well representing MIC and the previous three wells were sampled and cultured on blood agar and placed in anaerobic jar with a candle in a 37°C incubator for 24 h. The results were read according to bacterial growth.

Determination of MIC and MFC for *C. albicans*

First, several new yeast colonies were mixed with 5 ml of sterile physiological serum and adjusted to absorbance 0.15 at wavelength of 530 nm with spectrophotometer. The suspension was diluted 1/1000 and inoculated into a Roswell Park Memorial Institute Medium (RPMI). The broth microdilution method was applied to determine the MIC of the extract on the fungal

strain. For this purpose, in completely sterile conditions and under the laminar hood, 200 μ l of RPMI-1640 liquid medium was added to column 1 well, and 100 μ l of RPMI-1640 liquid medium was added to column 3–12 wells. Row A was considered for the alcoholic extract. Concentrations of 50 mg/ml of ethanolic extract were prepared, and concentrations prepared for different wells were 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, 0.09, 0.04, and 0.02 mg/ml, respectively. After adding the appropriate amount of alcoholic extract, the volume of well 2 using RPMI-1640 medium reached 200 μ l. 100 μ l was removed from the second well and added to the third one. Similarly, serial dilution of the investigated compounds was prepared in wells 2–11. Finally, 100 μ l of fungal suspension was added to all wells in rows 2–12. The first column wells of row A containing only 200 μ l of culture medium were used as blanks. The number 12 row A wells containing fungal suspension and RPMI medium without extract were used as the positive control. They were incubated at 32°C for 2 days. The concentration of the first clear well or well with a 90% reduction in turbidity compared to the control was determined as MIC. In all columns of rows C and D, a concentration of 1.100 of the extract was prepared so that the experiment should be repeated in case of inconsistency with the results obtained from the above two columns. To calculate the MFC, as much as the size of a sterile loop from a well representing MIC and the previous three wells, samples were taken and cultured at sabouraud 4% dextrose agar under the conditions mentioned above.

Disk agar diffusion method (DAD)

A different microbial strain was isolated from sterile swabs, inoculated on BHI agar, and cultured on grass. A sterile blank paper disk with a diameter of 6 mm was used to absorb the alcoholic extract of 50 L (Padtan Teb Laboratory Instruments, Iran). Each disc was placed 24 mm apart using sterile forceps and placed on the culture medium. Calculating the inhibition zone around the disc by mm was conducted after placing the plates at 37°C for 24 h [23].

Preventing biofilm formation

Microtiter plates were prepared by preparing turbidity 0.5 McFarland from 18- to 24-h cultures of pathogenic bacteria in TSB and sucrose and diluting at a ratio of 1/100. To affect the strength of biofilm formation, each extract was applied twice as much as MBC. An equal volume of bacterial suspension and extract solution was transferred to each microplate well. Physiological serum, chlorhexidine, and 200 ml of bacterial suspension formed the negative control wells. It was then incubated for 24 h at 37°C. The contents of the wells were removed after 24 h, and the wells were washed 3 times with phosphate saline buffer. A solution

of 33% glycolic acetic acid was added to 200 ml of crystal violet-stained cells. ELISA-Reader measured light absorption at 570 nm after 15 min and control wells compared percentage reduction in connection. The following equation was used to measure the biofilm formation rate:

$$\text{The biofilm formation rate} = \frac{\text{Samples(OD)}}{\text{Control(OD)}} \times 100$$

Biofilm degradation activity test

A microdilution method was used. TSB medium was added to synthetic saliva (McDougal solution) and 3% of glucose, and bacteria and fungi were inoculated. After 24 h incubation at 37°C, the compound was mixed. Following biofilm formation, extracts (MBC concentration) were combined with biofilm and incubated again for 24 h at 37°C. Phosphate buffer used to wash biofilms, and crystal violet used to stain the wells. About 95% of alcohol was added to the wells after three washes with sterile water. A new microplate used after 45 min of incubation. Optical absorption was measured in each well using a microplate reader at 570 nm to determine the extent of biofilm degradation. In the negative control, physiological serum was used instead of chlorhexidine 0.2%. Using the following equation, the percentage of biofilm reduction was calculated:

$$\text{The biofilm reduction rate} = \frac{\text{Samples(OD)}}{\text{Control(OD)}} \times 100$$

Cell culture and MTT test

HEK-293 cell line and human dermal fibroblasts were purchased from Pasteur Institute of Tehran and cultured at 37°C in an incubator with 5% CO₂ pressure and 95% humidity in RPMI-1640 medium containing 10% fetal bovine serum. MTT solution was prepared by dissolving in PBS, and the prepared solution was poured into small tubes and stored in a light-resistant container in the refrigerator. To each 96-well plate well, 90 μ l of culture medium containing 10⁵ cells of the intended cell line was added and incubated for 24 h. During incubation, the cells were examined for growth and non-contamination. The culture medium was then removed from the wells, and 150 μ l containing 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5, and 10 mg/ml of ethanol extract of *Citrullus colocynthis* were added to three wells from each concentration. Three wells were controlled without extract and only containing culture medium. The 96-well plate was then incubated for 24, 48, and 72 h so that the cells would be sufficiently in contact with the tested extracts. Finally, each well was examined by an ELISA reader at the wavelength range of 490-630 nm. The percentage of cell viability was measured using the following equation:

$$\text{The percentage of Cell viability} = \frac{\text{Samples(OD)}}{\text{Control(OD)}} \times 100$$

Data analysis

It was determined that the best way to compare the groups was to use a one-way ANOVA and a Tukey test based on SPSS software V-16.

Results

The total amount of phenolic and flavonoid compounds

The results of this study have shown that *C. colocynthis* fruit contains 9.73 mg/g of phenolic compounds and 6.47 mg/g of flavonoid compounds.

Results of MIC and MBC of ethanolic extract of *C. colocynthis* on the common bacteria in oral and dental infections

The MIC and MBC of the alcoholic extract of *C. colocynthis* on bacterial strains showed that the extract's most inhibitory and lethal activity were on *E. coli* and *S. aureus* bacteria at 0.78 mg/ml (Table 1).

Table 1: Minimum inhibitory concentration and minimum bactericidal concentration values of the ethanolic extract of *Citrullus colocynthis* on bacterial strains

Microorganism type	<i>Citrullus colocynthis</i>	
	MIC (mg/ml)	MBC/MFC (mg/ml)
<i>Escherichia coli</i>	0.78	1.56
<i>Staphylococcus aureus</i>	0.78	1.56
<i>Streptococcus mutans</i>	1.56	3.12
<i>Streptococcus salivarius</i>	1.56	3.12
<i>Lactobacillus acidophilus</i>	1.56	3.12
<i>Candida albicans</i>	3.12	6.25

All samples were tested 3 times in independent experiments. MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration.

Results of MIC and MBC of ethanolic extract of *C. colocynthis* on *C. albicans*

The MIC of the alcoholic extract of *C. colocynthis* was 3.12 mg/ml on *C. albicans*, and the MFC was 6.25 mg/ml.

Results of the MTT test of fibroblast cells

The results of the percentage of fibroblast viable cells at different concentrations showed that with increasing the concentration of alcoholic extract, the percentage of viable cells decreased compared to the control group. At a concentration of 10 mg/ml after 24 and 48 h and a concentration of 5 mg/ml in the first 24 h, more than 50% of cells were killed ($p < 0.01$), but cell viability at the concentration of 2.5 mg/ml was

significantly higher than 5 mg/ml after 24 and 48 h (Figure 1).

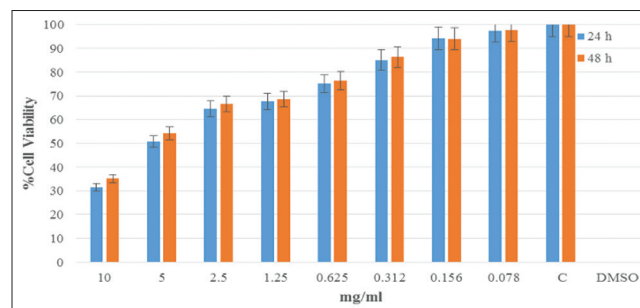


Figure 1: Viability rate of fibroblast cells in exposure to the ethanol extract of *Citrullus colocynthis* during 24 and 48 h of incubation

Results of MTT test of the HEK cells

The results related to the percentage of live cells in the HEK and fibroblast cells at different concentrations showed that with increasing the concentration of alcoholic extract, the percentage of surviving cells decreased compared to the control group, and at a concentration of 10 mg/ml (after 24 and 48 h) and a concentration of 5 mg/ml (in the first 24 h) more than 50% of the cells are killed ($p < 0.01$). After 24 and 48 h, no significant difference was indicated between 2.5 and 5 concentrations ($p > 0.05$) (Figure 2)

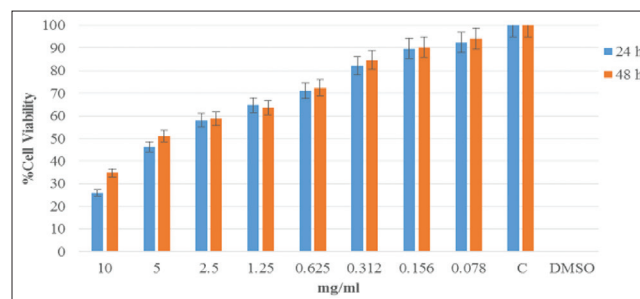


Figure 2: Viability rate of HEK line cells in exposure to the ethanol extract of *Citrullus colocynthis* during 24 and 48 h of incubation

Results of DAD test

Based on the results of the DAD test, there was no statistically significant difference between the growth inhibition zone of the alcoholic extract against bacteria and chlorhexidine compared to the growth inhibition zone of chlorhexidine ($p > 0.05$). There was, however, a significant difference in growth inhibition zones between the alcoholic extract and the chlorhexidine against *C. albicans* ($p < 0.01$) (Table 2).

Table 2: The value of the growth inhibition zone of the extracts and formulation in the disc agar diffusion test

Microorganism type	<i>Citrullus colocynthis</i> (mm)	CHX 0.2% (mm)
<i>Escherichia coli</i>	13	18
<i>Staphylococcus aureus</i>	11	19
<i>Streptococcus mutans</i>	10	17
<i>Streptococcus salivarius</i>	11	18
<i>Lactobacillus acidophilus</i>	9	17
<i>Candida albicans</i>	5*	17

*The significant difference ($P < 0.01$) with CHX 0.2%. All samples were tested three times in independent experiments. CHX: Chlorhexidine.

Results of preventive effects of the ethanolic extract on the biofilm formation

The results of the effect of alcoholic extract of *Citrullus colocynthis* on biofilm formation showed that the percentage of biofilm formed on all bacteria and *C. albicans* was significantly higher compared to chlorhexidine ($p < 0.01$) (Table 3).

Table 3: Percentage of biofilm formed after being exposed to the ethanolic extract of *Citrullus colocynthis*

Microorganism type	OD (570 nm) Sample 1 (%)	CHX 0.2% (%)
<i>Escherichia coli</i>	75*	20
<i>Staphylococcus aureus</i>	80*	22
<i>Streptococcus mutans</i>	80*	20
<i>Streptococcus salivarius</i>	85*	25
<i>Lactobacillus acidophilus</i>	85*	20
<i>Candida albicans</i>	90*	22

*A significant difference ($P < 0.01$) with CHX 0.2%. All samples were tested 3 times in an independent experiment. CHX: Chlorhexidine.

Results of degradative effect of ethanolic extract on biofilm

The effects of the alcoholic extract on biofilm degradation showed that the percentage of biofilm degradation of all bacteria and *C. albicans* in the investigated group was significantly lower compared to chlorhexidine ($p < 0.01$) (Table 4).

Table 4: Percentage of reduction of biofilms after formation and exposure to the ethanolic extract of *Citrullus colocynthis*

Microorganism type	OD (570 nm) Sample	CHX 0.2% (%)
<i>Escherichia coli</i>	17*	75
<i>Staphylococcus aureus</i>	15*	72
<i>Streptococcus mutans</i>	13*	75
<i>Streptococcus salivarius</i>	11*	70
<i>Lactobacillus acidophilus</i>	12*	70
<i>Candida albicans</i>	9*	72

*The significant difference ($P < 0.01$) with CHX 0.2%. All samples were tested 3 times in an independent experiment. CHX: Chlorhexidine.

Discussion

The increasing trend of fungal and bacterial diseases in susceptible people, including people with AIDS, diabetics, consumers of broad-spectrum antibiotics, and people undergoing chemotherapy on the one hand, and drug resistance (including both intrinsic and acquired) on the other hand, there has been an increasing tendency by researchers to investigate herbal medicines for their better efficacy and fewer side effects [24]. In this study, the inhibitory effect of ethanolic extract of *Citrullus colocynthis* on various microorganisms, including bacteria active in caries and *C. albicans*, was investigated to determine MBC/MFC and MIC. The MIC for *E. coli* and *S. aureus* bacteria was 0.78 mg/ml; for *S. mutans*, *S. salivarius*, and *L. acidophilus*, it was 1.56 mg/ml. The MBC of *E. coli* and *S. aureus* bacteria was 1.56 mg/ml, and for *S. mutans*, *S. salivarius*, and *L. acidophilus*, it was 3.12 mg/ml. As many as, 60% of fibroblasts and embryonic kidney cells survived at the concentration of the MICs mentioned above, but at MBC concentrations,

only about 50% of cells survived, indicating that despite the beneficial effect of the ethanolic extract of the plant on the investigated microorganisms, given the high cytotoxicity of this plant, living cells will be damaged as well. Moreover, by measuring the growth inhibition zone of this plant extract, it was found that this plant's antibacterial effect was lower than that of the control group. This difference was not significant. Moreover, in this study, by investigating the extract of this plant on *C. albicans*, the concentration of MIC was 3.125 mg/ml, and MFC was 6.25 mg/ml, indicating the need for higher concentrations of the extract of *C. colocynthis* for inhibitory and lethal activity on *C. albicans* against the bacteria. In addition, by investigating the growth inhibition zone, the effectiveness of this plant on *C. albicans* was properly indicated. However, this growth inhibition zone was significantly lower than that of the control group ($p < 0.05$). The study conducted by Shaikh *et al.* (2016) attempted to evaluate the antibacterial activity of methanolic extract of dried *Citrullus colocynthis* fruit on 30 types of bacteria and five species of fungi, including *C. albicans*. In their study, they used the disc agar diffusion method and observed that the methanolic extract of this plant had no antibacterial effect on bacteria and fungi. The cytotoxic effect of *Citrullus colocynthis* was also measured on rats, indicating damage and major changes in the histology of the heart, kidneys, and liver tissues [25]. Unlike the study conducted by Shaikh, the antibacterial and antifungal activity of ethanolic extract of *C. colocynthis* was observed on bacteria and *C. albicans*. However, its effect was less than that of the control group, and the effect of this plant on *C. albicans* was significantly less than that of the chlorhexidine group. However, in terms of cytotoxicity, similar results were obtained in two studies. Eidi *et al.* (2012) measured the antifungal effect of the ethanolic extract of *Citrullus colocynthis* on different species of *Candida* and *Aspergillus* using the DAD method. In their study, the MIC value was in the range of 3.125–12.5 mg/ml, and the MFC was in the range of 6.25–12.5 mg/ml. They concluded that this plant extract has an effective antifungal effect [26]. While the value of MIC and MFC of *C. albicans* in our study was 3.125 mg/ml and 6.25 mg/ml, respectively. Compared with the mentioned study with a concentration of 6.25 mg/ml and 12.5 mg/ml, respectively, at lower concentrations, we obtained an inhibitory and lethal effect on this fungus. Thus, the diameter of the growth inhibition zone was higher than that of the study mentioned above. In the study conducted by Marzouk *et al.* (2011), which investigate the antibacterial and antifungal effect of aqueous and acetic extracts obtained from different parts of this plant in three maturity levels of the fruit and seed of Tunisian *Citrullus colocynthis* on different Gram-positive and Gram-negative bacteria and different *Candida* species. The results of the study indicate that the fruit of this plant in comparison to other parts of plant had the lowest MIC, MBC, and MFC concentration and showed the highest inhibitory and lethal effect. In contrast, the root of this plant showed the least inhibitory and lethal

effect. Furthermore, the aqueous extract had a better effect on bacteria than the acetonic one. In contrast, the acetonic extract showed a greater effect on fungi [27]. The present study attempted to investigate the effect of ethanolic extract of *Citrullus colocynthis* on bacteria effectively in dental caries, including *Streptococcus* and *Lactobacillus acidophilus*; the results showed a good inhibitory and lethal effect on these bacteria. In Marzouk's study, the phytochemical method was used just like the present study, but unlike this study, no flavonoids were reported in the fruit of the plant. The reason behind this difference could be the use of different species of *Citrullus colocynthis*. In Marzouk's study, the species grown in Tunisia were used. Although, according to the results, flavonoids were not observed in the fruit of this plant, flavonoids were detected in the seeds of this plant. Still, the fruit of this plant showed a more inhibitory and lethal effect. It can be concluded that other important elements in this plant, including alkaloids, iridoids, and steroids, can also have antimicrobial effect. In the study conducted by Najafi *et al.* (2010), after conducting phytochemical experiments on *Citrullus colocynthis* fruit, the effect of alcoholic and aqueous extracts on standard and hospital strains of *S. aureus* was conducted through the DAD method, and compared with the antibacterial effect of Novobiocin antibiotic. It was reported that ethanolic extract has a greater inhibitory effect than aqueous extract, and this effect is completely dose-dependent [28]. In the present study, the highest inhibitory and lethal effect of ethanolic extract was on *S. aureus* and *E. coli* bacteria; this is in line with the results of the study conducted by Najafi. Gowri *et al.* (2009) investigated the antibacterial effect of chloroform and acetonic extract of *Citrullus colocynthis* leaf through the DAD method on *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *S. marcescens* bacteria. The results have indicated that the leaf extract of this plant (100 mg/ml) was significantly effective against all these bacteria, and the acetonic extract showed the highest effect on *P. aeruginosa* [29]. This is in line with the results of our study. Biofilm degradation and biofilm prevention experiments showed that *S. mutans* and *L. acidophilus* biofilm, the effective bacteria in causing caries, formed 80% and 85% after being exposed to ethanolic extract, respectively; compared to the chlorhexidine group, ethanolic extract was significantly weaker than chlorhexidine. Moreover, with the biofilm degradation effect, only 12% of *S. mutans* biofilm and 13% of *L. acidophilus* biofilm were destroyed. The ethanolic extract of *Citrullus colocynthis* was significantly lower than chlorhexidine.

Conclusion

The ethanolic extract of *Citrullus colocynthis* fruit has inhibitory and lethal effects on common pathogenic bacteria and fungi. However, according to

the results of cytotoxicity tests of this plant, at MIC and MBC concentrations, it destroys 50% of living tissue cells. Therefore, using this plant as a mouthwash is not recommended to suppress the occurrence and progression of caries and fungal infections of *C. albicans*.

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